Planta Medica

August 2011 · Page 1229 – 1472 · Volume 77

1229 Editorial

1230 Lectures

1232 Workshops
1232 WORKSHOP I: Young Researchers Workshop
   Rapid Strategies for (phyto) Chemical Characterization of Natural Products
   Chairs: J. L. Wolfender, J. Rollinger, A. R. Bilia
1235 WORKSHOP II: Young Researchers Workshop
   Rapid Strategies to Assess Bioactivity of Natural Products
   Chairs: D. Tasdemir, T. Efferth, A. Hensel
1238 WORKSHOP III: Permanent Committee on Regulatory Affairs of Herbal Medicinal Products
   Chairs: A. Vlietinck, S. Alban
1239 WORKSHOP IV: Traditional Chinese Medicine Workshop
   Chairs: D. Guo, R. Bauer, G. Franz
1240 WORKSHOP V: Biological and Pharmacological Activities of Natural Products
   Chair: V. Butterweck
1241 WORKSHOP VI: Quality/Agriculture joint Workshop
   Chairs: C. Franz, C. Erdelmeier

1242 Short Lectures

1263 Poster
1263 Topic A: Analytical Methods
1276 Topic B: Biotechnology
1288 Topic C: Clinical Studies
1290 Topic D: Cultivation and Breeding
1293 Topic E: Essential oils
1307 Topic F: Ethnopharmacology/Traditional and natural medicines
1330 Topic G: Isolation and structure elucidation
1357 Topic H: Marine natural product research
1359 Topic I: Molecular Biology
1362 Topic J: Nutraceuticals and Dietary Supplements
1368 Topic K: Pharmaceutical Applications
1373 Topic L: Pharmacognosy/Pharmaceutical Biology and Biodiversity
1399 Topic M: Pharmacology/Biological Activity
1456 Topic N: Veterinary Applications

1459 Authors’ Index

1472 Masthead

Cover picture: FTS Tourism
Dear Colleagues,

It is my great pleasure and honour to hold the 59th International Congress and Annual Meeting of the Society for Medicinal Plant and Natural Product Research on September 4–9, 2011 in Antalya, Turkey. This congress series has been organized annually since 1953 and has become the most important and popular congress in Europe in its respected field. It is the first time the congress is organized in Turkey. Turkey is a large peninsula bridging the east and the west at the junction of two continents and has been a passage way between Europe and Asia and even Africa. Due to its geographic location Turkey has been a melting pot of civilizations, cultures and nations, and is full of history and home to diverse traditions. It is a land of many firsts since history starts here. Thanks to its climatically and phytogeographically unique position and its transect ranging from sea level (0 m) to the peak of the Ararat mountain (5137 m) the flora of Turkey is rich and diverse with over 12,000 flowering plant taxa recorded of which 33% are endemic. Anatolia is the land of Galenus of Pergamon and Dioscorides of Anavarza. Pedanius Dioscorides, a physician in the Roman Army had written his famous Materia Medica in the 1st century AD. His birthplace Anavarza is in Kozan, Adana in Southern Turkey not too far from Antalya. The 59th Congress has attracted global attention and there are participants from all parts of the world. Its scientific level is high thanks to the efforts of the Scientific Committee. High rate of rejects were due to the meticulous work of the reviewers who gave it time and effort to keep the scientific level as high as possible.

Main topics of the Congress are as follows:
- New Trends in Pharmacognosy
- Traditional and Natural Medicines
- Leead Finding from Nature
- Antimicrobials – What’s next?
- Endophytes – Importance in Pharmacognosy
- Natural Immune Enhancers
- Nutraceuticals, Cosmeceuticals, Functional Foods – Prevention of Metabolic Diseases
- Essential Oils – Analysis, Bioactivities, Uses, Therapeutical Potential
- Biotechnology and Nanobiotechnology
- Advances in the Analysis of Natural Products

Ten plenary and two keynote lectures will be presented by distinguished scientists. 73 short lectures will be presented in three parallel sessions. Numerous researchers will be able to report their research findings in 900 poster presentations. In addition, young researchers will be able to present their papers at two parallel Young Researchers Workshops. There will also be three more Permanent Committee Workshops of the GA on regulatory affairs, pharmacology, agriculture and quality of natural products. An additional workshop will be held on Traditional Chinese Medicine (TCM). 31 lectures will be presented in the workshops. All in all over 1100 scientific presentation will be made at the congress.

I would like to thank the Executive and the Advisory Board members of the GA for their help and encouragement during the preparatory stages of the Congress. I wish to extend my grateful thanks to Georg Thieme Verlag KG for processing such a huge number of abstracts in a short time. My special thanks go to the members of the Organizing Committee and to the Congress Organizing Company FTS who have done their utmost to offer you a successful, satisfying and enjoyable congress.

I wish you all a fruitful congress which I hope will strengthen old friendships and develop new ones in a friendly, scientific and cultural atmosphere. I hope everybody enjoys their stay in sunny Antalya, gets the opportunity to discover hidden beauties of the region and Turkey, and takes home new scientific knowledge and unforgettable memories.

Prof. Dr. K. Hüsnü Can Başer
President of the 59th International Congress and Annual Meeting of the Society for Medicinal Plant and Natural Product Research
Abstracts | 59th International Congress of the GA | 4th–9th September 2011, Antalya, Turkey

1230

Abstracts

L2

Combination of ethnopharmacological knowhow with modern in silico tools
Bollinger JM
Institute of Pharmacy/Pharmacognosy, Center for Molecular Biosciences Innsbruck, University of Innsbruck, Innsbruck, Austria

Computational methods are valuable tools in current drug discovery and development processes. They aim at analyzing, understanding, and predicting the bioactivity of a compound with respect to a specific biological target, and have been applied successfully in medicinal chemistry. Their application in natural product research is however affected with some challenges, such as limited availability of high quality natural product databases, often restricted or laborious access to individual compounds for testing, and lack of chemoinformatics experience with secondary metabolites. This asks for a sensible application of data mining tools in this prospering field of lead finding from nature. The hyphenation of in silico strategies with knowledge from ethnopharmacology offers a unique opportunity to benefit from a combined theoretical and empirical approach. Herbal remedies, which are used since centuries, represent a particularly promising resource for drug leads. These tools are useful for target identification at the bioactivity of a compound with respect to a specific biological target, and have been applied successfully in medicinal chemistry. Their application in natural product research is however affected with some challenges, such as limited availability of high quality natural product databases, often restricted or laborious access to individual compounds for testing, and lack of chemoinformatics experience with secondary metabolites. This asks for a sensible application of data mining tools in this prospering field of lead finding from nature. The hyphenation of in silico strategies with knowledge from ethnopharmacology offers a unique opportunity to benefit from a combined theoretical and empirical approach. Herbal remedies, which are used since centuries, represent a particularly promising resource for drug leads. These tools are useful for target identification at the

L3

Biological Activities of Essential Oils
Buchbauer G
Department of Clinical Pharmacy & Diagnostics, Center of Pharmacy, Faculty of Life Sciences University of Vienna, Althanstrasse 14, A-1090 Vienna, Austria

Essential Oils (natural mixtures of single fragrance compounds) do possess biological properties. Besides psychological influences, these rather small and lipophilic molecules demonstrate distinct physiological activities upon inhalation and/or topical administration which can be shown in animal experiments as well as in human studies. After a short introduction about the correct definition of the term “Essential Oils” new research results on the application of fragrance compounds in therapy are presented. Examples of the biological properties of these natural compounds will be discussed and a great bow drawn from the effects on the human autonomic and central nervous system to “other effects”. These cover e.g. anti-inflammatory activities, anti-oxidative ones and penetration enhancing properties, anti-microbial and insect repellent activities and the possibility to use these small molecules in cancer prevention or therapy and for Alzheimer’s disease. Some studies on the biochemical mechanism of such effects are also presented as well as methods to investigate the above activities of essential oils.

L4

Infectious diseases and natural products. What is next?
Tasdemir D
Centre for Pharmacognosy and Phytotherapy, School of Pharmacy, University of London, London WC1N 1AX, UK

Infectious diseases caused by bacteria, viruses, parasites and fungi are results of complex interactions between the pathogen, the host and the environment. The early discovery of quinine, followed by the antibiotics and more recently artemisinin has brought a new, realistic hope in the control of infections that once ravaged the humankind. However, the widespread use of these drugs and globalization has led to the development of multidrug-resistant pathogens worldwide. On the other hand, the pharmaceutical industry that previously relied extensively on natural products (NPs) as source of small molecules for anti-infective drug discovery and development has undergone a significant de-emphasis in NP research. The main research activity currently falls on to academia and requires novel approaches for tackling infectious diseases. This lecture will emphasize the opportunities and the challenges in this area and present new application focus and areas for natural products. Specific examples from our own research will be presented in order to point out the potential of vast variety of natural products derived from plants, marine organisms and other sources in both prophylaxis and the chemotherapy of infectious diseases caused by parasites (e.g. malaria, trypanosomiasis, leishmaniasis, schistosomiasis and river blindness) and (myco)bacteria (e.g. tuberculosis). Target determination, molecular properties (lipophilicity, permeability, drug-likeness) and pharmacokinetic properties of some natural and natural product-derived synthetic leads will also be included. Keywords: Infectious diseases, natural products, drug discovery, prophylaxis, chemotherapy

L5

Natural products derived from traditional chinese medicine as novel inhibitors of the epidermal growth factor receptor in cancer cells
Efferth T
Department of Pharmaceutical Biology, Institute of Pharmacy and Biochemistry, Johannes Gutenberg University, Mainz, GERMANY

The success of established anticancer drugs is frequently hampered nonsufficient tumor specificity leading to side effects and drug resistance. Oncogene products and tumor suppressors are exquisite targets developing more specific drugs with improved features. The epidermal growth factor receptor (EGFR) became an important target for drug development. However, clinical application of EGFR tyrosine kinase inhibitors resulted in resistance to EGFR-targeting drugs due to the selection of EGFR-mutated variants. This phenomenon forced the search for novel inhibitors of EGFR and downstream signaling cascades. We investigated the therapeutic role of the EGFR in human tumor biopsies (lung cancer, glioblastoma, head and neck cancer) by comparative genomic hybridization and immunohistochemistry (1-4) and identified phytochemicals (dicheantine, camptothecin derivatives, artemesinate etc.) affecting EGFR signaling (5,7-9). Here, we report on recent achievements

The role of pharmacokinetics in natural products research

Butterweck V
University of Florida, College of Pharmacy, Gainesville, FL, USA

In recent years the number of studies investigating the pharmacodynamic effects of botanicals has increased exponentially, often reporting pharmacological effects of botanical extracts with insignificant bioactivities obtained in irrelevant in vitro bioassays. The data interpretation from these in vitro assays for their efficacy in animals and humans is based on the assumption that a sufficient concentration of active constituents can reach the target sites of action in the body. This interpretation can be misleading since the pharmacokinetic properties of a compound are completely ignored. Although important, there is still limited information available regarding herbal pharmacokinetics. This might be due to the following reasons: (i) the active constituents are not known; (ii) the study of herbal pharmacokinetics is extraordinarily complex because extracts are multicomponent mixtures which contain several chemical constituents. Therefore, concentrations of single compounds in the final product are in the lower mg range per dose. (iii) The resulting plasma concentrations are often in the μg to pg per liter range. As a consequence analytical methods determining bioavailability and pharmacokinetics of natural compounds have to be sufficiently sensitive. Advanced techniques such as GC-MS/MS or HPLC-MS/MS can be used nowadays to accomplish these goals. A better understanding of the pharmacokinetics and bioavailability of natural compounds can help in designing rational dosage regimen; and it can further help to link data from pharmacological assays with clinical effects. In this presentation, pharmacokinetic studies will be discussed that have been conducted for some of the top-selling botanicals worldwide, including artichoke, echinacea, mangosteen and valerian.

Overview of Dietary Supplements in USA

Khan IA
National Center for Natural Products Research and Department of Pharmacognosy, School of Pharmacy, The University of Mississippi, MS 38677 U.S.A.

Herbal product studies cannot be considered scientifically valid if the product tested was not authenticated and characterized in order to ensure reproducibility in the manufacturing of the product in question. In the case of botanicals, misidentification of the collected plant, adulteration with other species or contamination with extraneous ingredients are possibilities in which reproducibility may be affected unknowingly to the manufacturer. Many studies refer to the use of standardized material, but in reality they are referring to chemical standardization. While chemical standardization is important, its utility is limited when the starting material is not well characterized botanically. Although the resulting studies are sound with respect to the actual product tested, adequate authentication of the product cannot be compared to other products on the market. Also, a comparison of one study to another cannot be made due to inconsistencies in the identity of the botanical matrix. The tools needed for authentication of the field plant material also depend on the plant and process involved. This could be as straightforward as botanical/morphological identification or as elaborate as genetic or chemical profiling. These controls are also critical for the evaluation of pharmacological, toxicological and clinical studies of the botanical supplements. Keywords: Herbal products, botanical supplements, authentication

Discovery and applications of naturally occurring cyclic peptides

Crak D1, Poth A1, Colgrove M1, Akcan M1, Oku B1, Chan A1, Daly N1
1Institute for Molecular Bioscience, The University of Queensland, Brisbane, Australia; 2CSIRO, Division of Livestock Industries, St Lucia, Australia

Over recent years more than 200 examples of ribosomally synthesized head-to-tail cyclised proteins have been discovered in bacteria, plants and animals [1]. The cyclotides [2] are the largest family of these cyclic peptides and have applications in drug design [3] and agriculture [4]. They occur in plants from the Violaceae (violet), Rubiaceae (coffee) and Cucurbitaceae (cucurbit) families and have a diverse range of biological activities, including ertuxin A, anti-HIV, and insecticidal activities. The latter suggesting that their natural function is in plant defence. Individual plants express suites of 10 – 100 cyclotides. Cyclotides typically comprise ~30 amino acids, and incorporate three disulfide bonds arranged in a cystine knot topology. The combination of this knotted secondary structure with a circular backbone renders the cyclotides impervious to enzymatic breakdown and makes them exceptionally stable. This presentation will describe the discovery of cyclotides in plants, their structural characterization, evolutionary relationships and their applications in drug design. Their stability and compact structure makes them an attractive protein framework onto which bioactive peptide epitopes can be grafted to stabilize them. Keywords: Cyclic peptides; cyclotides; drug design; NMR; protein structure References: [1] Crak D J (2006) Science 311: 1561 [2] Gruber C W et al (2008) The Plant Cell 20: 2471 – 2483. [3] Henrique S T, Crak D J (2010) Drug Discovery Today 15: 57 64. [4] Barbeta B L et al (2008) PNAS 105: 1221 – 1225

Natural immunomodulators – A Drug Discovery Perspective

Gertsch J
Institute of Biochemistry and Molecular Medicine, University of Bern, Switzerland

The immune system is an adaptive complex system which poses a challenge to therapeutic intervention. Inflammation is known to be involved in numerous disease processes like autoimmune attack and carcinogenesis and can be targeted by immunopharmacological agents. However, it is far less clear how the immune system may be stimulated [1]. In this talk, a new view on how the immune system recognizes and reacts to noxious plant food shall be provided. During the evolution of the immune system different endogenous systems have evolved that modulate inflammatory processes, such as the pattern recognition receptors (TLRs) and the arachidonic acid lipidome. Studying the molecular interactions in these biochemical units by small organic molecules provides insight into how inflammation may be regulated and ultimately manipulated. The endocannabinoid system is a stress signal-integrating lipid signaling network and provides great opportunities to treat inflammatory diseases and bone degeneration with the potential to link nutrition and inflammation [2 – 4]. Key features: Inflammation, immune system, immunomodulation, drug discovery, endocannabinoid system References: [1] Gertsch J, Viveros-Paredes JM, Taylor P (2010) Plant immunomodulators-Scientific paradigm or myth? J Ethnopharmacol PMID: 20620205 [2] Gertsch J, Leonti M, Raduner S, Racz I, Chen JZ, Xie XG, Altmann KH, Karsak M, Zimmer A (2008) Beta-caryophyllene is a dietary cannabinoid. Proc Natl Acad Sci USA 105(26):9099 – 104 [3] Gertsch J (2008) Antiinflammatory cannabinoids in diet: Towards a better understanding of CB(2) receptor action? Commun Integr Biol 1(1):26 – 8. [4] Schuhy W., Viveros Paredes J M, Kleyer J, Huefner A, Anavi-Goffer S, Raduner S, Altmann KH, Gertsch, J. (2011)Mechanisms of Osteoclastogenesis Inhibition by a Novel Class of Biphenyl-type Cannabinoid CB2 Receptor Inverse Agonists. Chem Biol in press

Pharmacognosy in Turkey

Cahis I
Near East University, Faculty of Pharmacy, Department of Pharmacognosy, Lefkoşa, Turkish Republic of Northern Cyprus

This presentation will give an overview on the wide range of studies of Pharmacognosy in Turkey, since its establishment as a discipline during the Ottoman Empire, up until contemporary times. Studies of Pharmacognosy have begun in 1839 together with the official education of
The vast majority of pharmacognosy research is focused on drug discovery in higher plants. However, the isolation and characterization of natural compounds and their analogs as lead compounds in drug discovery is a major focus of current research. Pharmacognosy has the potential to provide novel natural products with high therapeutic and medicinal value. The use of natural products in drug discovery has increased significantly in recent years, and many natural products have been developed into new drugs. Pharmacognosy research has made significant contributions to the discovery of new natural products with potential medicinal value.

Pharmacognosy, particularly using plant-derived products, has been a vital source of new chemical entities for drug discovery. The study of plant secondary metabolites has led to the discovery of numerous compounds that exhibit a wide range of biological activities, including antitumor, antimicrobial, anti-inflammatory, and other medicinal properties. The use of traditional medicinal plants in the treatment of diseases has a long history, and the study of these plants can lead to the discovery of new drugs. The integration of traditional knowledge and modern research methodologies is crucial for the discovery of new natural products with potential medicinal value.
tivariate data analysis, brought us closer to the final goal of metabolomics, comprehensive evaluation of all metabolites in living organisms including plants. Of many analytical platforms NMR has been thought as one of the most promising techniques to cover all the metabolite pool in a short time despite its inherent low sensitivity compared with MS-based technology. In addition to the unambiguous advantages of NMR such as broad coverage of metabolite detection, the easiness of data handling for further statistic treatment and signal robustness have been attracting many metabolists. In this presentation we will display applications of NMR-based metabolomics for chemical characterization of plants, plant physiology, and screening method of bioactive metabolites will be shown as well as a possible protocol developed by our groups [4]. Keywords: Metabolomics, NMR, Chemicals, Characterisation References: 1. Kim H K et al. (2011) Trend Biotechnol In press. 2. Verpoorte R et al. (2008) Phytochem Rev 7: 523. 3. Verpoorte R et al. (2007) Phytochem Rev 6: 2 4. Kim HK et al. (2010) Nat Protoc 3: 536.

WSI 1
On-line coupling of Centrifugal Partition Chromatography (CPC) to High Performance Liquid Chromatography-Mass Spectrometry (HPLC-MS/MS)
Michel I, Destandau E, Eljawir C
Institute of Organic and Analytical Chemistry (ICOA), University of Orléans-CNRS UMR 6005, BP 67059, 45067 Orléans cedex 2, France.

Phytochemical analyses of food and medicinal plant extracts require rapid screening and detection strategies to identify bioactive natural products. Centrifugal Partition Chromatography (CPC), a free solid support liquid-liquid chromatography, is a well established method for the isolation of natural products and fractionation of complex samples at the preparative scale [1,2]. Nevertheless, even if the separation is monitored by detectors the composition of the different collected fractions must be evaluated by further High Performance Liquid Chromatography (HPLC) which is time consuming and give delayed information on the fraction composition. We present here the development of a versatile tool for fast screening and rapid detection of bioactive natural products from plant extracts: the on-line coupling of CPC-UV to HPLC-UV/MS/MS. The coupling of CPC and HPLC systems, via a six position switching valve, reduces the time of complete fractionation procedure by direct on-line analyses of collected fractions and permits a guided fractionation step [3]. HPLC columns suitable for fast analysis, monolith and focused core columns, were evaluated to allow rapid analysis of compounds separated by CPC. Furthermore, the use of MS in tandem mode allows to get a direct structural identification of separated molecules. This strategy was applied to the fractionation and purification of bioactive compounds from berries and roots of sea buckthorn (Hippophae rhamnoides L. Elaeagnaceae), a Eurasian medicinal thorny bush, known to have various pharmacological effects [4]. Keywords: On-line CPC-HPLC-MS/MS, fractionation, purification, Hippophae rhamnoides References: 1. Marston A, Hostettmann K (2006) J Chromatogr A 1112: 181 – 194 2. Ingkaninan K et al. (2000) J Liq Chromatogr Relat Technol 23: 2195 – 2208 3. Michel T et al. J Chromatogr A Available online 1 February 2011 4. Gulyiyan VB et al. (2004) J Chromatogr B 812: 291 – 307

WSI 2
A rapid LC-MS method for the simultaneous quantification of Oleuropein and its main metabolite, Hydroxytyrosol, in clinical samples after oral administration of commercial herb medicinal products
Lemonakis N1, Halabalaki M1, Giakas E2, Mougias V2, Skaltsounis A1
1Department of Pharmacognosy and Natural Products Chemistry, Faculty of Pharmacy, University of Athens, Panepistimiopolis, Zografou, Athens, Greece; 2Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Athens, Panepistimiopolis, Zografou, Athens, Greece; 3Department of Physical Education and Sport Sciences, Aristotle University of Thessaloniki, Thessaloniki, Greece

Oleuropein (OE) is a secoiridoid glycoside, which occurs mostly in the Oleaceae family presenting several pharmacological properties, including antioxidant, cardio-protective, anti-ischemic, anti-atherogenic effects etc [1]. Based on these findings OE is commercially available in many countries, as food supplement or Herb Medicinal Product (HMP). In the context of investigating the effects of OE on the human blood redox status, a commercial OE enriched HMP has been administered per- os to a selected population. A novel LC-MS methodology was developed and validated for the simultaneous quantification of OE and its main metabolite, Hydroxytyrosol (HT), for the aforementioned HMP prior to the clinical study. Modification of the above LC-MS method was performed in order to measure OE and HT in clinical serum samples of the study using a simple pretreatment protocol. Thus, separation of OE, HT and internal standard was achieved on a C18 Fused Core column with 3.1 min overall run time employing the SIM method for the analytical signal acquisition. The methods were validated according to ICH requirements and evaluated by measuring the selectivity, precision, accuracy, robustness, lower limit of quantification (LLOQ) and found to be linear (r² > 0.99) over a wide concentration range of 0.1 – 15 μg/mL (n = 12) for both analytes of interest with an LLOQ value of 0.1 μg/mL. The methodological approaches presented in this study allowed the standardization of the administered dose, whereas it was used for the high-throughput analysis of a list of HMP’s and clinical samples. In addition PCA-based similarity measurements were performed to the HMP’s. Keywords: LC-MS, Oleuropein, Hydroxytyrosol, herb medicinal products, serum References: 1. Lemonakis N, Halabalaki M, Skaltsounis AL, Kremastinos D (2010) The Use of Oleuropein on Myocardium, Olives and Olive Oil in Health and Disease Prevention. Academic Press. USA

WSI 3
Improved peptidomics screening protocol for the identification of cyclotide-containing plants
Kölbach J1, Dessein S2, Greger H2, Gruber CW1
1Medical University of Vienna, Center for Physiology and Pharmacology, Schwarzenbergstrasse 17, A-1090 Vienna, Austria; 2National Botanic Garden of Belgium, Demein van Bouchout, BE-1860, Meise, Belgium; 3University of Vienna, Department of Systematic and Evolutionary Botany, Renweg 14, A-1090 Vienna, Austria

Cyclotides are disulfide-rich plant peptides with unique structural features of a cyclic backbone and three conserved disulfide bonds in a knotted arrangement, known as cyclic cystine knot. So far their presence has only been reported for species of the families of Rubiaceae, Violaceae, Cucurbitaceae and recently Fabaceae [1], but it is very likely that cyclotides are more widely distributed since their predicted number in Rubiaceae alone is ~50,000 [2]. Their sequence diversity and range of bioactivities make them interesting templates for drug development [3]. In this study we investigated >120 plants in ~20 different families to get novel insights about the distribution of cyclotides within the plant kingdom. Further we improved the identification workflow for new cyclotide-containing species from crude plant extracts optimizing MALDI-TOF/TOF and LC-MS/MS experiments. The presence of 6 cysteines and their cyclic conformation gives distinct mass shifts of +348 Da for fully reduced and alkylated peptides and +366 Da for single-site protease cleavages. Novel cyclotide sequences were confirmed by manual and automated peptide sequence assignment using the ERA-tool [4]. We have identified many novel cyclotide-containing species and obtained several cyclotides. Amongst sequences from Psychotria spp. we identified Rauwolfia tetraphylla L. belonging to the Apocynaceae family, to be a ‘cyclotide-plant’ and for the first time we report sequences in this family confirming previous investigations [2]. This underpins earlier suggestions that cyclotides are one of the largest peptide classes within plants, offering access to a large natural peptide library for multiple biological and pharmaceutical applications. Keywords: cyclotides, plant screening, peptide sequencing Acknowledgement: This work is funded by the Austrian Science Fund-FWF P22889. References: 1. Poth et al. (2011) ACS Chem Biol, in press. 2. Gruber et al. (2008) Plant Cell 20: 2471 – 2483. 3. Henriques and Craik (2010) Drug Discov Today 15: 57 – 64. 4. Colgrave et al. (2010) Biopolymers 94: 592 – 601
**WSI 4**

Phytochemical profiling of *Opopanax persicus* Boiss.


1Department of Pharmacognosy, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran 141764411, Iran; 2Division of Pharmaceutical Biology, Department of Pharmaceutical Sciences, University of Basel, CH-4056 Basel, Switzerland; 3Division of Pharmaceutical Biology, Department of Pharmaceutical Sciences, University of Basel, CH-4056 Basel, Switzerland; 4Novartis Institutes for Biomedical Research, CH-4002 Basel, Switzerland; 5Division of Pharmaceutical Biology, Department of Pharmaceutical Sciences, University of Basel, CH-4056 Basel, Switzerland; 6Swiss Tropical and Public Health Institute, CH-4002 Basel, Switzerland; 7Department of Pharmacognosy, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran 141764411, Iran

*Opopanax* W. D. Koch is a genus of the Apiaceae family, with 11 species distributed throughout the Mediterranean and Iranica region (Iran, Afghanistan, Western Pakistan, Northern Iraq, Azerbaijan, Turkmenistan) [1]. With the exception of some phytochemical studies on *O. chironium* (L.) Koch growing in the Mediterranean and Balkan regions [2], the genus *Opopanax* has not been investigated from a chemical or pharmacological viewpoint. We carried out a phytochemical profiling of *O. persicus* Boiss., an endemic species growing in parts of Turkey, Iran and Transcaucasia [1]. From the dichloromethane extract, a total of 20 compounds were isolated by medium pressure liquid chromatography (MPLC), vacuum liquid chromatography (VLC), and preparative and semi-preparative HPLC. Structure elucidation was carried out by on-line ESI-MS and PDA data, HR-ESI-TOF-MS and off-line 1D- and 2-D-NMR spectra recorded in a 1-mm TXI microprobe. Compounds were identified as coumarins with predominantly linear and angular dihydrofuranocoumarin scaffold which 10 are reported for the first time as new derivatives. The absolute stereochemistry of isolated compounds was determined by X-ray crystallography and CD spectroscopy. Some of the compounds showed moderate activity against Plasmodium falciparum K1 strain and Trypanosoma brucei rhodesiense (IC50 s 3.6 to 6.9 μg/ml, and selectivity indices (SI) in L-6 cells of 5.7 to 25, respectively). Keywords: *Opopanax persicus* Boiss., phytochemical analysis, dihydrofuranocoumarin, absolute stereochemistry, X-ray crystallography, CD spectroscopy References: 1. Rechinger KH (1987) Flora Iranica, No. 162: 438 – 439. 2. Appendino G et al. (2004) Nat Prod 67(4): 532 – 536.

**Fungal co-culture as a new source of antifungal metabolites**

**Bertrand S**, Schumpp O', Bohni N', Monod M', Gindo K

1Laboratory of Pharmacognosy and Phytochemistry, School of Pharmaceutical Sciences, EPFL, University of Geneva, University of Lausanne, quai Ernest-Ansermet 30, CH-1211 Geneva 4, Switzerland; 2Swiss Federal Research Station Agroscope Changins-Wädenswil, Route de Duillier, P.O. Box 1012, CH-1260 Nyon, Switzerland; 3CHUV, Service de Dermatologie, CH-1011 Lausanne, Switzerland

Microorganisms are a very rich source of secondary metabolites with antimicrobial potential [1]. In order to produce original metabolites from this source, strategies have to be developed to induce synthetic pathways triggered by genes that are often silent [2]. Nutritional or environmental stress can be used to activate these orphan pathways, in particular jasmonic acid, salicylic acid, abscisic acid and ethylene, can be involved in plant responses to stress [1]. Our attention was focused on volatile compounds emissions (VOCs) from *Achillea collina* Becker, a medicinal plant, exposed to biotic and abiotic stressing conditions. Headspace Solid-Phase-Microextraction-Gas Chromatography-Mass Spectrometry (HS-SPME-GC/MS) “in vivo” method [2] was used to evaluate *A. collina* VOCs. Biotic stress was obtained by the infestation of *A. collina* plants with *Myzus persicae* or * Macrosiphoniella millefoli* (the generalist and specialist aphid species respectively. Mechanical stresses were obtained by applying a pressure to the plants using a specially designed equipment or by prickng the leaves with a needle. VOCs emissions are reported in figure 1. As shown, some compounds were induced by both the biotic and abiotic stresses (eg. 1-Hexanol, Pinocarvone, α-Fenchene) while some other VOCs were specific to the type of stress applied. As an example Spathularenol was only induced by M. millefoli and β-Linalool was induced only by the mechanical damage. Aromadendrene, Terpineol-cis-β, Tetradecanal were only induced by the biotic stresses. *A. collina* shows a great plasticity in the VOCs biosynthesis, highly modulated by the external stimuli, a possible good model for future investigations at a molecular level. **Figure 1**: fungal co-culture on agar plates of Trichophyton rubrum (1) and Bionectria ochroleuca (2) showing long distance repulsion

Keywords: **fungital metabolites, co-culture, confrontation, anti-fungals**


**WSI 5**

Plants have evolved wide range of mechanisms to cope with biotic and abiotic stresses. It’s suggested that hormone signaling pathways, in particular jasmonic acid, salicylic acid, abscisic acid and ethylene, can be involved in plant responses to stress [1]. Our attention was focused on volatile compounds emissions (VOCs) from *Achillea collina* Becker, a medicinal plant, exposed to biotic and abiotic stressing conditions. Headspace Solid-Phase-Microextraction-Gas Chromatography-Mass Spectrometry (HS-SPME-GC/MS) “in vivo” method [2] was used to evaluate *A. collina* VOCs. Biotic stress was obtained by the infestation of *A. collina* plants with *Myzus persicae* or * Macrosiphoniella millefoli*, the generalist and specialist aphid species respectively. Mechanical stresses were obtained by applying a pressure to the plants using a specially designed equipment or by prickng the leaves with a needle. VOCs emissions are reported in figure 1. As shown, some compounds were induced by both the biotic and abiotic stresses (eg. 1-Hexanol, Pinocarvone, α-Fenchene) while some other VOCs were specific to the type of stress applied. As an example Spathularenol was only induced by M. millefoli and β-Linalool was induced only by the mechanical damage. Aromadendrene, Terpineol-cis-β, Tetradecanal were only induced by the biotic stresses. *A. collina* shows a great plasticity in the VOCs biosynthesis, highly modulated by the external stimuli, a possible good model for future investigations at a molecular level.

**Figure 1**: fungal co-culture on agar plates of Trichophyton rubrum (1) and Bionectria ochroleuca (2) showing long distance repulsion

Keywords: **fungital metabolites, co-culture, confrontation, anti-fungals**

A validated HPLC method for standardization of the most active fraction of the antihyperglycemic drug Cleome droserifolia using bioactive markers


The aqueous and ethanolic extracts of the aerial parts of Cleome droserifolia (Forssk.) Del. were assessed for their antihyperglycemic effects in male albino rats at the same dose level of the biguanide metformin (150 mg/kg body weight). Diabetes was induced intraperitoneally with a single dose of alloxan (150 mg/kg body weight) [1]. The blood glucose level was monitored after 2 and 4 weeks from zero time (Table 1). The four sub-fractions (n-hexane, chloroform, ethyl acetate and n-butanol) of the more active aqueous extract were tested at the same dose level. A validated RP-HPLC method for standardization of the most active ethyl acetate fraction (70% as potent as metformin after 4 weeks of oral administration) was developed. Three flavonoid glycosides;isorhamnetin-3-O-β-D-glucoside (F1), quercetin-3′-methoxy-3-O-(4''-acetylrhamnoside) (F2) and kaempferol-4′-methoxy-3,7-dirhamnoside (F3) (Fig. 1) were isolated from the ethyl acetate fraction and proved to increase basal glucose uptake, 2-folds as insulin, in C2C12 skeletal muscle cells [2]. These compounds (Fig. 1) were used for the standardization (Fig. 1). The parameters of validation of the method (linearity, repeatability, reproducibility, ruggedness, robustness and accuracy) were evaluated. A standard calibration curve, established for the major compound F3 at a concentration range of 44 – 174 μg/ml, showed good linearity with a correlation co-efficient (R2) of 0.998. The recovery of the method was 100.5%. A high degree of repeatability and reproducibility (relative standard deviation values less than 5%) were also achieved.

Table 1. Antihyperglycemic effect of the different extracts of Cleome droserifolia

<table>
<thead>
<tr>
<th>Extracts</th>
<th>Time</th>
<th>Zero</th>
<th>2 weeks</th>
<th>4 weeks</th>
<th>% of change</th>
<th>% of change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Db + aqueous extract</td>
<td>Diabetic rats (Db) non treated</td>
<td>243.7 ± 8.2</td>
<td>256.8 ± 9.6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Db + ethanolic extract</td>
<td>257.3 ± 11.4</td>
<td>216.2 ± 7.6</td>
<td>49.5</td>
<td>81.9 ± 2.2</td>
<td>68.2</td>
</tr>
<tr>
<td></td>
<td>Db + aqueous extract</td>
<td>256.8 ± 10.1</td>
<td>173.2 ± 6.2</td>
<td>32.5</td>
<td>141.9 ± 5.5</td>
<td>44.7</td>
</tr>
<tr>
<td></td>
<td>Db + n-hexane fraction</td>
<td>246.9 ± 7.8</td>
<td>214.3 ± 8.6</td>
<td>13.2</td>
<td>198.6 ± 7.1</td>
<td>19.5</td>
</tr>
<tr>
<td></td>
<td>Db + chloroform fraction</td>
<td>251.9 ± 8.6</td>
<td>186.8 ± 7.4</td>
<td>25.8</td>
<td>138.9 ± 5.8</td>
<td>44.8</td>
</tr>
<tr>
<td></td>
<td>Db + ethyl acetate fraction</td>
<td>258.4 ± 7.1</td>
<td>187.4 ± 6.3</td>
<td>27.5</td>
<td>135.3 ± 4.1</td>
<td>47.6</td>
</tr>
<tr>
<td></td>
<td>Db + n-butanol fraction</td>
<td>258.3 ± 10.2</td>
<td>224.9 ± 8.4</td>
<td>13.3</td>
<td>203.7 ± 6.5</td>
<td>21.1</td>
</tr>
</tbody>
</table>

Extracts, fractions and the standard metformin were given at a dose of 150 mg/kg body weight. * Statistically significant difference from zero time at P < 0.01. M, mean; S.E., standard error (n = 6).


Fig. 1: HPLC chromatogram of the ethyl acetate fraction

HPLC chromatogram of the ethyl acetate fraction showing the flavonoid glycosides F1, F2 and F3. The method involved the use of a Lichrosphere 100 RP-18 column with a guard column. Gradient elution was from 10 to 75% v/v acetonitrile/0.3% orthophosphoric acid in water, in 25 min, at a flow rate of 1.0 ml/min and UV detection at 325 nm.
WSII 1
Risk assessment of hERG channel inhibition by natural products – screening and activity directed analysis of spices, food and medicinal plants

Schrann A1, Boburin I1, Hering S2, Hamburger M1
1Division of Pharmaceutical Biology, University of Basel, Klingelbergstrasse 50, 4056 Basel, Switzerland; 2Institute of Pharmacology and Toxicology, University of Vienna, Althanstrasse 14, 1090 Vienna, Austria

The most prominent determinant of acquired long QT syndrome is inhibition of the hERG potassium channel. Drug-induced QT prolongation can cause undesirable cardiac side effects and has led to several drug withdrawals in the past. In contrast to synthetic drugs, little is known about hERG channel inhibitors of natural origin. For assessing the risk of natural products on hERG channel inhibition, extracts obtained from frequently consumed spices, food, and medicinal plants were submitted to a broad in vitro screening. We established an HPLC-based activity profiling approach for this target by combining HPLC-microfractionation with on-line and off-line spectroscopy, and an automated two-micro-electrode voltage clamp assay with transfected Xenopus laevis oocytes. Among the extracts tested, the methanolic extract of the TCM herbal drug Evodiae Fructus (Evodia rutaecarpa [Juss.] Benth., Rutaceae) reduced the peak tail hERG current by 60.9 ± 6.9% at 100 μM. HPLC-based activity profiling of the crude extract led to the identification of dehydroevodiamine and hortiamine, two indoloquinazoline alkaloids, as the active principles. First information on structure-activity relationships revealed that both the methyl group at position 14 and the double bond between C13(12b) and N14 are essential for the inhibitory effect of this compound class. We developed a method for removal of both inhibitory alkaloids by filtration over a cation exchange resin (Lewatit® MonoPlus SP 112). The resulting extract was devoid of hERG channel blocking activity. Moreover, we determined the dehydroevodiamine content in different commercial batches of Evodiae Fructus and in various processed TCM products. Keywords: hERG channel inhibition, herbal extracts, Evodia rutaecarpa, Rutaceae, HPLC-based activity profiling, indoloquinazoline alkaloids

WSII 2
New cerebrosides and hydroxylated fatty acids from TCM drugs

Rozema E1, Popescu R1, Sondergerg H2, Uhrschmidt C2, Fakhruddin N1, Reznick G1, Atanasev AG1, Heiss EH1, Bourtj M1, Schuster D1, Urban E1, Huck CW1, Dirsch VM1, Kopp B1
1Department of Pharmacognosy, University of Vienna, Althanstrasse 14, 1090 Vienna, Austria; 2Institute of Analytical Chemistry and Radiochemistry, University of Innsbruck, Innrain 52c, 6020 Innsbruck, Austria; 3Institute of Pharmacy/Pharmaceutical Chemistry and Center for Molecular Biosciences Innsbruck (CMBI), University of Innsbruck, Innrain 52c, 6020 Innsbruck, Austria; 4Department of Pharmaceutical Chemistry, University of Vienna, Althanstrasse 14, 1090 Vienna, Austria

In recent years, increasing attention has been drawn towards the role of lipids in cell signalling pathways and regulation.1 Plants are a rich source of bioactive lipids. We, therefore, focused on (complex) lipids from plants used as traditional Chinese medicinal (TCM) drugs and their influence on cellular processes. Four new cerebrosides, which belong to the compound class of sphingolipids, were isolated and characterized from Arisaema amurense (Arisaemataceae) and Arthrostemma sp. (Persicaceae). The fatty acids showed high PPARγ agonist function of the salicyl alcohol moiety. Promising results were obtained on the dengue RNA polymerase inhibition assay and preliminary structure-activity relationships were deduced. CHIKV assays are in progress. Keywords: Arthrostemma, Arisaema, dengue, emerging viruses

WSII 3
Potential inhibitors of chikungunya and dengue viruses isolated from Malagasy plants

Bourtj M1, Leyssen P1, Eydoux C1, Guilmot J1, Canard B1, Rassoanaivo M2, Guitte W1, Litouaden M1
1Centre de Recherches de Gif, Institut de Chimie des Substances Naturelles (ICSN), CNRS, avenue de la terrasse, 91910 Gif sur Yvette, France; 2Rega Institute for Medical Research, Minderbroedersstraat, B3000, Leuven, Belgium; 3Laboratoire d’Architecture et de Fonction des Macromolécules Biologiques (AFMB), avenue de Luminy, 13288 Marseille, France; 4Institut Malgache de Recherches Appliquées (IMRA), 102 Antananarivo, B.P.3833, Madagascar

Chikungunya virus (CHIKV) and dengue virus (DENV) are two emerging arboviruses. CHIKV has recently re-emerged, causing massive epidemics that have moved from Africa throughout the Indian Ocean to India and Southeast Asia. In humans, it is responsible for an acute disease, characterized by high fever, arthralgia and maculopapular rash.1 Regarding the dengue fever, it affects more than 50 million people annually.2 Increasing mortality and geographical expansion are the drastic changes noted in the recent epidemiology of the disease. No specific antiviral therapy is currently available, on the market, for these two diseases. A screen on a cellular activity using CHIKV-infected 400 plants from the Indian Ocean Islands. This screening has led to the selection of Flacourtia ramoschii L’Hér. (Salicaceae), a tree distributed in the south of Asia and in Madagascar. Fruits and seeds are used in folk medicine for the treatment of rheumatic arthralgia, cholora and dysentery.3 Eight new phenolic glycosides and one caffeic acid derivative, together with three known phenolic glycosides and one betulinic acid derivative were obtained by a bioassay-guided isolation from the stem bark. The phenolic glycosides have a salicylic core structure; this core may be esterified with benzoic acid and/or 1-hydroxy-6-oxycholen-2-en-1-carboxylic acid, on the glucose moiety in 2’, 3’ and 4’ positions and on the primary alcohol function of the salicyl alcohol moiety. Promising results were obtained on the dengue RNA polymerase inhibition assay and preliminary structure-activity relationships were deduced. CHIKV assays are in progress. Keywords: Flacourtia ramoschii, Salicaceae, phenolic glycosides, chikungunya, dengue, emerging viruses

WSII 4
Toxicological risk assessment of Aristolochia species

Michl J1, Simmonds M2, Ingrouille M1, Heinrich M1
1The Centre for Pharmacognosy and Phytotherapy, the School of Pharmacy, University of London, London, United Kingdom; 2Biological Interactions, Royal Botanic Gardens, Kew, Richmond, United Kingdom; 3The Centre for Pharmacognosy and Phytotherapy, the School of Pharmacy, University of London, London, United Kingdom; 4The Centre for Pharmacognosy and Phytotherapy, the School of Pharmacy, University of London, London, United Kingdom; 5Southern Cross Plant Science, Centre for Phytochemistry and Pharmacology, Southern Cross University, Lismore, Australia

Aristolochia species are known to contain aristolochic acids, nitropeptantrone derivatives responsible for their nephrotoxic and genotoxic effects [1]. There are numerous aristolochic acid analogues, including aristolats, biosynthesized on binding to the ligands with even higher cytotoxic potency than aristolochic acid I [2]. Previous research mainly focused on Aristolochia species used in traditional Chinese medicine, but ethnopharmacological studies indicate that other members of the genus are frequently used medicinally [3]. The aim of our research is to assess the
toxicological risk associated with the use of different Aristolochia species as herbal medicines. Metabolomic analysis allows us to take into account all compounds that might be responsible for the nephrotoxic effect. LC-DAD-MS analysis was carried out on A. manshurienis Kom., A. kankauensis Siebold & Zucc. and related species and AA I, AA II, AL I and AA C were quantified. A. kankauensis contained the highest levels of AA I and AA II, whereas A. manshurienis contained the highest variety of different AA analogues (AA I, AA II, CA, AA C, AA G, DD-glucoside and AA D-\(\beta\)-D-glucoside). The results show that the content of aristolochic acid analogues varies greatly between different parts of the plant, with highest amounts found in the flowers. Extraction of the plant material with aqueous ethanol results in high yields of AA I and AL I, whereas extraction with hot water only yields in small amounts of AA I and AL I, and can therefore be associated with lower toxicological risk. Keywords: Aristolochia, aristolactam, aristolochic acid, metabolomics.


Malassezia yeasts are part of human skin microbiota and can become pathogenic under currently unclarified conditions. HPLC/UV combined with LC-MS/MS analysis of the extracts of several Malassezia species, revealed the production of compounds indole[3,2-b]carbazol (ICZ) [1], indirubin [2] and tryptanthrin[3], which are among the most active Aryl-hydrocarbon Receptor (AhR) inducers known and, interestingly, are preferentially biosynthesized by Malassezia furfur isolates from dis eased skin. A previously reported by our group biomimetic synthesis, from indole-3-carboxaldehyde (I3A), the main metabolic product of tryptophan found in all Malassezia studied species, to indirubin and trypranthrin simultaneously [3], showed a common biosynthetic pathway for these two metabolites. This reaction is a one-step oxidation, using hydrogen peroxide and diphenyliselenide as a catalyst. The synthesis of the above indole alkaloids allowed us to proceed not only to their quantification by HPLC analysis, but also to the further examination of this biomimetic reaction. Surprisingly, the formation of the same metabolites was achieved even when simple indole was used as starting material. This gave us the opportunity to synthesize a series of symmetric indirubin and trypranthrin analogues, beginning from the appropriately substituted indoles. Although AhR is an orphan receptor, there are increasing data about its relation with skin homeostasis and skin nosology. Based on our previous work on the activation of AhR in HaCaT cells by Malassezia extracts [3] we could propose that the presence on the human skin of microorganisms able to constantly synthesize potent AhR ligands may play a crucial role in the development of skin diseases.


Figure 1: positive reaction b) negative reaction

Keywords: Allium, Melanocrommyrum, alliinase, basic-native polyacryla mide gel, electrophoretic mobility, in agarose gel as an isolating technique and correlation with synergistic cytotoxicity enhancement

WSI 6

A rapid method for detection of alliinase activity in Allium, especially in the subgenus Melanocrommyrum

Miekle M, Reugen M

Philips-Universität Marburg, Institute of Pharmaceutical Chemistry, Marbacher Weg 6, D-35032 Marburg, Germany

In the genus Allium about 800 species are currently known belonging to several subgenera [1]. Wild Allium species can be found on the northern hemisphere with a main habitat in Central Asia. The enzyme alliinase occurs in Allium plants catalyzing the cleavage of cysteine sulfoxides leading to typical odorous compounds [2]. Whereas the alliinases of common Allium species like garlic and onion [3] are well analyzed, the properties of other alliinases occurring in further Allium species have not been examined yet. Especially the species of subgenus Melanocrommyrum do not express an alliinase like garlic, as can be shown on SDS-PAGE. The separation of alliinase from other proteins is sometimes difficult because of similar protein properties due to their size and probably also due to glycosylation. Therefore, a new method for direct alli nase activity detection has been developed. A crude alliinase preparation can be separated on a basic-native polyacrylamide gel. This method results in functional enzymes, separated into different spots. These prototype spots can be cut out of the gel and screened for their alliinase activity. The L-\(\tau\)-S-\(\beta\)-phenylpropionylcysteine sulphoxide used as indicator turns into a red dye after the enzymatic cleavage by alliinase (Figure 1) [4]. Although the alliinase is still incorporated inside the gel matrix, a positive reaction can be detected after a few minutes (Figure 2). This new test allows an easy and quick detection of alliinase-like enzymes. Furthermore, the amount of sample needed is very small, allowing tests out of a single bulb.

Figure 2: indicator reaction

Keywords: Allium, Melanocrommyrum, alliinase, basic-native polyacrylamide gel, electrophoretic mobility in agarose gel as an isolating technique and correlation with synergistic cytotoxicity enhancement

WSI 7

Electrophoretic mobility in agarose gel as an isolating technique and correlation with synergistic cytotoxicity enhancement

Thakur M 1, Weng A 1, Melzig M2, Fuchs H1

1Institute for Clinical Chemistry and Pathobiology, Berlin, Germany; 2Department of Pharmacy, Free University, Berlin, Germany

In previously published work, we reported that Saponinum album dramatically improves the inhibition of tumor growth by targeted toxins in mice in a synergistic way. Herein, we report a simplified slab gel based electrophoretic isolation technique to determine the highly effective fraction of Saponinum album with a relative electrophoretic mobility (RF) of 0.44 from the mixture. In total, four different fractions were separated at a preparative scale, and evaluated by ESI-MS, HPLC and thin layer chromatographic analysis. Electrophoretic mobility and electrochemical properties of the different fractions of saponins from Saponinum album were set into relation to their ability to enhance the cytotoxicity of epidural growth factor (EGF)-based targeted toxins. We here treated HER-14 cells, which are NIH-3T3 Swiss mouse embryo cells transfected with the human EGF receptor. Untransfected NIH-3T3 cells

Abstracts | 59th International Congress of the GA | 4th-9th September 2011, Antalya, Turkey | 1237

Planta Med 2011; 77: 1229–1472 Georg Thieme Verlag KG Stuttgart · New York · ISSN 0032-0943
served as control. The major bulk of Saponarium album (72.3%) (Rf 0.62) migrated to the farthest and was found to be significantly ineffective (p < 0.05) in enhancing the cytotoxicity of the targeted toxin, while the second fraction (Rf 0.45) showed an enhancement factor of 3200-fold. The third (EM 0.30µ) had an enhancement factor of 3200, the fourth (RF 0.08) was again significantly ineffective (p < 0.05) in exhibiting any enhancement of cytotoxicity. This is the first report for the use of slab gel electrophoresis as a convenient isolation technique for saponins.

References:

WORKSHOP III: Permanent Committee on Regulatory Affairs of Herbal Medicinal Products

WSIII 1
Quality of herbal medicinal products and food supplements – EU regulation and practical experience
Sievers H
PhytoLab GmbH & Co. KG, Dutendorfer Straße 5 – 7 91487 Vestenbergsgreuth, Germany

Herbs and herbal preparations have been playing an important role for both dietary and medicinal use from prehistoric times to 21st century urban culture. People have always been aware that the variability of the properties of herbal raw materials, e. g., on the exact plant part, vegetal state, weather conditions, harvesting time and – essentially – the mode of preparation by, e. g., drying, peeling, cooking (or not), fermentation, treatment with inorganic substances, extraction etc., are not less important for the tolerability, digestibility or – in case of medicinal use – success of treatment than the choice of the plant itself. Over the past decades scientific findings have provided a rationale for what our ancestors had established on an empirical basis. We understand why, based on the absence/presence and dose of certain secondary compounds some plants/preparations are beneficial or harmful. Since both beneficial and potentially harmful properties of herbal ingredients in food supplements are being increasingly assigned to specific secondary compounds it is evident that certain standards are necessary in order to provide for safety and effectiveness of such products. While legal limits have been established in the EU for a large spectrum of possible contaminants in food and thus food supplements including, e. g., pesticides, heavy metals or mycotoxins, no common standards exist for the overall quality assessment of herbal raw materials/preparations for food supplements. While rules established for the quality control of herbal medicinal products and their respective herbal raw materials/preparations are not transferable for various reasons, they provide orientation regarding practicable technical standards and methodology. A comparison will be given of the EU regulatory quality standards in both areas in the light of practical experience.
Herbal medicinal products are regulated in Europe under the drug law. If only health claims are stated, some may be considered as dietary supplements. For drug approval, efficacy, safety and quality have to be demonstrated. A special European regulation exists for traditional herbal medicinal products (Directive 2004/24/EC), which does not require clinical studies, but only 30 years of traditional use, out of them 15 years in Europe [1]. A committee for Herbal Medicinal Products (HMPC) at the European Medicines Agency (EMA) currently prepares a list of traditional and well-established herbal drugs/preparations/combinations which can be used for drug approval in the European Union. Also in America, small herbal drugs were recognized by the Food and Drug Administration (FDA) [2]. Therefore, chromographic techniques, like TLC, HPLC and GC are the approach to consider the complex composition of herbal products. Hence, the following aspects related to TCM will be briefly overviewed in the lecture, including TCM theory, TCM resources, related research institutions and education systems, Chinese Pharmacopoeia and TCM volumes, TCM new drug registration regulations, by SFDA, TCM-based drug discovery, government funding sectors, TCM hospitals, etc. The effects of TCMs are, of course, induced by their chemical constituents. For effective quality control and efficacy evaluation, we should know: What exist in TCMs? Which are biological active? And how the components induce integrative functions? During the last decade, our research team used multidisciplinary theory and methodology to develop novel methods for multi-component and multi-target evaluation of TCMs. First, a diagnostic ion filtering strategy was proposed for rapid screening and identification of non-target compounds in complex TCM samples by UPLC-Q-TOF/MS. Next, a novel chromatographic technique, like TLC, HPLC and GC are the methods of choice for quality control [3]. Keywords: Chinese herbal medicine, regulation, Europe, quality, efficacy, safety References: [1] Silano M et al. (2004) Phytotherapy 75: 107 – 116 [2] Bauer R, Franz G (2011) Current status and future perspective of traditional Chinese medicine in China Guo D, Liu X, Guan S, Wu W, Yang M, Jiang B Shanghai Research Center for TCM Modernization, Shanghai Institute of Materia Media, Chinese Academy of Sciences, Shanghai 201203, P.R. China

Traditional Chinese medicine (TCM) has over 3000 years of history to treat diseases in China and has played an essential role in the Chinese healthy system. More and more western scientists are now interested in TCM research and hope to learn about the basics of TCM in China. Hence, the following aspects related to TCM will be briefly overviewed in the lecture, including TCM theory, TCM resources, related research institutions and education systems, Chinese Pharmacopoeia on TCM volume, TCM new drug registration regulations, by SFDA, TCM-based drug discovery, government funding sectors, TCM hospitals, recent research progress made on TCM research, which may help the audience to understand the basic current situation of TCM in China. In addition, the current research on the quality standard construction of traditional Chinese medicines will also be elaborated with some concrete examples. The future directions on TCM modernization will also be insighted as a personal viewpoint. Acknowledgements: Author like to thank Ms. Gangqiang SU (Director General of Department of Scientific Research, State Administration of TCM) for her providing some basic data for the lecture.
Chinese medicine processing is a traditional Chinese pharmaceutical technology, and it is also the main characteristic property that distinguishes traditional Chinese medicine and natural medicine. There are complex chemical changes during the process of Chinese medicine processing, and these chemical constituents may be the basis of clinical efficacy changes. To clarify the changes of the chemical constituents in Chinese medicine is the main purpose of the mechanism research of Chinese medicinal processing. In recent years, many research institutions at home and abroad have done deep research in chemical mechanism during the process of Chinese medicine processing, and clearly highlight the chemical reactions and chemical changes in the process of Chinese medicinal processing. The main chemical reactions occurred in the Chinese medicine processing are hydrolysis reaction, oxidation reaction, replacement reaction, isomerization reaction, decomposition reaction and so on. This paper reviewed the main achievements in the chemical mechanism research during the process of Chinese medicine processing, and prospected the research directions and future in the search of Chinese medicine processing. Key words: Chinese medicine processing; process reaction; chemical constituents; mechanism

Identification of PPARγ agonists from natural sources

Christensen KB
Institute of Chemical Engineering, Biotechnology and Environmental Technology, University of Southern Denmark, Niels Bohrs Alle 1, 5230 Odense M, Denmark

The peroxisome proliferator-activated receptors (PPARγ) have been in focus for more than a decade for the development of drugs to treat and/or prevent diseases associated with the metabolic syndrome (MS). The PPARγ agonists are nuclear receptors (NRs) highly involved in lipid and energy metabolism and hence, targets for treating MS-related disorders. In particular, PPARγ is a key regulator of insulin sensitivity and is the target of many conventional drugs, although this may result in severe side effects. However, such side effects could be avoided using selective modulators or partial agonists instead [1,2]. Natural products have proven to be a valuable source of PPAR activators [3] of which some have demonstrated interesting partial agonist activities in vivo [4]. In our studies on identification of PPAR modulators from natural sources we used different approaches such as bioassay-guided fractionations, structure-activity relationships, and pharmacophore-modeling [5,6,7,8]. This has led to the identification of new potential PPARγ agonists from purple coneflower (Echinacea purpurea) (L.) Moench [5], a plant not traditionally used to treat MS. For traditional anti-diabetic remedies such as sage (Salvia officinalis) L. and elderflowers (Sambucus nigra) L., PPAR activating properties have been identified suggesting a mechanism of action involving these NRs [6,7]. This was also the case when a pharmacophore-driven approach led to the identification of novel PPARγ partial agonists from mastic gum (Pistacia lentiscus L.). Hence, there is a large potential for finding modulators of these versatile NRs amongst natural products and hence important information about their mechanisms of action and their use as drug candidates. Keywords: Metabolic syndrome, PPAR, natural products, medicinal plants, bioassay-guided fractionation. References: 1. Auwerx J et al. (2003) Nuclear Receptor Signalling 1: e006. 2. Berger JP et al. (2005) Trends Pharmacol Sci 26: 244 – 251. 3. Huang TH et al. (2009) Pharmacol Res 60: 195 – 206. 4. Christensen KB et al. (2009) Phytother Res 23: 1316 – 1325. 5. Christensen KB et al. (2009) Nat Prot 72: 933 – 937. 6. Christensen KB et al. (2009) Phytother Res 24: S129-S 132. 7. Christensen KB et al. (2010) J Ethnopharmacol 132: 127 – 133. 8. Petersen RK et al. (2011) Comput Aided Mol Des 25: 107 – 116.

Plant derived therapeutics for the treatment of Metabolic Syndrome

Cefalu W
Chief of the Division of Nutrition & Chronic Diseases, Pennington Biomedical Research Center, Louisiana State University, Baton Rouge, USA

Type 2 diabetes is a progressive disorder whose pathophysiology consists of impaired insulin secretion, hepatic glucose overproduction and insulin resistance in peripheral tissues such as adipose tissue and muscle. Currently, clinical strategies to achieve improved metabolic control endorse the use of a combination of agents from multiple pharmacologic classes and which target specific pathophysiologic defects. Given the role of insulin resistance in the pathophysiology, the improvement of insulin sensitivity for treatment of the metabolic syndrome or type 2 diabetes remains as a primary clinical strategy. Recent data, however, have questioned the safety of the current pharmacologic agents used to enhance insulin sensitivity. As such, alternative strategies, e.g., nutritional supplementation with targeted nutrient agents, are being promoted by a large number of patients and are frequently done so without the knowledge of the provider. Based on historical human use, there has been great interest in plant extracts (botanicals) as a source for nutritional supplements intended as adjunctive therapies for human diseases. Specifically, isolated compounds identified from plant sources, i.e. phytochemicals or bioactives, have served as a source for therapeutic agents for many diseases, including malignancy, infectious diseases and diabetes. Interestingly, the development of one of the most commonly used anti-hyperglycemic agents in the world today, i.e. metformin, can be traced back to a botanical source. However, the concern with most nutritional supplements, including those considered “natural” (e.g. botanicals) by the consumer, is the paucity of data in humans in regard to efficacy to improve metabolic abnormalities. Thus, there remains considerable controversy regarding the use of botanical supplements for human health. Despite the stated concerns for botanical supplements, there are a number of botanicals that have shown considerable promise for human use but they have to be carefully validated in controlled clinical trials.
With the toolbox of the “omics”, nutritional science has become a new advent in understanding the processes that make up mammalian metabolism in health and disease. Essentially unlimited when taken into studies in human cells in culture or in vivo studies in animal models, omics applications in human trials are limited by the availability of biosamples restricted to body fluids, blood cells or biopsy materials. Nutrigenomics research in the last years has delivered some important insights into mechanisms by which dietary constituents affect metabolism and health risks. I shall be presenting findings from human studies in which transcript-, proteome- and metabolite-profiling techniques have been applied – mainly in peripheral mononuclear cells and plasma/urine as biosamples. It also will include some studies on the effects of plant secondary components. Despite enormous efforts, genome-wide association studies as a top-down approach have so far only shown very weak effects of genetic heterogeneity in individual genes and their association with disease initiation or progression. Amongst the reasons that science crossed itself in identifying causal molecular links between diet and disease is that usually only a “snapshot” is taken when applying any of the profiling techniques. But, it is an intrinsic feature of metabolism that it is highly dynamic in time (acute and chronic adaptation) and space (within cell compartments or i.e. the interorgan metabolism). In the future, we therefore should first define the variability in the phenotypic response of mammals as a function of time and space and redefine the homeostatic control as a transient equilibrium. To obtain robust phenotypic alterations it therefore may be advised to challenge the biological system to drive it in a critical state. Such a “critical state” may in terms of nutrition be defined as a severe state of starvation or diets providing extreme nutrient compositions. I shall demonstrate findings from a metabolomics application in humans with such defined challenges. Taken together, nutrigenomics has extended our knowledge on how diets or individual ingredients affect human metabolism on the background of a given genetic make-up, but it is far from delivering predictive parameters for personalized health and/or disease prevention.

References:

WORKSHOP VI: Quality/Agriculture joint Workshop
Chairs: C. Franz, C. Erdelmeier

Environmental Contaminants – Heavy Metals
Origin – Analytical Methods – Points to Consider
Hofmann A
PhytoLab GmbH & Co. KG, Leibnizstrasse 9, D-89231 Neu-Ulm, Germany

Environmental relevant heavy metals are lead, cadmium, mercury, arsenic, copper, nickel, zinc and iron. Since Tschernobyl and actually Fukushima caesium 137 and even plutonium 239 might be relevant parameters for some proveniences. The input of these elements into the herbal materials is diverse. Some of them like nickel, arsenic or lead are from direct geogenic sources and therefore not avoidable at all. Other inputs are made via air by traffic, industry and combustors also. Even the agricultural industry itself takes part in that scenario by using heavy metal loaded pesticides like some dithiocarbamates (zinc in zineb and others) or anorganic mineral fertilizers (accumulation of cadmium in the soil). Some plants are so called hyperaccumulators for heavy metals at all. Even some of the herbal drugs used for phytopharmacy are found in that group (e.g. Sativ and Populus). In that group results for cadmium found often exceed the specified limits. Methods and limits are described in the European Pharmacopoeia in the chapters 2.4.27, 2.4.31 and in the monograph “ Herbal Drugs” (monograph number 1433). The official testing methods for heavy metals are atomic absorption spectrometry (AAS) or inductively coupled plasma-atomic emission spectrometry (ICP-AES) or inductively coupled plasma-mass spectrometry (ICP-MS). All methods need to be validated on the herbal materials at least on distinct plant organs like roots or fruits etc. Points to consider in the management of GACP to avoid OOS results for heavy metals are control of the soil, specified pest management, specified selection of plants and varieties.

Key Words: Heavy Metals, absorption spectrometry (AAS), or inductively coupled plasma-atomic emission spectrometry (ICP-AES), inductively coupled plasma-mass spectrometry (ICP-MS)

WSV 2

The Application of Pesticides in the Production of Medicinal Plants in China
Yang M
Key Laboratory of Bioactive Substances and Resources
Utilization of Chinese Herbal Medicine, Ministry of Education, Institute of Medicinal Plant Development, Peking Union Medical College, Beijing, 100193, P.R. China

Traditional Chinese Medicine (TCM) is getting more and more attention all over the world, due to its systematic approach and clinical effectiveness [1]. The quality of TCM determines the safety and efficacy of Chinese-made TCM products. However, the improper use of pesticides in the medicinal plant production has seriously affected the quality of TCM. In the early 1980s, Chinese scientists had already recognized this problem and put in an effort to standardize the pesticide use. During that time, the main focus was mainly involved in the half life of organochlorines and those pesticides that easily caused cumulative toxicity (e.g., hexachlorocyclohexane, DDT, dieldrin, endrin) in Chinese herbal preparations, herbal patents and Chinese patent products. Accordingly, “Pharmacopoeia of People’s Republic of China” (CP, the edition of 2000, the first section) set up the assay methods and residual limit of the organochlorine pesticide [2]. During the “9th Five-Year project”, the government organized Chinese medicine technology research key project “Standardize the quality of Chinese Materia Medica “, which included a systematic way of Research and determination of 71 kinds of Chinese herbal medicines. This project not only improved the research level on the pesticides resides in TCM, but also made us have basic understanding the polluted situations of organic pesticides in the commonly used TCM, providing a substantial basis for Good Agriculture Practice (GAP) on Chinese herbal materials in China. During the “10th Five-Year project “, the project, “Researches on the detection and limited standards of 50 kinds of Chinese medicine “, was classified as a major task by the Ministry of National Science and Technology of China. On the basis of this project, we have completed these items, such as: “Detection methods of the pesticide residue of pyrethroid in TCM “; “Detection methods of the pesticide residues of organic phosphorus in TCM “. And those above have been officially recorded in 2005 version of Chinese Pharmacopoeia. In recent years, the detection technology of pesticide residues in traditional Chinese herbal medicine has developed rapidly. Application of technologies such as GC, LC, LC-MS, LC-MS, CE-MS, and multiple-stage mass spectrometry techniques, greatly increase the qualitative capability, the detection sensitivity, detection limit and detection coverage. At present, some new analysis methods and techniques are making great progress, especially in the analysis of multi-pesticide residues for many types of pesticides, the analysis of multi-pesticide residues for the same type of pesticides, the analysis in the trial sample for new single pesticides, and rapid analysis and other aspects. According to GAP for TCM herb requirements, pesticide residues should comply with the Green standards of Medicinal Plants and Pesticide Residue Control of the Ministry of Commerce of the P. R. China [3]. At present, China Pharmacopoeia (Edition 2010) supplemented the respective analytical methods of 9 organochlorines (OCPs), 3 pyrethroids and 12 organophosphor- ous pesticides (OPPs), which aim to standardize the cultivation of TCM herbs and bring TCM in line with the international practice. Now the Pharmacopoeia stipulated the MRLs (maximum residue level) of 9 OCPs including BHC, DDT and pentachloronitrobenxene (PCNB) for medicinal materials, among which only two were involved, Radix et Rhizoma Glycyrrhizae and Radix As- tragali, but no MRLs for pyrethroids and OPPs have been established for Good Agriculture Practice for TCM. During the project, “Researches on the detection and limited standards of 50 kinds of Chinese medicine “, the project, “Researches on the detection and limited standards of 50 kinds of Chinese medicine “, was classified as a major task by the Ministry of National Science and Technology of China. On the basis of this project, we have completed these items, such as: “Detection methods of the pesticide residue of pyrethroid in TCM “; “Detection methods of the pesticide residues of organic phosphorus in TCM “. And those above have been officially recorded in 2005 version of Chinese Pharmacopoeia. In recent years, the detection technology of pesticide residues in traditional Chinese herbal medicine has developed rapidly. Application of technologies such as GC, LC, LC-MS, LC-MS, CE-MS, and multiple-stage mass spectrometry techniques, greatly increase the qualitative capability, the detection sensitivity, detection limit and detection coverage. At present, some new analysis methods and techniques are making great progress, especially in the analysis of multi-pesticide residues for many types of pesticides, the analysis of multi-pesticide residues for the same type of pesticides, the analysis in the trial sample for new single pesticides, and rapid analysis and other aspects. According to GAP for TCM herb requirements, pesticide residues should comply with the Green standards of Medicinal Plants and Pesticide Residue Control of the Ministry of Commerce of the P. R. China [3].

Abstracts | 59th International Congress of the GA | 4th–9th September 2011, Antalya, Turkey

Planta Med 2011; 77: 1229–1472 Georg Thieme Verlag KG Stuttgart · New York · ISSN 0032-0943
Legal requirements for the control of contaminants in herbal medicinal products

Steinhoff B
German Medicines Manufacturers’ Association (BAH), Uhberstrasse 71–73, D-53173 Bonn, Germany

Herbal medicinal products require a pre-marketing approval like all other medicinal products and have to prove their quality, safety and efficacy. In order to guarantee optimum consumer and patient protection according to the European legislation. Taking into a consideration the natural origin and potential environmental influences, special emphasis is put on tests for contaminants such as heavy metals, pesticide residues, mycotoxins and microorganisms. For heavy metals, the European Pharmacopoeia has set limits for lead, cadmium and mercury within the general monograph on herbal drugs. Similar rules are being developed for extracts. In this respect, a German industry’s working group has collected a large amount of data on heavy metals occurring in herbal drugs. The respective data base has been evaluated and published [1]. For pesticide residues in plant material, the respective European Pharmacopoeia monograph sets limits for 70 substances. For further substances reference is made to Regulation (EC) 396/2005 on pesticide residues in food. For aflatoxins, specific limits exist for medicinal products which are comparable to the food area. With regard to microbiological purity of herbal medicinal products, the European Pharmacopoeia describes requirements for three different categories of these products as well as determination methods. Raw materials of herbal origin which are intended for use in food supplements have to fulfill the respective European legal requirements for the food area. Besides the above mentioned Regulation (EC) 396/2005 on pesticide residues, limits for further contaminants are described in Regulation (EC) 1881/2006, e.g. for mycotoxins or heavy metals. The United States Pharmacopoeia (USP) is currently developing specific rules on heavy metals in dietary supplements of botanical origin. References: [1]Gasser U, Klier B, Kühn AW, Steinhoff B (2000) Current findings on the heavy metal content in herbal drugs. Pharmeuropa Scientific Notes 2009–1:37–40.

Pesticide testing according to the European Pharmacopoeia (Ph.Eur.) – legal requirements and practical approach

Klier B
PhytoLab GmbH & Co. KG, Dutendorfer Str. 5–7, 91487 Vestenbgreuth, Germany; Phone: 0649 – 9163 – 88342

In 1996 the monograph “Pesticide residues (2.8.13.)” in herbal drugs has been implemented to the European Pharmacopoeia (Ph.Eur.). There were “definition”, “limits”, “sampling”, “qualitative and quantitative analysis of pesticide residues” and a “Test for pesticides” described in the monograph. Referring to the publication in PHARMEUROPA Vol.17 No 1, January 2005 (Pesticide Residues in medicinal Drugs and Preparations) the Ph.Eur. Pesticide Expert Group has been mandated to update the monograph Ph.Eur. 2.8.13. The revised monograph (Ph.Eur. 6.2) has been published in 2008. The revision includes following changes: The new harmonised European Pesticide Regulation (EU 396/2005) replaced old European Directives (EC 76/895 and EC 90/642); the list of pesticides has been extended from 34 to 115 substances frequently observed in herbal drugs; limits has been set in view of toxicology and according to a 90 per cent percentile approach; the formula for the calculation of pesticide limits in extracts and other herbal drug preparations has been modified; more details for method validation procedure has been given (cross reference to document SANCO/10232/2006); the method for determination of pesticides (“Test for pesticides”) has been deleted. With the updated monograph a framework for quality control of pesticide analysis in herbal drugs has been given. For the frequently found 115 substances pesticides in herbal drugs and herb drug preparation limits could be found or calculated easily. The allocation from product to limit of all other (not listed) pesticides according to the new harmonised European Pesticide Regulation (EU 396/2005) remains difficult. The scope of testing depends on the methods of analyses used. Using additionally new analytical techniques based on LC-MS/MS detection more than 500 substances could be detected analysing pesticide residues in herbal drugs.

Pheromone glycosides: Naturally occurring apoptosis inducers

Saracoğlu I, Harput U
Department of Pharmacognosy, Faculty of Pharmacy, Hacettepe University, 06100, Sihhiye, Ankara, Turkey

Natural products have long been regarded as excellent sources for drug discovery given their structure diversity and wide variety of biological activities. Phenylethanoid glycosides are naturally occurring compounds of plant origin and are structurally characterized with a hydroxyphenyl-ethyl moiety to which a glucopyranose is linked through a glycosidic bond and esterified by a cinnamic acid moiety. There can be one to four sugars in their composition and cinnamic acid esterification generally occurs on a glucose directly bound to phenylethanoid moiety (1,2). To date several hundred compounds of this type have been isolated from medicinal plants and further pharmacological studies in vitro or in vivo have shown that these compounds possess a broad array of biological activities including antibacterial, antioxidant, antitumor, antiviral, anti-inflammatory, neuro-protective, hepatoprotective, immunomodulatory, and enzyme inhibitory actions [1,2]. In this study, we have investigated in vitro anticancer (cytotoxic) activity and structure-activity relationships of 10 different phenylethanoid glycosides against human and murine cancer cell lines, Hep-2 (human epidermoid carcinoma), RD (human rhabdomyosarcoma), and L-20B (transgenic murine L-cells), using MTT method [3,4]. Acteoside, forsythoside B, samioside and teucrisoside were exhibited significant cytotoxic activity against tested cancer cell lines in the concentration range of 8–50 µg/mL. To determine the selectivity of cytotoxicity, VERO (African green monkey kidney) cell line was used for the comparison and no cytotoxicity was determined. In addition, apoptotic cell death was observed in the histological analysis of tested cancer cell lines. Keywords: Phenylethanoids, cancer cells, cytotoxicity, structure-activity relationship Acknowledgement: Activity studies were supported by Hacettepe University Research Foundation (Project No: 0302301010). References: 1. Funes L, et al. (2010) Chemistry and Physics of Lipids 163: 190 – 199. 2. Korkina LG (2007) Cellular and Molecular Biology 53(1): 15 – 25. 3.Saracoğlu I, et al. (1995) Biological and Pharmaceutical Bulletin 18(10): 1396 – 1400. 4.Saracoğlu I, et al. (1997) Fito-terapia 68(5): 434 – 438.

With a different approach it is possible to develop commercially useful antimicrobial products from plants

Eloff J, Pauw L
Phytomedicine Programme, Faculty of Veterinary Science, University of Pretoria, South Africa

Many compounds from plants play a major role as pharmaceutical products in several therapeutic applications in human and animal health. Despite thousands of publications in the field there has been a remarkable absence of therapeutic products from plants to combat microbial infections. Data have been presented to show that the following factors play a role in this absence: the extractant used, the bioassay used, the unexpectedly low activity of isolated antimicrobial compounds, the presence of synergism in crude extracts and focussing on plants traditionally used. The antibacterial activity of acetone leaf extracts of more than 700 South African tree species (mainly important nosocomial pathogens Staphylococcus aureus, Enterococcus faecalis, Escherichia coli and Pseudomonas aeruginosa) were determined. A high proportion of these extracts had antibacterial activities with MICs lower than 0.08 mg/mL. Unfortunately in many cases the extracts are toxic to mammalian cells. Many of these species have a good potential to be used as crude extracts to treat topical infections in humans. Examples will be demonstrated where these extracts have been as effective as commercial products in controlling microbial infections in animals and plants. The limitations associated with commercializing plant extracts as antimicrobials such as quality control, availability of material and potentiating the extracts to yield patentable products will also be discussed. It appears that if the focus is on using extracts rather than isolated pure compounds there is a considerable opportunity to use the compounds present in plants to combat microbial infections. Keywords: antibiotic activity, synergism, extractant, MIC, plant extract, nosocomial bacteria, commercial product Acknowledgement: The National Research Foundation provided financial support.
A one-tube assay for four Hypericum species – PlantID
Howard C, Socratous E, Williams S, Graham E,
Teunier MH, Scott NW, Bremer PD, Slater A
1Biomolecular Technology Group, De Moffart University, Leicester, U.K. LE1 9BH; *East Midlands Forensic Pathology Unit, Leicester University, Leicester Royal Infirmary, U.K. LE2 7LX

The benefits of DNA-based identification methods for medicinal plant products have been shown – negligible amounts of starting material, high resolving power, increased taxonomic specificity, and fast results (1). However, the simultaneous detection of multiple species in one sample has not until now been possible. We report the design of PlantID for St John’s Wort (SJW) (Hypericum perforatum L.); a technique capable of detecting both the target species (SJW) and a number of likely adulterants in one sample using a multiplex PCR approach coupled to high resolution DNA fragment analysis. The method is based on the creation of fluorescently labelled amicloids of different lengths which can be resolved via capillary electrophoresis. Each amicloid confirms the presence one of four Hypericum species; H. androsaemon L., H. atwood Boiss. & Orph., H. ascyron Siebold ex Blume and H. perforatum L. These amicloids are produced in a multiplex PCR, with all four reactions occurring simultaneously. The target species for design used in this study represent a worst case scenario, with only a few base differences between the ITS regions for each target. It is likely that a selection of target medicinal plants would not be as closely related, and would therefore have significantly more sequence differences. This would dramatically increase the number of species which could be detected in one assay. This technique has the power to both confirm the presence of expected plant material and detect adulterant material in one reaction. The method of design could be replicated for any other medicinal plant, and its problem adulterants. Keywords: Molecular Identification, Hypericum perforatum, PlantID References: 1 Howard C et al. (2009) Planta Med 75: 864 – 869.

Design and synthesis of natural product-based ligands with high affinity to the kappa-opioid receptor
Zawiony JK, Polepaky PR, Roth BL2, Setola V2, Vardy E2
1Department of Pharmacognosy, and Research Institute of Pharmaceutical Sciences, School of Pharmacy, University of Mississippi, University, MS 38677, USA; 2Department of Pharmacology, School of Pharmacy, NIH Psychoactive Drug Screening Program, University of North Carolina, Chapel Hill, NC 27599, USA

Psychoactive natural products play an important role in the discovery and development of new drugs for the treatment of central nervous system (CNS) disorders. Our studies are focusing on identification of plant metabolites responsible for CNS activity and designing new ligands with high affinity to CNS receptors. Salvinorin A, the most potent naturally occurring hallucinogen isolated from the plant Salvia divinorum Epling & Játiva (Lamiaceae), has received great attention since the kappa-opioid receptor (KOR) was identified as its principal molecular target. Previously, extensive efforts were made to understand how salvinorin A binds to and activates KOR [1 – 4]. Our goal was to design a series of ligands with high affinity to KOR to further explore the ligand-receptor interactions at the molecular level. Following the synthesis of 22-thiocyanatosalvinorin A [5], the first irreversible KOR ligand, we now report the synthesis and biological evaluation in vitro of new salvinorin A derivatives with Michael acceptor-type functional groups.

Figure 1: Synthesis of salvinorin A derivatives with Michael acceptor-type functional groups

Keywords: Salvia divinorum, salvinorin A, kappa-opioid receptor, synthesis of new ligands, affinity, ligand-receptor interactions

Acknowledgements
This work was financially supported by National Institutes of Health grants R01DA017204 and R01DA025520.

References:

In silico approaches to identify FXR-inducing constituents from Ganoderma lucidum - the Chinese mushroom of immortality
Grienke U1, Mihldy B, Schuster D2, Guo D3, Guan S4, Cheng C5, Bochko VN5, Binder B2, Wolber G2, Stuppler H2, Egger M1
1Institute of Pharmacy/Pharmacognosy, University of Innsbruck and Center for Molecular Biosciences Innsbruck, University of Innsbruck, Innrain 52c, 6020 Innsbruck, Austria; 2Center of Biomedical Medicine and Pharmacology, Department of Vascular Biology and Thrombosis Research, Medical University of Vienna, Schwarzenbergstr. 17, 1090 Wien, Austria; 3Computer-Aided Molecular Design Group, Institute of Pharmacy/Chemical Medicine and Pharmacology, University of Innsbruck, Innrain 52c, 6020 Innsbruck, Austria; 4Shanghai Institute of Materia Medica, Chinese Academy of Sciences, 555 Zu Chong Zhi Road, Zhang Jiang Hi-Tech Park, Pudong, 201203 Shanghai, China

The ligand-dependent transcription factor farnesoid X receptor (FXR), belonging to the nuclear receptor superfamily, plays a regulative role in glucose and lipid metabolism. Due to its revealed structural information FXR represents an attractive target for computer-aided drug design. Pharmacophore models based on publicly accessible FXR crystal structures were generated as in silico tools to identify novel bioactive components with the ability to control endogenous pathways in close relation to inflammatory diseases, like metabolic syndrome, dyslipidemia, atherosclerosis, and type 2 diabetes [1, 2]. Lanostane-type triterpenes, as typically found in the fruit body of the famous TCM fungus Ganoderma lucidum (Curtis) P. Karst (Ganodermataceae) were identified as putative FXR ligands by virtual screening of our in-house Chinese Herbal Medicine database [3]. To verify the in silico predictions, 25 constituents isolated from G. lucidum were tested on their FXR-inducing potential in a reporter gene assay at 10 µM. A dose-dependent activity could be confirmed for five lanostane triterpenes, i.e. ergosterol peroxide, lucidumol A, ganoderic acid TR, ganodermanontriol, and ganoderiol F, with EC50 values between 1 µM and 30 µM [4]. In depth structural insights were gained by molecular docking studies allowing a first structure

References:

Keywords: Sesquiterpene Lactones and Pentamethoxylated Flavone from Artemisia kalbada Boiss. & Buhse Rustaiyan A, Ezzataddeh E
Department of Chemistry, Science & Research Branch, Islamic Azad University, P.O.Box 4515 – 773, Tehran, Iran

Artemisia is a genus of small herbs or shrubs found in Northern temperate regions. It belongs to the important family Compositae (Asteraceae), one of the most bulky vegetal groupings, which comprises about 1000 genera and over 20000 species. Within this family, Artemisia is included into the tribe Astemideae and comprises itself over 500 species. The 500 species of Artemisia are mainly found in Asia, Europe and North America. They are mostly perennial herbs and dominating the vast steppe communities of Asia. Asia seems to show the greatest concentration of species with over 150 accessions for China, 174 in the ex U.S.S.R. about 50 reported to occur in Japan and 35 species of the genus are found in Iran, of which two are endemic: A. melaleuca Boiss. and A. kermanensis Pold. Artemisia species, widespread in nature, are frequently utilized for the treatment of disease such as malaria, hepatitis, cancer, inflammation, and infections by fungi, bacteria and viruses and this prompted us to conduct a phytochemical investigation of Artemisia kalbada. The aerial parts of Artemisia kalbada afforded a germacranolide and guaianolide type sesquiterpene lactones together with a pentamethoxylated flavone. The structures were elucidated by spectroscopic methods, including 1D and 2D NMR analysis. Keywords: Artemisia kalbada, Compositae, Sesquiterpene lactones, Guianolide, Germacranolide, Flavone

References:
activity relationship. Furthermore the five active compounds were tested for general anti-inflammatory effects. At a concentration of 5 μM they significantly inhibited the TNF or LPS induced expression of IL-8 and E-selectin in human endothelial cells [4]. The observed FAD induction indicates a possible involvement of this nuclear receptor in the mechanism of the inflammatory regulation by these compounds. Keywords: farnesoid X receptor, Gaonodema lucidum, lanostane triterpenes, molecular modeling, virtual screening, natural products Acknowledgement 1. This work was supported by the National Research Network (NRF) – project “Drugs from Nature Targeting Inflammation” S107/035/S1071/S1073 granted by the Austrian Science Fund (FWF). References: 1. Schuster D et al. (2011) submitted. 2. Lin H R et al. (2006) Bioorg Med Chem Lett 16: 4178. 3. Falkhrudin N et al. (2010) Mol Pharmacol 77: 559. 4. Grienke U et al. (2011) submitted.

SL7

Biological Activities of Andrographolide and Some Semisynthesis Nonbitter Andrographolides

Aromdee C1, Sriabomluk N2, Ekalsaksananon T3, Pientong C4, Seubsean S5, Wiyakrutta S5, Khunkitti W6

1Pharmaceutical Chemistry, Faculty of Pharmaceutical Sciences, Khon Kaen University, Khon Kaen 40002, Thailand; 2Department of Microbiology, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok, Thailand; 3Department of Microbiology, Faculty of Medicine, Khon Kaen University, Khon Kaen 40002, Thailand; 4Department of Microbiology, Faculty of Science, Mahidol University, Bangkok, Thailand; 5Pharmaceutical Technology, Faculty of Pharmaceutical Sciences, Khon Kaen University, Khon Kaen 40002, Thailand

Andrographis paniculata Nees, Acanthaceae, is known as the king of the bitter due to its main constituent, andrographolide. The activities of A. paniculata and andrographolide were antiviral, analgesics, antipyretics, antibacterials and anti-inflammatory [1]. Andrographolide is an ent-labdane containing an α-alkylidene-γ-butyrolactone moiety; two double bonds Δ(17), Δ(12,13); and three hydroxyls at C-3 (a secondary), C-19 (a primary), and C-14 (an allylic). The functional group/s responsible for bitterness of the compound is not yet confirmed, however, esterification of the free hydroxyls of the compound with short chain and long chain fatty acids resulted in diminishing of the bitterness as well as improving those claimed activities of A. paniculata. In our studies 14-acetylation increases the antibacterial against some Gram positive bacteria which resulted in the cell division of B. subtilis [2]. The 3,19-isopropylidenandrographolide, 14-deoxy-3, 19-dipalmitoylandrographolide gave the highest analgesic, antipyretic and antiinflammatory activities [3]. Whereas, only 3,19-isopropylidenandrographolide gave absolute anti-replication of herpes virus [4] and interfering HSV-1 glycoproteins synthesis which was more effectively normalized by STW 5 than by sulfasalazine.


SL8

Different effects of the herbal combination STW 5 in small and large intestine of rats

Klein K1, Angst J2, Merkel K1, Keber O1, Okpanyi SN2, Weiser D3, Heine H2

1Institut für Physiologie der Universität Tübingen, Tübingen, Germany; 2Scientific Department, Steigerwald Arzneimittelwerk GmbH, Darmstadt, Germany

Herbal medicinal products belong to the most successful treatment options in irritable bowel syndrome (IBS), a functional disorder of the gastrointestinal tract which is still not completely understood in its pathogenesis. The aetiology includes disturbed mechanical transport, caused by hyper- or hypomotility of intestinal smooth muscles. For the herbal combination STW 5 (Iberogast®), the influence on spontaneous and induced contractions of ring preparations from murine jejunum, ileum and colon was determined in an organ bath device. The results show that small intestine shows very regular spontaneous contractions under in vitro conditions (amplitude 1.8 ± 0.4 μm, frequency 19.8 ± 3.5 per min) which are inhibited in dose dependent manner by STW 5 and its constituent extracts. (e.g. 50% inhibition by STW 5 in a dilution of 10 μg/mL physiological solution, 90% inhibition by peppermint extract).

The spontaneous activity of large intestine is less pronounced and reveals stronger contractions but a very low frequency. With these specimens the inhibitory effect of STW 5 is less pronounced and corresponds to 10 – 25% inhibition at the same given concentration. When contractions were stimulated by acetylcholine (10 μM) or by KC-Induced depolarization and were inhibited by 90 mM KCl the inhibitory effects of STW 5 were similar in both types of intestine. It can be concluded, that STW 5 has stronger effects in the more proximal parts of the intestine rather than in distal ones under in vitro conditions. However, spasmylosis after stimulated contraction can be achieved in small and large intestine. Keywords: Irritable bowel syndrome, functional dyspepsia, Iberis amara, Peppermint, Mentha piperita, Intestine.
different subgenera of the Allium. L-arginine is a valuable nitrogen storage compound in many angiosperms [2]. But this amino acid has also been found in some Allium species in high concentrations. As an example, the amount of L-arginine in Allium cepa L. was increased during the maturational process [3]. Further on, the amount of L-arginine in samples belonging to the subgenus Allium and Cepa was significantly higher (p < 0.05) as the amount of L-arginine found in the subgenus Melanocormum suggesting different mechanisms of nitrogen storage. These differences should allow chemotaxonomical classification of subgenera. Keywords: Allium, cyclic sulphoxides, o-phthalaldehyde, amino acids, References: 1. Friths RM et al. (2010) Phyton 45: 145 – 220, 2. Van Etten AH et al. (1963) Agr Food Chem 11: 399 – 409, 3. Schupan W, Schwertfeger E (1972) Qual Plant Mater Veg 21: 141 – 157

What can phylogeny tell us about chemical diversity?

Ranstved N, Stafford G1, Meewor AW2, Petersen GC1, Van Staden J1, Jager AK2
1Natural Products Research, Department of Medicinal Chemistry, University of Copenhagen, Universitetsparken 2, DK-2100 Copenhagen, Denmark; Present address: Botanical Gardens, Natural History Museum of Denmark, Salgade 83, Opg. S., DK-1307 Copenhagen, Denmark; 2USDA, 13680 Old Cutler Road, Miami, Florida, USA; Botanical Gardens, Natural History Museum of Denmark, Salgade 83, Opg. S., DK-1307 Copenhagen, Denmark; 4University of KwaZulu-Natal, Pietermaritzburg, South Africa

Plant secondary metabolites are produced and selected by evolution for their biological activity. Such natural products played a major role in traditional medicine and as leads for modern medicine. Only a small fraction of the Worlds biodiversity has been explored for chemical and biological activity. A correlation between phylogeny and biosynthetic pathways is often assumed and could offer a predictive approach enabling more efficient selection of plants for traditional medicinal lead discovery. However, formal tests of correlations between phylogeny and chemistry are rare, and the potential predictive power is consequently unknown. As a case in point, we are exploring the Amaryllidaceae subfamily Amaryllidoideae sensu APG, which is known for its subfamily specific alkaloids with activity in the central nervous system (CNS). Galanthamine registered for the treatment of Alzheimer’s disease was first isolated from the Caucasian snowdrop Galanthus woronowii Losinsk. We present a phylogenetic hypothesis of the Amaryllidaceae subfamily Amaryllidoideae based on nuclear, plastid and mitochondrial DNA sequences of over 100 of the currently accepted tribes and geographical regions. All major lineages are now well supported and the extended sampling uncovered several genera as non-monophyletic, emphasizing the importance of using phylogenetic rather than classical classification for interpretation of character distribution. Alkaloid profiles and CNS-related bioactivity profiles are significantly correlated with phylogeny using formal tests. Relationships between phylogenetic and chemical diversity are further explored. The predictive power can be used to select candidate taxa for lead discovery and to make recommendations for traditional use. Keywords: Amaryllidaceae, phylogeny, chemical diversity, lead discovery. This research was supported by a Steno grant (N/C30272 – 07 – 0281) to NR from the Research Council of Zhejiang University and Skyherb Ingredients. Acknowledgement: This research work was supported by the Research Council of Zhejiang University and Skyherb Ingredients.

Determination of bioactive coumarins in Radix angelicae pubescentis by HPLC

Cheng Y1, Zha M2, Yan W1
1Department of chemistry, Zhejiang university, hangzhou, 310027, China; 2Department of TCM, Zhejiang institute for food and drug control, hangzhou, 310004, China

Radix angelicae pubescentis (Duhuo in Chinese) was collected in Chinese Pharmacopoeia 2010 and widely used to treat thrombosis, arthritic disease, and anti-inflammatory. Pharmacological studies and clinical practice have demonstrated that Radix angulicae pubescentis possesses bioactive coumarins, including osthole, O-acetyl-columbianetin, and columbianadin. The HPLC method for determination of O-acetyl-columbianetin, osthole and columbianadin in Radix angulicae pubescentis has been developed in this paper. HPLC separations were carried out with a Diomons C18 column (250 mm x 4.6 mm, 5 μm). The mobile phase was consisted of water and acetonitrile. The gradient elution was as follows: 0 to 15 min, isocratic (52:48, v/v); 15 to 45 min linear gradient (52:48 to 42:58, v/v). The column temperature operated at 40 °C. The detection wavelengths was 322 nm. The system suitability tests, including the linearity, limit of detection, limit of quantification, precision, repeatability and accuracy have been made. The developed method provides high selectivity and sensitivity with good accuracy and reproducibility. The established method was applied to simultaneous determination of the three analytes in seven samples of Radix angelicae pubescentis obtained from different companies and batches. The content ranges of three bioactive coumarins are 3.60 to 1.36 mg/g for O-acetyl-columbianetin, 6.03 to 4.44 mg/g for osthole, and 4.04 to 9.04 mg/g for columbianadin. The results demonstrated the influence of the treatment method, and the storage time on the contents of O-acetyl-columbianetin, osthole, and columbianadin in Radix angelicae pubescentis. Acknowledgement: This research work was supported by the Research Council of Zhejiang University and Skyherb Ingredients.

Novel heparanase activity assay based on a fluorescence sensor technology

Alban S, Schiemann S
Pharmaceutical Institute, Christian-Albrechts-University of Kiel, Cunenburgstr. 76, 24118 Kiel, Germany

Since tumors and other diseases are characterized by increased heparanase (HEP) levels, HEP is considered a diagnostic marker and a target for tumor therapy. Therefore, different HEP detection methods are necessary to assay HEP and to examine inhibitors. Based on previous findings that HEP degrades not only heparan-sulfate, but also the also the sulfated pentasaccharide fondaparinux (FPX) [1] and that this can be quantified by its effect on the fluorescence intensity (FI) of the sensor molecule Polymer-H [2], we aimed to develop a fluorimetric HEP activity assay. Since the FPX degradation products proved to have no effect on the FI, the remaining FPX can be measured without separation. Optimization of various assay parameters led to the following two-step procedure: (1) Incubation of HEP containing solution with FPX (10 μg/mL). (2) Sample dilution and FPX detection by adding Coom-Polymer-H (7.5 μg/mL) and measuring the FI (λex=330 nm, λem=510 nm). The FI degradation showed to increase with the concentration of HEP. After 30 min at 37 °C, 1.5mU/mL HEP led to complete FPX-degradation. By varying incubation time and FPX concentration, the LOD of 0.2mU/mL HEP can be considerably decreased. Various HEP inhibitors demonstrated the suitability of the assay for inhibitor screening. In conclusion, a rapid, simple and robust microplate HEP activity assay was developed. A major advantage is the use of FPX as substrate. In contrast to heparan-sulfate, it is chemically defined, well available and has not to be labeled with radioactive or other markers for detection. Moreover, its fluorimetric detection is much more convenient than its anti-FXI-activity or HPLC-MS. Keywords: pharmacology, heparanase, assay development, fluorescence sensor technology, heparanase inhibitors References: 1. Alban S et al (2009) J Thromb Haemost 7, Suppl 2: PP-WE-506. 2. L/Cehn S, Schrader T, Sun W, Alban S (2010) Pharm Biomed Anal 52: 1 – 8.

Determination of curcumin in turmeric using magnetic iron oxide nanoparticles as solid phase extractor and HPLC

Hadjmohammadi M, Salamat G, Sharifi V
Department of chemistry, University of Mazandaran, Babolsar, Iran

Curcumin, a derivative of Curcuma longa L., is used extensively in the food industry and researches have shown the health benefits of this compound [1]. In this work, a novel, simple and rapid method for extraction and determination of curcumin in turmeric was performed using magnetic iron oxide nanoparticles (MIONs) as solid phase extractor and HPLC. The unique properties of nanoscale materials offer excellent prospects for designing new methods and instrumentation for chemical analysis [2]. The MIONs were synthesized according to the method proposed by Laurent et al. [3]. The average size of nanoparticles was in the range of 90 nm which was determined by using atomic force microscopy (AFM) (Fig. 1). Extraction of curcumin is based on the complex Fe3O4-cumin complex on MIONs. Desorption of analyte was performed by NaOH solution containing methanol in order to dissolve the desorbed analyte. Various parameters affecting the extraction recovery such as: pH, volume and concentration of NaOH as desorbing reagent, and con-
centration of Fe (+3) and percentage of methanol were investigated and optimized. These optimized parameters were: pH = 2.0, 1.5 mL of 0.2 M NaOH containing 30% methanol and 0.1 M of Fe (+3), respectively. The intra-day precision (R.S.D.) was 4.0% and inter-day R.S.D. was less than 7.0%. The preconcentration factor of 100 was achieved in this method. The proposed procedure has been successfully applied to the determination of curcumin in turmeric.


**Figure 1:** size distribution of MIONs determined by AFM


**Figure 1:** size distribution of MIONs determined by AFM


New cytotoxic pregnane glycosides from Caralluma sinaica growing in Saudi Arabia

A. Berger, growing wild in the western region of Saudi Arabia, is used by locals as a remedy to treat diabetes [1]. Pregnaneglycosides, the key phytochemical ingredients in Caralluma, are drawing much attention in recent years because of their antitumor and anticancer activities [2]. Thirteen pregnane glycosides, including six new (Fig. 1), were isolated from the cytotoxic chloroform extract of the titled plant using repeated normal and reversed phase chromatographic techniques. The structures of the isolated compounds were characterized using extensive spectroscopic techniques including 1D and 2D microflow-NMR methods for compounds available in restricted amount. A detailed profiling of the constituents was obtained by UHPLC-ESI-TOF/MS data [3]. Some isolated compounds were evaluated for their in vitro cytotoxic activity, and quinone reductase induction was also assessed. Abbreviations: Benzoyl: Bz, Tigloyl: Tig, Acetate: Ac, Thevetose: Thev, Cymarose: Cym.

Figure 1: Main structure of pregnane glycoside

List of new compounds

<table>
<thead>
<tr>
<th>R</th>
<th>R1</th>
<th>R2</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>The-(1-4)-cym-(1-4)-cym</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Glc-(1-4)-cym</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Glc-(1-4)-dig-(1-4)-cym-(1-4)-cym</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Glc-(1-4)-thev-(1-4)-cym-(1-4)-cym</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Glc-(1-4)-cym</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Glc-(1-4)-cym-(1-4)-cym</td>
<td></td>
</tr>
</tbody>
</table>


Isolation of Novel Cytotoxic Compounds from a Bangladeshi Medicinal Plant Acrostichum aureum

Natural products and related drugs are used to treat 87% of all categorized human diseases including cancer and immunological disorders [1]. This study reports on the isolation and characterisation of novel cytotoxic compounds from Bangladeshi medicinal plants. Following LC-MS metabolic profiling and cytotoxic screening of 16 Bangladeshi medicinal plants against normal mouse fibroblast (NIH3T3) and three human cancer cell lines (AGS, HT-29 and MDA-MB-435S), Acrostichum aureum L. was selected for further phytochemical and pharmacological investigations. A total of 13 compounds were isolated from this plant using SPE and reversed-phase HPLC. The structures of compounds were elucidated by NMR MS and other spectroscopic methods. Three compounds (1, 2 and 5) were identified as novel natural products. Eight known compounds were isolated for the first time from A. aureum: di-(2-methylthyl) phthalate (3), (2S, 3S)-retinol (4), (2R)-retinol (7), tetracosan (6), quercetin-3-O-β-D-glucosyl-(6-1)-O-L-rhamnose (9), quercetin-3-O-L-rhamnose (10) and quercetin-3-O-α-L-rhamnose-7-O-β-D-glucoside (11) and patriscabratine (12). Two known flavonoids, quercetin-3-O-β-D-glucoside (8) and kaempferol (12) have previously been isolated from A. aureum. The cytotoxic activity of all compounds was assessed against the cell lines mentioned above using the MTT assay. Four compounds (3, 5, 6 and 12) showed moderate to potent cytotoxic activity. Compound 3 displayed the most potent cytotoxicity against gastric and colon adenocarcinoma cell lines (IC50 75 – 216 μg/mL). The mode of action was revealed as induction of apoptosis. This research has demonstrated that Bangladeshi plants are an exciting source of bioactive novel compounds and as such a rich basis for ongoing drug discovery research. References: 1. Newman DJ and Cragg GM (2007) J Nat Prod 70(3): 461 – 77.

Figure 1: Biofeedback
Neuropeptide Y (NPY) is a stress hormone widely distributed in the central and peripheral nervous system. Human studies have revealed a role for NPY in “buffering” the harmful effects of stress (adaptation to stress) [12]. There is a plethora of pre-clinical and clinical evidence suggesting a mood and cognitive performance improving action for NPY [3, 4]. Higher levels of NPY have been observed in soldiers who either present reduced psychological distress or belong to special forces [2]. In contrast, decreased levels of NPY were observed in depression and in brain tissues of suicide victims [1]. Our study for the first time provides evidence that adaptogens, specifically ADAPT-232 – a fixed combination of Eleutherococcus senticosus Maxim. root extract SHE-2, Schisandra chinensis K.Koch berry extract, Rhodiola rosea L. SHR-5, temporarily lower the expression of NPY, heat shock factor-1 (HSF-1) and release of the heat shock protein (Hsp72) in isolated neural cells. Pre-treatment of human neuroglia cells with NPY-siRNA or HSF1-siRNA (which silences the expression of intracellular NPY and HSF-1 respectively), before treatment with ADAPT-232 resulted in a significant suppression of Hsp72 release. References: 1. Morales-Medina JC et al. (2009) Brain Res 1314:194–205. 2. Morgan III CA et al. (2002) Biol Psychiatry 52: 136 – 142. 3. Morgan III CA et al. (2000) Biol Psychiatry 47: 902 – 909. 3. Redrobe JP et al., (2002) Life Sci 71:2921 – 37. 4. Fletcher MA et al. (2010) Behav Brain Funct 6:76 – 83.

Arabinogalactan-proteins (AGPs) are macromolecular glycoproteins belonging to the putative active compounds of Echinacea preparations [1]. (β-D-Glc)-Yariv phenylglycoside specifically binds to most plant AGPs and has been used to isolate AGPs from pressed juice of the aerial parts and from suspension cultures of Echinacea purpurea L. Moench (Asteraceae). These AGPs have been structurally characterized and compared concerning their protein- and polysaccharide moiety. The main components of the carbohydrate moiety of AGP from herbal material are 1,6-Galp, 1,3-Galp, and in the side chains 1,5-Araf, terminal Araf and terminal GlcpAp. Side chains of AGP from cell cultures are structurally different with only traces of 1,5-Araf. The protein part of both AGPs mainly consists of Hyp, Axs, Glx, Ser, Thr and Ala. Interestingly, AGP from herbal material showed an amino acid sequence rather untypical for AGPs with predominantly contiguous arrangement of three to four Hyp residues in blocks [2]. For microscopic localization of AGPs in fresh plant tissue, a new method has been developed. Antibodies against Yariv’s reagent have been generated in rabbits and used for immunofluorescent labeling of plant tissue. Xylem tracheary elements showed very strong labeling of the cell wall, especially at the inner side of the wall and in the area of pit canals. Preparations of pressed juice from Echinacea purpurea are used as herbal medicinal products with immunomodulating properties. In vitro, AGP from the pressed juice of herbal material showed complement stimulating activities [3] as well as binding to human leukocytes [4]. Keywords: Echinacea purpurea, arabinogalactan-protein, structure elucidation, immunofluorescence, natural immune enhancer. Acknowledgement: The authors thank Rottapharm/Ma- daus GmbH, Köln, Germany, for financial support of this work. References: 1. Classen B et al. (2006) Phytomedicine 13: 688 – 694. 2. Classen B et al. (2005) Planta Med 71: 59 – 66. 3. Alban S et al. (2002) Planta Med 68: 1118 – 1124. 4. Thude S et al. (2006) Phytomedicine 13: 425 – 427.
SL25 Metabolomic profiling of saw palmetto products using proton-NMR spectroscopy and multi-variate analysis
Booker AJ1, Zloh M1, Said M1, Suter A2, Heinrich M1
1Centre for Pharmacognosy and Phytotherapy, University of London, School of Pharmacy, UK; 2Product Development and Medical Affairs, Bioforce AG, Switzerland

Saw palmetto products with an often poorly known and variable chemical composition are used in the treatment of Benign Prostatic Hyperplasia.(1, 2) Here we present a method for the metabolomic analysis of saw palmetto products using NMR spectroscopy and multi-variate analysis in order to determine if there are significant differences in metabolites of the given products and if marker compounds can be identified. Spectra were obtained on a Bruker 500 MHz spectrophotometer. TOPSPIN was used for spectra acquisition and processing. Deuterated methanol and chloroform were selected as solvents. NMR spectra were transferred to AMIX. The spectra were divided into 251 regions and the signal intensity was integrated. Unscrambler was used for PCA analysis. The analysis showed that saw palmetto products can be grouped according to their metabolite profile. Multi-variate analysis showed significant variations between powders, soft extract and tincture products. The largest variation in any product tested was observed for a hexane extract. Oleic acid and capric acid ethyl ester were identified as potential marker compounds. Additional information regarding TLC analysis and clinical outcomes was supplied by Andy Suter from Bioforce AG, Switzerland. Variations in chemical content were identified using NMR spectroscopy, however, multivariate analysis of the products suggested that there was no significant difference in metabolites between the European extracts tested but differences were observed when compared to non-European products or products that used hexane as the extraction solvent. It is tested but differences were observed when compared to non-European products. The largest variation in any product tested was observed for a hexane extract. Oleic acid and capric acid ethyl ester were identified as potential marker compounds. Additional information regarding TLC analysis and clinical outcomes was supplied by Andy Suter from Bioforce AG, Switzerland.

SL26 Herb-based functional foods: from laboratories to the market
Tsai H
Institute of Biochemical Sciences and Technology, Chaoyang University of Technology, Wufang, Taichung, Taiwan

Consumption of alternative herbal folk medicine has had a tremendous increase in the last decade. A number of medicinal plants contain secondary metabolites which have many biologically active compounds. They are used against hepatic fibrosis and heart ischemia-reperfusion and proved to have antioxidant, antithrombosis, antihypertension, antitress, antivirus, antitumour, antidiabetic, antiaging and anti-inflammatory activities. Non-availability of quality planting materials, slow germination, slow plant growth, disease and pest incidence are the major obstacles in conventional medicinal plant cultivation. In Taiwan, many economically important medicinal plants and herbs are produced using various explant materials by tissue culture technique to meet the increasing demand for their medicinal properties. Rapid multiplication through in vitro tissue culture can be advantageous for the continuous supply throughout the year. We have developed and standardized efficient, simple and rapid tissue culture regeneration protocols of many medicinal plants, optimized the conditions in green house and successfully established the regenerated plantlets in the field for the large scale commercial production. Availability of tissue culture protocol is the first step towards the development of the genetic transformation. Keywords: Medicinal plant, Tissue culture, Functional foods Acknowledgement: Thanks to the National Science Council of Taiwan for financial support

SL27 Safety evaluation of licorice consumption from dietary and phytotherapeutic sources
Wohlmuth H1, Meireiros P2, Lee R3, Brushett D4, Arellano J3
1Southern Cross Plant Science, School of Health and Human Sciences, Southern Cross University, PO Box 157, Lismore, NSW, Australia; 2University of Redlands, 1200 East Colton Ave P.O. Box 3080, Redlands, CA, 92373, USA; 3School of Health and Human Sciences, Southern Cross University, PO Box 157, Lismore NSW, Australia

Licorice (Glycyrrhiza glabra L.) contains the triterpenoid glycyrrhetinic acid (GA), which has an intensely sweet taste and is used as a flavouring agent, mostly in confectionary products and tobacco. Licorice also has a long history of medicinal use and is listed in current editions of the British, European and Chinese pharmacopoeias. GA and its aglycone, glycyrrhetinic acid, competitively inhibit 11β-hydroxysteroid dehydrogenase type 2, which converts active glucocorticoids to their inactive metabolites. Mineral corticoid-like effects can result, giving rise to pseudoaldosteronism characterised by hypokalaemia, sodium retention, oedema and suppression of the renin-angiotensin-aldosterone system with resulting hypertension.1,2 Licorice-induced pseudoaldosteronism is well documented with most cases involving confectionary licorice. Although it is not possible to accurately identify a NOEL for GA most human studies suggest that a daily intake of more than 100 mg GA over a period of weeks is necessary to produce pseudoaldosteronism. In order to evaluate the potential risk associated with the consumption of Australian made confectionary licorice products and liquid extracts dispensed in phytotherapy, we determined the GA content of ten confectionary products, five liquid extracts and two licorice root samples by reverse-phase HPLC. All but one liquid extract contained GA in concentrations that could readily result in a daily intake in excess of 100 mg if taken in a therapeutic dose. In contrast, confectionary products contained GA in concentrations that varied three orders of magnitude and only a few of them would provide 100 mg/d of GA if consumed in amounts up to 100 g daily. Keywords: glycyrrhetic acid; licorice; safety

SL28 The molecular cloning of dihydroartemisinic aldehyde reductase and its implication in artemisinin biosynthesis in Artemisia annua
Kaysier O1, Ryden A1, Bouwmeester H1, Ruyter Spira C2, Osada H4, Muranaka T4
1Technical University Dortmund, Technical Biochemistry, 44227 Dortmund, Germany; 2Laboratory of Plant Physiology, Wageningen University, 6700 AR Wageningen, the Netherlands; 3University of Groningen, Pharmaceutical Biology, 9713 AV Groningen, the Netherlands; 4Chemical Biology Department, Antibiotics Laboratory, Advanced Science Institute, RIKEN, 2 – 1, Hirosawa, Wako, Saitama 351 – 0198, Japan

A key point in the biosynthesis of the antimarial drug artemisinin is the formation of dihydroartemisinic aldehyde which represents the key difference between chemotype specific pathways. This key intermediate is the substrate for several competing enzymes some of which increase the metabolic flux towards artemisinin and some of which – as we show in the present study – may have a negative impact on artemisinin production. In an effort to understand the biosynthetic network of artemisinin biosynthesis, extracts of A. annua L. flowers were investigated and found to contain an enzyme activity competing in a negative sense with artemisinin biosynthesis. The enzyme, Red1, is a broad substrate oxidoreductase belonging to the short chain dehydrogenase/reductase family with high selectivity for dihydroartemisinic aldehyde and valuable monoterpenoids. Spatial and temporal analysis of cDNA revealed Red1 to be trichome specific. The relevance of Red1 to artemisinin biosynthesis is discussed.
**Keywords:** Artemisia annua, red1, dihydroartemisinic aldehyde, dihydroartemisinic acid, trichome, oxidoreductase. **References:** Rydén A-M, Ruyter-Spira C, Osada H, Muranaka T, Kayser O, Bouwmeester H (2010) Planta Medica 76:1–6

---

**Figure 1:** Biosynthetic pathway of artemisinin and the activity of Red1

---

**SL29**

HPLC-M5 and NMR spectroscopy: two integrative analytical tools for the quality control of plant extracts. The case of a commercial blend sold as dietary supplement

Karioti A1, Giocarelli E2, Pieraccini G2, Vannacci A3, Galli E4, Bilia A1

1University of Florence, Department of Pharmaceutical Sciences, Via Ugo Schiff 6, 50139 Sesto Fiorentino Florence, Italy; 2University of Florence, Mass Spectrometry Center (CISM), Viale Pieraccini 6, 50139 Sesto Fiorentino Florence, Italy; 3University of Florence, Department of Preclinical and Clinical Pharmacology, Centre for Molecular Medicine (CIMMBA), Viale Pieraccini 6, 50139 Florence, Italy

In this study, HPLC-DAD-ESI-MS, HPLC-ESI-MSn and 2D NMR techniques were applied for the quality control of a herbal supplement. Several reports were received by the pharmacovigilance system by patients who fainted after consuming “Olivis” (L.) Medik. () sample was subjected, after lyophilization, to direct 2D NMR and HPLC-DAD-ESI-MS analyses to identify the marker constituents of the different Herbal Drugs. Comparison of the NMR and chromatographic profiles of the product with those of the marker compounds of the declared plant species, (such as oleuropein, protopine), showed the lack of the latter. Samples of the declared plant species were taken into consideration as species, (such as oleuropein, protopine), showed the lack of the latter. Further, HPLC-DAD and HPLC-ESI-MS analyses showed the presence of reserpin in the product with those of the commercial product revealed marked differences in their content. Further, HPLC-DAD and HPLC-ESI-MS analyses revealed the presence of rauwolfia sp. type indole alkaloids, while HPLC-ESI-MSn analyses showed the presence of reserpin. Parallel phytochemical fractionations led to the isolation of ajmaline. Quantitative studies showed that the content of reserpin in the product was in the therapeutic range and therefore responsible for the collapse of the patients; (ii) ajmaline prevailed against reserpin indicating that a Rauwolfia species other than R. serpentina was used. The present study shows the importance of extensive controls using combined analytical tools of the botanical products on the market to assure their quality and as a consequence their safety profiles. Keywords: HPLC-UV-DAD-MS profile, NMR profile, dietary supplement, adulterant, reserpin, ajmaline

---

**SL30**

Isolation and characterization of biosurfactant producing bacteria having antimicrobial activity isolated from oil contaminated sites

Qazi MA1, Ahmed S2, Malik ZA2, Hameed A3

1Institute of Pharmacy/Pharmacognosy, Center for Molecular Biosciences Innsbruck, University of Innsbruck, Innsbruck 52c, A-6020 Innsbruck, Austria; 2Department of Microbiology, Via Ugo Schiff 6, 50139 Sesto Fiorentino Florence, Italy; 3University of Florence, Mass Spectrometry Center (CISM), Viale Pieraccini 6, 50139 Sesto Fiorentino Florence, Italy

Biosurfactants are surface active amphiphilic compounds that reduce the surface tension of liquids, thereby increase the miscibility of hydrophobic compounds. One of the isolate from oil contaminated site was tested for its ability to produce biosurfactants. The bacterium was identified on the basis of morphological and biochemical characterization and found to be Pseudomonas putida SOL-10. The isolate was tested for biosurfactant production under shake flask fermentation and found to be potent biosurfactant producer. The biosurfactant production was analyzed by surface tension and emulsification index (E24%) measurements. In this study the biosurfactant was produced by a newly isolated Pseudomonas putida SOL-10 at optimized conditions. The biosurfactant produced by the isolate reduced the surface tension of culture broth from 43.6–29.9 mN m⁻¹ achieving a maximum biosurfactant concentration of 4.5 g/L after 72 hours of incubation. The biosurfactant also demonstrated a good antimicrobial activity as well as the capability to enhance the antimicrobial effect of some antibiotics. The current study describes the effect of biosurfactant with Ampicillin, Ciprofloxacin and Cefixime, against Escherichia coli, Bacillus subtilis, and Klebsiella pneumonia. The results demonstrated promising antimicrobial activity enhancing effect of the biosurfactant and suggested a possibility of the biosurfactant to be used with antibiotic formulations in order to increase the effectiveness of antimicrobials against multidrug-resistant pathogenic bacteria. Keywords: Pseudomonas putida, biosurfactant, characterization, antimicrobial activity, antibiotics, surface tension

---

**SL31**

Egyptian Herbal Drug Industry: Challenges for the Future

Abdel Azim NS

Phytochemistry Dept., National Research Centre, Cairo, Egypt

According to the World Health Organization (WHO), the goal of ‘Health for All’ cannot be achieved without herbal medicines. While the demand for herbal medicines is growing in developing countries including Egypt, there are indications that consumers in developed countries are becoming disillusioned with modern healthcare and are seeking alternatives in traditional medicines. There is, therefore, an increasing consumer demand for herbal medicines in developed countries. Medicinal plants have been used as a source of remedies since ancient times in Egypt. Many plants are still used today in folklore medicine and are sold at herbal venders and shops. Egypt is characterized by abundant production of medicinal and aromatic plants that are exported all over the world and is considered as one of the most important sectors can be relied upon to increase the volume of Egyptian exports due to the growing global demand but several factors pose constraints to their entry into the international market and put them in a disadvantageous position. This lecture explores the situation of the Egyptian herbal drug industry, the economic value, the needs and recommendations for developing this important sector. Keywords: herb medicine, Egypt, drug industry, challenges References: References 1. Batanouny K H (1999) Wild Medicinal Plants in Egypt (with contribution: E. Aboutabl, M. Shabana & F. Soliman). 2. Dagmar L (2006) International Trade in Medicinal and Aromatic Plants, Actors, volumes and commodities, Plants.- In Bogers, R.J., Craker, L.E. & Lange, D. (eds.).; Medicinal and Aromatic Plants, 155 – 170. 3. Das M. (2005) Medicinal Plants: An Approach toward sustainable development, available at http://www iamot.org/conference/index.php/ocs/4/paper/viewFile/467/18 4. Saleh NAM (2003) Phytochemistry 62: 239 – 241.

---

**SL32**

Bioactivity-guided isolation of GABAA Receptor Modulating Constituents from the Rhizome of Actaea racemosa Cick S1, Khom S1, Taferner B1, Hering S2, Suppern H1

1Institute of Pharmacy/Pharmacognosy, Center for Molecular Biosciences Innsbruck, University of Innsbruck, Innsbruck 52c, A-6020 Innsbruck, Austria; 2Department of Pharmacology and Toxicology, University of Vienna, Vienna, Austria

Black cohosh (Actaea racemosa Walter ex Steud.) is a frequently used herbal remedy for the treatment of mild climacteric symptoms. The plants active principle was extensively studied, but its mechanism of action remained unclear [1]. In this study the modulation of GABA-induced chloride currents (I GABA) through GABAA receptors by black cohosh extracts and isolated compounds was investigated. GABAA-receptor consisting of α1, β2 and γ2S subunits were expressed in Xenopus laevis oocytes and potentiation of I GABA was measured using the two-microelectrode voltage clamp technique. In a bioactivity-guided isolation procedure the positive modulation of I GABA could be restricted to the terpenoid fractions, resulting in the isolation of 11 cycloartenyl glycosides, of which 4 compounds significantly enhanced I GABA by more than 150% (p < 0.05). The strongest effect was observed for 23-O-acetyl-
shengmanol-3-O-β-D-xylpyranoside (100 μM) at 1692 ± 201 %, while actein, cimigenol-3-O-β-D-xylpyranoside and 25-O-acetylshengmanol-3-O-α-L-arabinopyranoside were significantly less efficient (range of I_{50} = 378 ± 64 %). 23-O-acetylshengmanol-3-O-β-D-xylpyranoside which exhibited the greatest potentiation of I_{50} was additionally investigated in a mouse model. In these studies a decrease in motor activity as well as a clear reduction in anxiety behaviour of animals treated with 23-O-acetylshengmanol-3-O-β-D-xylpyranoside was observed, indicating this compound to induce strong sedative effects with concomitant anxiolytic activity. We hypothesise that the established positive allosteric modulation of GABA_A receptors may contribute to beneficial effects of black cohosh extracts in the treatment of climacteric symptoms. References: 1. Palacio C, Masri G (2009) Drugs Aging 26:23 – 36.

**Pharmacokinetic interactions between botanicals and drugs: in vitro investigations**

SL33

Herb-drug pharmacokinetic interactions include interferences of plant constituents with drug bioavailability by means of altered absorption, metabolism, distribution and/or elimination [1]. Although in vitro pharmacokinetic investigations are not always clinically significant in the in vivo situation [2], it may in many cases indicate potential important interactions that can influence the bioavailability of co-administered drugs. This work reports on the effects of extracts from different botanicals such as whole leaf extracts and gels (Aloe vera, Aloe ferox) [3], corns (Hypoxis hemerocallidea), aerial parts (Sutherlandia frutescens, Aspalathus linearis) [4], fruit (Sclerocarya bierrea, Pseudium guajava, Dovyalis caffra, Prunus persica, Drosophyllum lingua, Prunus domestica) and vegetative tubers (Daucus carota, Beta vulgaris) [5].

The mechanisms of drug transport alteration was determined for some of the botanicals by means of either measuring transport in two directions (to indicate efflux inhibition/induction) or by measuring the transepithelial electrical resistance (to indicate opening of tight junctions).


**Antimicrobial investigations with impact – what can researchers do to ensure continuity?**

SL35

When reflecting on past antimicrobial studies undertaken on medicinal plants, it is clear that those having the most impact have been when a targeted disciplinary approach has been adopted. In order to elaborate on this, past and present studies will be presented with different approaches (pathogen specific, ethnopharmacological correlations, combination studies, structure activity relationships, formulations etc.) in order to achieve outcomes that address recommendations made from previous antimicrobial reviews [1,2,3,4]. Medicinal plant use by habitants from Maputaland, KwaZulu Natal (South Africa) were undertaken, whereby the in vitro antimicrobial investigations against diseases associated with diarrhoea, respiratory and sexually transmitted infections were validated. The outcomes of these studies address the need to investigate specific pathogens, interactional efficacies between different plant species and toxicity profiles. When popular essential oils (Melaleuca alternifolia Cheew, Thymus vulgaris L., Mentha piperita L. and Rosmarinus officinalis L.) were combined with conventional antibiotics (ciprofloxacin and amphotericin B), a predominantly antagonistic interaction was noted, highlighting the need to not only focus on synergistic interactions. Structure activity relationships are important when reporting both the chemistry and antimicrobial activity and pharmacokinetic profiles of Leptospermum petersonii F.M.Bailey and the major compounds demonstrate the cidal effect of cital within 1 h exposure to Staphylococcus aureus. Future formulation studies look promising, as demonstrated by the enhanced antimicrobial efficacy of Melaleuca alternifolia when encapsulated into polymeric liposomes. With analysis of past shortfalls and future recommendations, researchers should be encouraged to continue producing as much information as possible to allow for a better understanding of plant-based antimicrobial research. Keywords: antimicrobial, pathogen specific, ethnopharmacological correlations, combination studies, structure activity relationships, formulations References: 1. RHS JL, Recio MC (2005) Ethnopharmacol 100: 284 – 288. 2. Cos P et al. (2006) J Ethnopharmacol 109: 302 – 302. 3. Van Vuuren SF (2008) J Ethnopharmacol 119: 462 – 472. 4. Van Vuuren SF, Viljoen AM (2011) Plant Med DOI http://dx.doi.org/10.1055/s-0033-1250736.
The high impact of Natural Products (NP) in medicine is proven and well sustained [1]. NP are characterized by unique structural diversity and critical drug-like features which rank them as the most promising candidates for possible future drugs [2]. However, several factors constrain their development related mostly to the time-consuming and labor procedures required for their isolation, the high cost and the dereplication problem [3]. In the present work, we propose a structure-oriented approach using UHPLC-HRMS/MS (LTQ-Orbitrap) methodologies trying to release the entire procedure from repeated and useless isolation steps and pursuing the targeted determination of the possible bioactive compound. This approach is carried out in crude extracts and is based on fast UHPLC methods, high resolution mass spectra and ms/ms accurate mass measurements. As a proof of concept, the Cameroononian tree *Amphimias pierocarpoides* Harms (Leguminosae) was selected and flavonoids – isoflavonoids were chosen as the chemical class of interest. Based on a chromatographic (Rt, polarity) and spectrometric features (UV, accurate m/z, proposed ECs, RDB values and RIAS) as well as ms/ms spectra, the compounds of interest were defined and structurally elucidated. 12 of the 17 traced flavonoids were selectively isolated and characterized using 1 and 2D NMR techniques verifying our concept. Applying this approach, the identification of target-compounds is achieved early in the discovery procedure facilitating the dereplication of known compounds. Consequently much time required for the isolation, purification and separation is saved while the possibility of the discovery of novel structures and subsequently novel actives is elevated. References: [1] Newman DJ, Crigg GM (2007) Nat Prod 70: 461 – 477. [2] Yuliana ND, Khatib A, Choi YH and Verpoorte R (2011) Phytother Res 25: 157 – 163. [3] Pojerot O and Hamburger M, (2006) Current Organic Chemistry 10: 899 – 920.

**SL37**  
Monodimensional and comprehensive liquid chromatography linked to mass spectrometry for unravelling bioactive components in natural plants

**Monello L**, Donato P, Cacciola P, Dugo P  
1Departmento Farmaco-chimica, University of Messina, 98168 – Messina, Italy; 2University Campus Bio-Medico, 00128 – Rome, Italy; 3Chromaleont S.R.L. c/o Studio Raffa-Gattuso, 98123 – Messina, Italy

There is considerable evidence nowadays that dietary flavonoids and other phenolic compounds may exert preventive and/or therapeutic effects in a role of human diseases. Despite the great interest in determining the role of phytochemicals as potential therapeutic agents, and the rising demand of natural sources with nutraceutic benefits, the antioxidant content of many medicinal plants and foodstuffs is unknown, the rising demand of natural sources with nutraceutic benefits, the antioxidant content of many medicinal plants and foodstuffs is unknown, the role in preventing and curing diseases [1]. Also, they have a broad range of applications in foods, perfumes, cosmetics and human nutrition. Gas chromatography-mass spectrometry (GC-MS) is the most important technique for the analysis of essential oils [2]. However, there are some fundamental problems in their analysis including baseline drift, spectral background, noise, low S/N, changes in the peak shapes and co-elution (overlapped, embedded ions) [3]. Mathematical chromatography (MC) as a branch of chemometrics [4] attempts to develop new tools to handle these problems. In this work, first, we have extracted the essential oils of the peels of eighteen citrus fruits such as lemon, lime, mandarin, grapefruit and hydrodistillation and then analyzed with GC-MS. Then, their signals were analyzed by MC. Using this strategy, the numbers of identified components were extended and quality of the results was improved significantly. As a positive consequence of using the proposed strategy human time and work are saved. Also, some new components were identified for the first time. In addition, we used our recently developed software, called MCRC Software for performing these techniques [5]. After resolving the volatile components in different samples, principal component analysis (PCA) was used for monitoring the pattern of volatile components in different samples. It is concluded that GC-MS/MC/PCA can open a new window to the comprehensive analysis of essential oils. Keywords: Chromatography, Mathematical chromatography, Essential oil, Gas chromatography-mass spectrometry, Citrus fruits


**SL38**  
Gas chromatography-mass spectrometry combined with mathematical chromatography as a powerful tool in the analysis of citrus fruits essential oils

**Parastar H**, Jalali Heravi M, Sereshti H  
1Department of Chemistry, Sharif University of Technology, Tehran, Iran; 2Iran National Elite Foundation, Tehran, Iran; 3Department of Chemistry, Sharif University of Technology, Tehran, Iran; 4Department of Chemistry, Faculty of Sciences, University of Tehran, Tehran, Iran

Citrus fruits essential oils are valuable natural products that are more popular nowadays in the world due to their effects on health conditions and their role in preventing and curing diseases [1]. Also, they have a broad range of applications in foods, perfumes, cosmetics and human nutrition. Gas chromatography-mass spectrometry (GC-MS) is the most important technique for the analysis of essential oils [2]. However, there are some fundamental problems in their analysis including baseline drift, spectral background, noise, low S/N, changes in the peak shapes and co-elution (overlapped, embedded ions) [3]. Mathematical chromatography (MC) as a branch of chemometrics [4] attempts to develop new tools to handle these problems. In this work, first, we have extracted the essential oils of the peels of eighteen citrus fruits such as lemon, lime, mandarin, grapefruit and hydrodistillation and then analyzed with GC-MS. Then, their signals were analyzed by MC. Using this strategy, the numbers of identified components were extended and quality of the results was improved significantly. As a positive consequence of using the proposed strategy human time and work are saved. Also, some new components were identified for the first time. In addition, we used our recently developed software, called MCRC Software for performing these techniques [5]. After resolving the volatile components in different samples, principal component analysis (PCA) was used for monitoring the pattern of volatile components in different samples. It is concluded that GC-MS/MC/PCA can open a new window to the comprehensive analysis of essential oils. Keywords: Chromatography, Mathematical chromatography, Essential oil, Gas chromatography-mass spectrometry, Citrus fruits


**SL39**  
Allium species of the subgenus Melanocrommyum are a rich source for cysteine sulfoxides

**Keusgen M, Kusterer J**  
Philippus-Universitat Marburg, Institute of Pharmaceutical Chemistry, Marbacher Weg 6, D-35032 Marburg

The plant genus *Allium* (onions) is highly diverse. About 800 species are currently known belonging to several subgenera [1]. The main centre of distribution is the northern hemisphere, especially the area of South-west and Central Asia. Inside this area, the subgenus *Melanocrommyum* is most prominent. Methin 1 seems to be a nearly ubiquitous cysteine sulfoxide for the genus *Allium* (Figure 1). Propiin 2 is a minor compound, which was found in *A. ubiquitense* R.M. Fritsch. The L-(+)-(3-pyrrolidyl)cysteine sulfoxide 3 is the major cysteine sulfoxide of *A. stipitatum* Regel (0.22%, related to the fresh weight of bulbs) as well as the closely related *A. altissimum* Regel (0.23%) and *A. jesdianum* Regel (0.20%).

The plant genus *Allium* (onions) is highly diverse. About 800 species are currently known belonging to several subgenera [1]. The main centre of distribution is the northern hemisphere, especially the area of South-west and Central Asia. Inside this area, the subgenus *Melanocrommyum* is most prominent. Methin 1 seems to be a nearly ubiquitous cysteine sulfoxide for the genus *Allium* (Figure 1). Propiin 2 is a minor compound, which was found in *A. ubiquitense* R.M. Fritsch. The L-(+)-(3-pyrrolidyl)cysteine sulfoxide 3 is the major cysteine sulfoxide of *A. stipitatum* Regel (0.22%, related to the fresh weight of bulbs) as well as the closely related *A. altissimum* Regel (0.23%) [2]. It seems to be possible that the corresponding N-oxide 4 is also present in these plants. Most species of the subgenus do also contain the L-(+)-(3-pyrrolidyl)cysteine sulfoxide 3. High amounts of 4 were found in *A. jesdianum* Boiss. & Buhse subs. remediorum R.M. Fritsch (0.52%). A. macleanii Baker (0.29%), A. tichiganicum (0.23%) and A. roseanum (0.20%). Highest amounts of marasmin 5 were detected in *A. susworowii* (2.25%). Compound 5 is also present in *A. altissimum* and *A. stipitatum*. Especially the latter species is widely used as vegetable, spice and traditional medicine.

**Acknowledgement:** The authors gratefully acknowledge Shimadzu and Sigma-Aldrich/Supelco for the continuous support.
SL40

Anticancer, antimicrobial and antiviral potentials of selected medicinal plants from the Island Soqotra

Mothana RA1, Lindequist U2, Bednarski PJ3, Mentel R4
1Department of Pharmacognosy, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia; 2Department of Pharmaceutical Biology, Institute of Pharmacy, Greifswald University, Greifswald, Germany; 3Department of Pharmaceutical and Medicinal Chemistry, Institute of Pharmacy, Greifswald University, Greifswald, Germany; 4Friedrich-Loeffler-Institute of Medical Microbiology, Greifswald University, Greifswald, Germany

Soqotra is considered the “jewel” of biodiversity in the Arabian Sea. Surveys have revealed that more than a third of the plant species of Soqotra are found nowhere else [1]. Fifty plants were collected, extracted with methanol and hot water and evaluated for their in vitro anticancer activity against three human cancer cell lines (A-427, 5637 and MCF-7) and for their antimicrobial activity against Gram-positive and Gram-negative bacteria as well as multiresistant Staphylococcus strains. Moreover, the antiviral activity of 25 plants has been assayed in two in vitro viral systems, influenza virus type A/MDCK cells and herpes simplex type 1/vero cells, at non-cytotoxic concentrations. The methanolic extracts of Ballochia atrovirgata Balff., Hypecoymum, A. stipitatum, A. altissimum, A. harmsworthii and A. tuberosum var. obovatum had the highest activity. The methanolic extracts of Ballochia atrovirgata Balff., Hypecoymum, A. stipitatum, A. altissimum, A. harmsworthii and A. tuberosum var. obovatum had the highest activity. The methanolic extracts of Ballochia atrovirgata Balff., Hypecoymum, A. stipitatum, A. altissimum, A. harmsworthii and A. tuberosum var. obovatum had the highest activity. The methanolic extracts of Ballochia atrovirgata Balff., Hypecoymum, A. stipitatum, A. altissimum, A. harmsworthii and A. tuberosum var. obovatum had the highest activity.

SL41

Toxicological evaluation of DAS-777 – a herbal preparation

Department of Pharmacology, Faculty of Basic Medical Sciences, College of Medicine, University of Lagos, P.M.B. 12003 Lagos, Nigeria.

DAS-777 is a herbal preparation that contains the milled bark of Mangifera indica L., and root of Carica papaya L. (1:1). It is used for the treatment of various ailments in Nigeria hence this toxicity assessment. In the acute toxicity study, DAS-777 (constituted in dH2O) was administered to mice p.o. up to 10 g/kg and i.p. at 250 – 3000 mg/kg. Mortality within 24 h was recorded. In the chronic toxicity study, rats were orally treated for 90 days at doses of 80, 400 (therapeutic dose, TD) and 2000 mg/kg. Rats were weighed and observed for feeding and drinking habits. By 90 days, animals were sacrificed and blood samples collected for haematological and biochemical analysis. Organs were harvested for weight determination, antioxidants and histopathological assessments. DAS-777 did not produce any lethality administered p.o. up to 10 g/kg but the i.p. LD50 was 1222 mg/kg. At TD, DAS-777 produced significant changes (p < 0.05) only in body weight and food intake, ovary weight, testes weight, kidney weight, liver enzymes (GOT and GPT) and serum parameters (creatinine, urea, total proteins, lipids (cholesterol) and cholesterol ratios). DAS-777 did not produce any lethality administered p.o. up to 10 g/kg but the i.p. LD50 was 1222 mg/kg. At TD, DAS-777 produced significant changes (p < 0.05) only in body weight and food intake, ovary weight, testes weight, kidney weight, liver enzymes (GOT and GPT) and serum parameters (creatinine, urea, total proteins, lipids (cholesterol) and cholesterol ratios).

SL42

The design of DNA barcode-specific PCR primers for medicinal plant authentication

Howard C, Smith S, Bremmer P, Fowler M, Scott N, Slater A
Biomolecular Technology Group, Faculty of Science and Life Sciences, De Montfort University, Leicester LE1 9BH, United Kingdom

The DNA Barcode of Life initiative aims to obtain designated “barcode” sequences for every known flowering plant. Despite DNA barcoding having cited medicinal plant authentication as one of the potential applications of barcode information. However, DNA sequencing of barcode regions may not be suitable for routine quality assurance testing of plant materials, which could contain mixtures of plant species and/or degraded DNA. The value of DNA barcoding in this arena may in fact lie in its role as a platform for the design of standardised DNA-based tests. We have studied three groups of plant species (Hypericum spp, Actaea spp. and Rhodiola spp.), each comprising a target commercial medicinal plant and known or potential adulterant species. The suitability of five “barcode” regions for the design of species-specific PCR primers was determined; the designated plastid rbcL and matK barcode regions, the candidate plastid trnH-psbA spacer barcode region and the nuclear ribosomal ITS region. Problems were encountered with the plastid barcodes (low inter-specific variation of rbcL, lack of reliable generic primers for matK, repetitive sequences in trnH-psbA) and for all three groups of plants the nrITS region proved to be most appropriate for primer design. Targeting the nuclear genome also allows discrimination of hybrids that may not be detected using plastid barcodes. Whilst DNA barcoding may prove to have a role in plant species identification, the current choice of universal plant barcodes may not be ideal for the development of routine authentication tests. Keywords: authentication, DNA barcode, PCR primer, ribosomal ITS.

SL43

Traditional use as a regulatory category – experiences in Europe

Peschel W
European Medicines Agency, 7 Westferry Circus, Canary Wharf, London E14 4HB, UK

Directive 2004/24/EC established a regulatory pathway for traditional herbal medicinal products in the European Union that allows the registration and marketing without the standard clinical data packages on safety and efficacy as required for other medicinal products. Five main
Euphol, a novel cannabinoid agonist, prevents inflammatory and neuropathic persistent pain in rodents

The cannabinoids have been considered a relevant target for pain management, but their effects on antidiabetic states are prevalent and debilitating diseases, which remain without safe and adequate treatments. Inflammatory and neuropathic pain was induced by carrageenan, CPA, PSNL, cancer/chemotherapeutic agent, PGE2 and PKCε agonist. Pro-inflammatory mediators were measured by immunohistochemistry, enzyme-linked immunosorbent assay (ELISA) and real-time-PCR. Here, we reported that euphol exhibited pronounced and long-lasting oral analgesia in several rodent behaviour models of inflammatory and neuropathic persistent pain. These effects were markedly blocked by CB1 or CB2-selective antagonists and oligonucleotide antisense. Our data indicate that euphol activates cannabinoid receptor binding experiments showed that euphol directly bound with high affinity to both CB1 (Ki = 71.090 nM) and CB2 (Ki = 0.037 nM), being 1,880-fold more selective for CB2 receptors. Euphol at similar dose inhibited the levels/mRNA expression, either at the spinal cord or the dorsal root ganglia level. Of relevance, euphol did not display significant central nervous system alterations. Acute toxicological studies carried out in rodents showed that euphol is safe and well tolerated. Therefore, euphol represents a novel orally active and safe natural analgesic for the management of inflammatory and neuropathic pain states. Keywords: Euphol, cannabinoid receptors, pain, inflammation, Acknowledgement: This work was supported by grants from the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), the Programa de Apoio aos Núcleos de Excelência (PRONEX), and the Fundação de Apoio a Pesquisa do Estado de Santa Catarina (FAPESC), all of Brazil. R.C.D., K.A.B.S.S., A.F.B., A.E.P. and R.M. are Ph.D. students in pharmacology receiving grants from CNPq.
Analogue of a lupane-type triterpene as potential anti-HIV agents

**SL47**

Infection with human immunodeficiency virus (HIV), the etiologic agent of acquired immunodeficiency syndrome (AIDS), continues to be ranked high on the list of the most important health issues facing the world. Although significant progress has been made since the introduction of highly active antiretroviral therapy, it has also led to increased adverse effects and the emergence of multidrug-resistant viral strains. Therefore, there is a need for new classes of drugs involving novel molecular mechanisms [1]. Many classes of natural products and some of their analogues have been tested for their anti-HIV activity [2]. In fact, modification of betulinic acid led to the discovery of bevirimat, a first-in-class drug candidate as a viral maturation inhibitor [3]. The goal of our study was to get new insights into the antiviral potential of lupane-type triterpenes that could inhibit HIV-1 replication and would be useful for the design of new drugs with clinical application [4]. Therefore, we prepared 17 derivatives based on the betulin scaffold, whose structures were determined by spectroscopic studies, and comparison with data previously reported. These derivatives and betulin were tested for their ability to inhibit the HIV replication. Two compounds of this series exhibited a promising activity at 10 μM with replication inhibition percentages of 26 and 31%, respectively. A study of the influence of the substitution pattern on the lupane skeleton revealed that oxidation at C-3, acetylation at C-28 and modification of the isoprenyl moiety play an important role in the activity. Keywords: Lupane triterpene; betulin analogues; anti-HIV agents

Acknowledgement: We are indebted to the Agencia Canaria de Investigación, Innovación y Sociedad de la Información (C200801000049) project for financial support. CO thanks the CajaCanarias for the fellowship.

**References:**

---

**SL48**

**Hoodia gordoni** Quality control and biopharmaceutical aspects

**Vermaak I^1, Viljoen AM^1, Hamman JH^1**

**^1Department of Pharmaceutical Sciences, Faculty of Science, Tshwane University of Technology, Pretoria, South Africa;**

**^2Unit for Drug Research and Development, School of Pharmacy, North-West University, Potchefstroom, South Africa**

**Hoodia gordoni** Sweet is a popularly consumed commercially available weight loss product. Therefore, developing rapid quality control methods for raw material and products, and investigating key biopharmaceutical aspects of the perceived active ingredient P57, is of utmost importance. High performance thin layer chromatography (HPTLC) and vibrational spectroscopy coupled with chemometric analysis are attractive alternative quantification methods for P57. The in vitro transport of pure P57 and P57 from crude plant extracts across porcine intestinal and buccal tissue was also investigated. The HPTLC system produced good band separation including the P57 band [1] and linear calibration curves with good correlation coefficient (R2) values of 0.9706 – 0.9993 were developed for P57 quantification. For the NIR spectroscopy data, the partial least squares projections to latent structures (PLS) model with 2nd derivative pre-processing predicted P57 content with an R2 value of 0.9629 and a root mean square error of prediction (RMSEP) of 0.03% [2]. Pre-processing of the Raman data with orthogonal signal correction yielded a PLS model with an R2 value of 0.9986 and an RMSEP of 0.004% [3]. Pure P57 was transported across porcine intestinal tissue at a much higher rate than from the buccal tissue, but pure P57 was transported across the buccal mucosa when applied in the form of a crude plant extract but no transport was detected for pure P57 [4]. The availability of rapid alternative quantification methods may positively influence the quality of distributed raw materials and products.

---

**SL49**

Application of liquid chromatography and mass spectrometry methods in pharmacognostic investigations of Traditional Chinese Medicines (TCM)

**Yang Y^1, Ge L^2, Ping TC^3, Qian KC^2, Zhen XZ^2, Ying PS^2**

**^1Natural Products Chemistry Department, & State Key Laboratory of Drug Research, & SIMM/CUHK Joint Laboratory for Promoting Globalization of Traditional Chinese Medicines, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, 555 Zu-Chong-Zhi Road, Shanghai 201203, P. R. China;**

**^2School of Biological Sciences, Faculty of Medicine, & SIMM/CUHK Joint Laboratory for Promoting Globalization of Traditional Chinese Medicines, The Chinese University of Hong Kong, Hong Kong SAR, P. R. China**

TCM has a long history to treat human diseases in China. In our investigations to depict medicinal functionalities of TCM using LC-MS related technologies, a diversity of new chemical structures including novel skeletons were acquired [1]. These molecule-orientedated technologies have been integrated effectively into the pharmacognostic investigations of medicinal herbs. In our chemical study of Stemona species, a HPLC-MS method was developed for the characterization of alkaloids with a pyrrolidol[1,2-a]azepine A, B-ring core from S. saxorum Gagnep. based on the ESI-MS results of five reference compounds [2]. 41 components were separated, of which 12 compounds (4 new) were identified as Stemona alkaloids with such a core. A practical HPLC method was designed to detect the content of shikimic acid, the start material of Tamiflu, in Chinese *Illicium* plants from 21 different species or habitats. The minor toxic asinin and its analogues were also monitored by an UPLC-MS/MS method. Our results provided scientific evidences for safe usage of fruits of *Illicium* plants. Additionally, new series of sesquiterpenoids and phe nylopropanoid flavonoid polymers were identified from these species [3]. *Marsdenia tenacissima* (Roxb.) Moon is rich in C21 steroidal glycosides, of which some were proved to reverse the multi-drug resistance through inhibiting P-gp, ABCG2, and MRP1 transporters. An UPLC-LTQ method was established to quantitatively monitor the biotransformation of its extract by human intestinal bacteria. The results showed that three major compounds were degraded to their corresponding de-sugar active derivatives within 4 hours. This provided well evidence for TCM as a pre-drug, and a possible new way to verify its efficacy. Keywords: HPLC-MS, Stemona saxorum, *Illicium* plants, *Marsdenia tenacissima*, *Stemona* alkaloids, shikimic acid, asinin, C21 steroidal glycosides References: 1. Lin LG, et al. (2007) Tetrahedron Lett 48: 1559 – 1561. 2. Peng SY, et al. (2009) J Nat Prod 72: 1420 – 1423. 3. Zhu SY, et al. (2009) Rapid Commun Mass Spectrom 23: 3621 – 3631 4. Vermaak I et al. (2010) S Afr J Bot 76: 119 – 124. 2. Vermaak I et al. (2010) Food Chem 120: 944. 3. Vermaak I et al. (2010) Phytochem Lett 3: 156 – 160. 4. Vermaak I et al. (2011) J Med Chem 53: 3233 – 3141.
The genus Hypericum, which comprises about 450 species of herbs, shrubs, and trees, belongs to the family Clusiaceae (Guttiferae), formerly Hypericaceae. One of the topmost and commercially renowned species of the genus Hypericum is Hypericum perforatum L., commonly known as St. John’s Wort. Hypericum perforatum, is well known for its profound pharmacological activities as antidepressant, anxiolytic, anti-inflammatory, antimicrobial, and wound healing. In this study, using HPLC-ED system, quantitative analysis of hypericin was carried out in different water-methanolic extracts of St. John’s Wort. Hot extract of water-methanol extracts of St. John’s Wort was prepared. The herba (1 g) was powdered and extracted with water-methanol (1:1:10 mL). Afterward 1 mL of that extract was centrifuged, obtaining supernatant which was used for analysis. The standard was hypericin purchased from HWI Analytic GmbH, Germany. Mobile phase: methanol-acetonitrile, water, acetic acid (20+10+70+1), electrochemical detector with range 50 nA, potential +0.84 V, filter, 0.02 Hz, flow rate, 1 mL/min and temperature 25 °C. Injection volume: 20 μL Concentrations of standard were 0.1 μg/20 μL, 0.2 μg/20 μL and 0.4 μg/20 μL. Retention time of hypericin in the standard solution and herbal extracts were 28.4 minutes. Limit of detection was 20 ng of hypericin. Amount of hypericin in extracts of St. John’s Wort was 25 – 26 mg/g dry weight. The very high content of hypericin in Bosnian St. John’s Wort give more importance to this plant as traditional drug for its use in official medicine. Keywords: hypericin, Hypericum perforatum, HPLC-ED, determination. References: 1. Rosson N, (2003) Hypericum botany. In: Ernst, E. (Ed.), Hypericum. Taylor and Francis, New York, pp. 196 – 241. 2. Wissenschaftliche Staatsan für Brauerei in München e. V., Dr. Biendl Haltert. Hopfenveredelungsgesellschaft m.b.H. References: 1. Milligan SR et al. (2000) Clin Endocrin Metab 85: 4912 – 4915. 2. Diller RA et al. (2007) Planta Med 73: 755 – 761. 3. Butterweck V et al. (2007) J Pharm Pharmacol 59: 549 – 552.
The genus *Blechnum* has 13 common and well-distributed species in Chile [1]. *B. chilense* plants have been used for various purposes [1,2]. *Tetranychus urticae* Koch is highly polyphagous species with 1094 host plant species reported worldwide to date [3]. In this research, we report a phytochemical analysis of *B. chilense* and its mite growth regulatory effects. From the n-hexane fraction four phytoecdysones were isolated: ecdysone, ponasterone, shidastereone and 2-deoxoyectysone. We conducted a bioassay with *T. urticae* eggs placed on *Phaseolus vulgaris* L. leaf discs previously treated and the development stage was recorded every 24 hours during thirteen days. All treatments showed statistical significant differences regard to Control in emerged and living adults. The EtOAc fraction at 250 ppm and the n-hexane fraction at 250 ppm and 100 ppm caused the greatest mortality of nymphs and almost the total mortality of the low quantity of adults that emerged (Fig. 1). Our results shows that even low concentrations of 10 ppm in both fractions affects the life cycle of *T. urticae* causing a significant decrease in its population. Studies on the identification and physiological role of ecdysteroids in mites are minimal [4], especially in the order Prostigmata. Our results suggest that early exposure of eggs and larvae to phytoecdysones from *B. chilense* may interfere in the natural ecdysteroid metabolism in *T. urticae* leading to the death of nymphs and adults. Our results suggest too that the deaths could be caused by starvation due to the deterrent effects of some phytoecdysones [5,6].

**Figure 1:** Percentage of *T. urticae* individuals in each stage treated with different fractions of *B. chilense*

- HB250: n-hexane fraction at 250 ppm, HB100: n-hexane fraction at 100 ppm, HB10: n-hexane fraction at 10 ppm, AB250: EtOAc fraction at 250 ppm, AB100: EtOAc fraction at 100 ppm, AB10: EtOAc fraction at 10 ppm.

Keywords: *Blechnum chilense*, phytoecdysones, *Tetranychus urticae*  

Figure 1: Model of hyperspectral cube

Figure 2: (a) Visible image from top; (b) Near infrared image from top; (c) Infrared image from top; (d) 3-D model of in vivo images


Gastrointestinal inflammation caused by pathogen infection may lead to the overexpression of pro-inflammatory proteins and cytokines in immune system. Lemon pepper fruits (Zanthoxylum aethiopicum DC.; Rutaceae) have been used as a traditional source against stomach ache by Batak people in North Sumatera province, Indonesia. However, its scientific evidence for treatment of inflammatory disorders has not been reported. Here, we investigated the inhibitory effects of lemon pepper fruit extract (LPFE) against inflammatory biomarkers by conducting cell culture experiments in vitro. The fruits of lemon pepper were dried and extracted twice in 70% ethanol followed by evaporation and freeze-drying. The concentrated extract was further tested for its potential inhibition on the protein and gene expression of several inflammatory biomarkers, i.e. tumor necrosis factor (TNF-α), interleukin (IL)-6, inducible nitric oxyde synthase (iNOS), cyclooxygenase (COX)-2, and matrix metalloproteinase (MMP)-9 in the cell system. Our results suggest that LPFE dose dependently decreased the expression of TNF-α and COX-2 proteins, and MMP-9 activity in macrophages treated with LPS. At the gene level, LPFE were effectively found to block the mRNA expression of TNF-α, IL-6, iNOS, COX-2, and MMP-9 in the cell system. Our results suggest that LPFE significantly inhibits selected inflammatory biomarkers at the protein and gene levels in LPS-induced macrophages. Further in vivo study using animal models is still needed to determine the exact anti-inflammatory potential of LPFE. Keywords: lemon pepper fruit, anti-inflammatory activity, Zanthoxylum aethiopicum, lipopolysaccharide, macrophages Acknowledgement: This work was funded by the 2010 Ministry for Science and Research and the Ministry for Health, Family and Youth (Vienna, Austria) (Novel Analytical Tools for Quality Control in Traditional Chinese Medicine. Project No. 80855), the Leopold-Franzens University. Innsbruck (Nachwuchsförderung) for financial support. References: 1. Pezzete C, Pallua JD, Schaefer G, Seifarth C, Huck-Pezzei V, Bittner LK, Klockier H, Bartsch G, Bonn GK, Huck CW (2010) Mol Biosyst 6: 2287. 2. Pallua JD, Pezzete C, Huck-Pezzei V, Schönbichler S, Bittner LK, Bonn GK, Saeed A, Maedo S, Farooq A, Najam-ul-Haq M, Abel G, Popp M and Huck CW (2011)Curr Bioactive Comp 7: in press.

Figure 1: Model of hyperspectral cube


Gastrointestinal inflammation caused by pathogen infection may lead to the overexpression of pro-inflammatory proteins and cytokines in immune system. Lemon pepper fruits (Zanthoxylum aethiopicum DC.; Rutaceae) have been used as a traditional source against stomach ache by Batak people in North Sumatera province, Indonesia. However, its scientific evidence for treatment of inflammatory disorders has not been reported. Here, we investigated the inhibitory effects of lemon pepper fruit extract (LPFE) against inflammatory biomarkers by conducting cell culture experiments in vitro. The fruits of lemon pepper were dried and extracted twice in 70% ethanol followed by evaporation and freeze-drying. The concentrated extract was further tested for its potential inhibition on the protein and gene expression of several inflammatory biomarkers, i.e. tumor necrosis factor (TNF-α), interleukin (IL)-6, inducible nitric oxyde synthase (iNOS), cyclooxygenase (COX)-2, and matrix metalloproteinase (MMP)-9 in the cell system. Our results suggest that LPFE dose dependently decreased the expression of TNF-α and COX-2 proteins, and MMP-9 activity in macrophages treated with LPS. At the gene level, LPFE were effectively found to block the mRNA expression of TNF-α, IL-6, iNOS, COX-2, and MMP-9 in the cell system. Our results suggest that LPFE significantly inhibits selected inflammatory biomarkers at the protein and gene levels in LPS-induced macrophages. Further in vivo study using animal models is still needed to determine the exact anti-inflammatory potential of LPFE. Keywords: lemon pepper fruit, anti-inflammatory activity, Zanthoxylum aethiopicum, lipopolysaccharide, macrophages Acknowledgement: This work was funded by the 2010
SL60

**Histomorphometric analysis of bone marrow constitutents in ovariectomized Cimicifuga racemosa (L.) Nutt. (CR) treated animals**

SediOva Wuttke D, Wuttke W
University Medical Center Göttingen, Robert-Koch-Str. 40, D-37075 Göttingen, Germany

Following ovariectomy (ovx) rats lose trabecular bone and hematopoietic tissue whereas the amount of fat tissue increases. These fat cells secrete cytokotoxik cytokines which cause local inflammation in the bone marrow which augments development of osteoporosis. Whether this can be prevented by estradiol-17β (E2) or CR BNO 1055 was tested. Rats (3 months old) were ovx and fed with E2 (0.156 mg/animal/day, positive controls) or with CR BNO 1055 (8.22 mg/animal/day) which was shown in previous experiments to prevent osteoporosis. The surface of trabecles and their 3-dimensional structure was determined histomorphometrically. In addition, the amount of fat tissue was quantified. The trabecular surface and the 3-dimensional integrity of the trabecular apparatus in the metaphysis of the tibia was severely disturbed following ovx and this was almost totally prevented by E2 and CR BNO 1055. In addition ovx animals had high fat load in the bone marrow which was largely prevented by E2 and the BNO 1055 extract. These results indicate that the high amount of fat tissue in the bone marrow correlates with decreased trabecular integrity. This can be largely prevented by E2 and by compounds present in CR BNO 1055. Hence, E2 and CR BNO 1055 (which is devoid of estrogenic compounds) protect against the development of osteoporosis in part by decreasing the bone marrow fat load.

Keywords: Cimicifuga racemosa, osteoarthritis, osteoporosis

**Acknowledgement:** This work was funded by the Bayerische Forschungsstiftung AZ-838 – 08, Germany.

SL61

**Economically Motivated Adulteration of Botanical Raw Materials, Herbal Extracts, and Essential Oils in the Global Marketplace**

Blumenthal M
American Botanical Council, P.O. Box 144345 Austin, TX 78714 – 4345, USA

The trade of botanical ingredients for the production of herbal drugs and phytomedicines, dietary supplements, and natural cosmetics is global, with supply and quality issues in one geographical region affecting other areas. Chemical complexity of botanicals requires added quality control diligence for raw material suppliers and manufacturers. In recent years there have been numerous cases of accidental misidentification of botanical materials due to nomenclatural confusion, lack of adequate quality control measures, etc. Also, there have been persistent cases of inadvertent contamination with heavy metals, agricultural chemicals, excessive microbial load, excessive solvent levels in extracts, etc. But there is also the disturbing trend of intentional adulteration – economically motivated adulteration (EMA) – as well as the “spiking” of extracts with undisclosed lower-quality and lower-cost ingredients. This includes the spurious and illegal addition of active pharmaceutical ingredients (conventional pharmaceutical drugs), e.g., sildenafil in dietary supplement products for erectile dysfunction and sibutramine in weight-loss products. This presentation reviews many of these quality control challenges and notable cases of safety concerns and economic fraud created by them as is being compiled American Botanical Council and the American Herbal Pharmacopoeia from information supplied by botanical ingredient suppliers, manufacturers, and laboratories in the United States and in other countries.

The antimicrobial activity of honey in relation to the composition of pollen (Bosnia-Herzegovina, W. Balkan)

Redzic SJ, Kartogic H, Przina N, Tuka M, Andagic T
*Department of Biology of the Faculty of Science University, 33 – 35 Zmaja od Bosne St., 71 000 Sarajevo, Bosnia and Herzegovina; **Federal Institute of Agriculture, Sarajevo, Bosnia and Herzegovina; ***Private Pharmaceutical institution “Apoteka VITA”, Kiseljak, Bosnia and Herzegovina

Honey has significant antimicrobial activity (AMA) [1, 2]. As the quality of honey depends on the composition of pollen, and it can expect a different AMA. Honey samples were collected from 12 different locations in the continental part of Bosnia. Honey samples were taken at the end of the season (September – October 2006). Microscopic preparations were made for the standard method of pollen analysis. For every sample 300 pollen grains were counted. Each pollen grain has been determined. Antimicrobial activity was tested by diffusion method at Muller-Hilton agar. For that clinical isolates of Staphylococcus aureus, Escherichia coli and Pseudomonas sp. were used. On the edge of prepared pools from strains of microorganisms placed 200 μl prepared honey. All 12 samples of honey caused high inhibition zone for Staphylococcus aureus (11 – 21 mm), eight samples of honey caused growth inhibition of Escherichia coli (11 – 13 mm) and three samples of honey caused a zone of inhibition at Pseudomonas sp. (12 mm). The highest antimicrobial activity has poly-floral mountain honey dominated by pollen of the following species: Trifolium repens L. (17%), Taraxacum officinale F.H.Wigg. (13%), Filipendula ulmaria Hill (13%), Centaurea jacea L. (11%), Trifolium pratense L. (19%), Plantago lanceolata L. (9.5%), Lotus corniculatus L. (9%), Orchardum vulgare L. (5%), Erica carnea L. (5%) and others (total 15 plant species). The greatest effect on Pseudomonas sp. causes honey dominated of Lotus corniculatus (23%). Tested honey shows no effect of the bacterium Salmonella enteritidis and Candida albicans. Most of these plants are honey and medicinal plants and edible plants [3, 4]. Keywords: Honey plants, Biodiversity of plants, pathogenic bacteria, Taraxacum, Trifolium References: 1 Ulusoy E et al. (2010) J Food Biochem 34 (Suppl1): 321 – 335. 2. Bogdanov S et al (2008) J Am Coll Nutr 27: 677 – 689. 3. Redzic SS (2007) Coll Antropol 31: 869 – 890. 4. Redzic SJ (2006) Ecol Food & Nutr 45(3):189 – 232.

SL62

**Immunostimulatory and protective effects of Aloe vera components against coccidiosis in broilers**

Akhtar M1, Ha I1, Muhammad P1, Awais MM1, Anwer MI1
*Department of Parasitology, University of Agriculture, Faisalabad, Pakistan; **Department of Physiology and Pharmacology, University of Agriculture, Faisalabad, Pakistan

Present study reports immunostimulatory effect of Aloe vera L. extracts in chickens and their protection against coccidiosis. Study was divided into two experiments. Experiment-I was conducted for evaluation of immunostimulatory activity of Aloe vera extracts and experiment-II for protective efficacy against coccidiosis. Results of experiment-I revealed significantly higher (P<0.05) lymphoproliferative responses in chickens administered with ethanolic extract as compared to chickens administered aqueous extract and control group. Microplate haemagglutination assay for humoral response on day 7th and 14th post primary and secondary injections of sheep red blood cells (SRBC) revealed significantly higher (P<0.05) anti SRBC antibody (total Ig, IgG and IgM) titers in experimental groups as compared to control; although both the Aloe vera extracts showed no significant effects on the development of lymphoid organs. Results of experiment-II revealed maximum percent protection (60%) in chickens administered with aqueous extract as compared to ethanolic extract administered chickens (45%). Mean oocysts per gram of droppings in control group was significantly higher (P<0.05) as compared to chickens in both experimental groups. Chickens administered with aqueous extract (40%) showed severe lesions (3.0 – 4.0); whereas 55 and 75 percent severe lesions were recorded in ethanolic extract administered and control chickens, respectively. Daily weight gains from day 3 rd-12th post-challenge were significantly higher (P<0.05) in chickens administered with aqueous extract as compared to those administered with ethanolic extract and control. It was concluded that Aloe vera may be potential and valuable candidate to stimulate the immune responses and can be used successfully in immunosup-
Effects of nutrient medium strength on cardiotonic glycoside accumulation in Digitalis lamarckii Ivan, an endemic medicinal species

Sahin G, Verma SK, Girél E

Department of Biology, Faculty of Science and Letters, Abant Izzet Baysal University, Bolu, Turkey

The effects of different strengths of Murashige and Skoog (MS) (1) medium on cardiotonic glycoside accumulation in Digitalis lamarckii Ivan were investigated. D. lamarckii, commonly known as dwarf foxglove (yuksukütu in Turkish), is an Turkish endemic medicinal plant and belongs to the family of Plantaginaceae. The Digitalis species are biennial or perennial herbs containing important cardioactive compounds (glycosides) that are used to treat heart problems. D. lamarckii has been marked as vulnerable (VU) in the Red Data Book of Turkish Plants (2). Contents of five different cardenolides (namely, digoxigenin, gitoxigenin, lanatoside C, digoxin and digitoxin) in the callus developed from hypocotyl explants, which were cultured for 10 days on different strengths of MS media supplemented with 33 sucrose, 0.5 ppm TDZ and 0.25 ppm IAA, were determined by HPLC. Three different strengths of MS media were tested: quarter-, half- and full-strength. Of the five cardenolides, only lanatoside C could be detected in the callus cultured on all medium strengths. The highest concentration of lanatoside C was observed on half-strength MS medium (690 μg/g dry weight, dw), while the callus cultured on full- and quarter-strength media producing 231 and 332 μg/g dw lanatoside C, respectively. In conclusion, the protocol described here is expected to have an important contribution to the future efforts for a large scale production of cardenolides in Digitalis species. Keywords: Cardenolide accumulation, Digitalis lamarckii, medium strength, Murashige and Skoog basal medium strength.


Caribenolide revisited. Reisolation of caribenolide-I together with new congeners

Kamagai K1, Akakabe M2, Minamida M2, Nishisaka T1, Tsuda M2, Konishi Y1, Tsuda M1, Tominga A4

1Science Research Center, Kochi University, Kochi, Japan; 2Department of Pharmaceutical Science, Kochi University, Kochi, Japan; 3Center for Advanced Marine Core Research, Kochi University, Kochi, Japan; 4Graduate School of Medicine, Kochi University, Kochi, Japan

Caribenolide-I [1] was originally discovered from the cell extract of a free-swimming Caribbean dinoflagellate Amphidinium gibbosum by Shimizu and coworkers. The structure was interpreted to be a 26-membered macrolide containing a 6-membered hemiacetal ring, a tetrahydrofuran ring, an epoxide, a ketone carbonyl, four C1 branches, and five hydroxyl groups. On the other hand, Kobayashi et al. reported the isolation of amphidinolide N [2] isolated from a symbiotic dinoflagellate Amphidinium species earlier than the report of caribenolide-I. The structure of amphidinolide N was elucidated to be the ring-opening form at the C-21-C-24 tetrahydrofuran ring for caribenolide-I. Caribenolide-I was reported to exhibit strong cytotoxic activity against tumor cell lines and in vivo antitumor activity. Amphidinolide N also showed extremely potent cytotoxic activity. Caribenolide-I as well as amphidinolide N would therefore also appear to be a promising anticancer therapeutic lead. Nevertheless, the scarcity of materials has prevented more detailed studies. Because stereochemistries of caribenolide-I and amphidinolide N have not determined yet, it is difficult to supply the sample by synthesis. In our investigation for anticancer drug leads from the Amphidinium dinoflagellates, we have isolated caribenolide-I together a three new caribenolide-I congeners from two benthic Amphidinium strains collected off Iriomote Island, Japan. In this symposium, we will discuss the isolation of these four compounds, structural relationship between caribenolide-I and amphidinolide N, and structure elucidation of three new compounds. References: 1. Bauer I, Maranda Y, Young K A, Shimizu Y, Fairchild C, Cornell I, MacBeth J, Huang S (1995)J Org Chem 60: 1084 – 1086. 2. Ishibashi M, Yamaguchi N, Sasaki T, Kobayashi J (1994)J Chem Soc, Chem Commun,1445 – 1446.
This study aimed to introduce Artemisia annua L. plant to the Egyptian cultivation and to achieve the technological package for its production under Egyptian conditions. The seeds were introduced from Germany and propagated. Several experiments were carried out during two successive seasons in two different locations (clay loamy soil and sandy loam soil). The first experiment aimed to study the effect seasonal variation on growth, yield, essential oil and chemical composition using organic farming system under the Egyptian conditions. The essential oil content and essential oil yield of A. annua significantly increased with increasing plant age to reach their maximum values after 180 days from transplanting. The second experiment was carried out in loamy clay soil to study the effect of the mineral fertilization on the growth, yield and the active constituents of A. annua. The highest value of artemisinin was obtained from plants treated with 75 kg N/fed + 50 kg K/fed. The third experiment was carried out in loamy clay soil to study the effect of organic fertilizer and/or biofertilizer on the growth and active constituents of A. annua. The highest yield of Artemisinin was obtained from plants treated with 30 m³ compost/fed. without biofertilizer followed by the application of 30 m³ compost/fed. with biofertilizer. The fourth experiment was carried out to study the effect of soil type on the growth and active constituents of A. annua. Plants grown in sandy soil gave a positive increase in the essential oil yield and artemisinin content and yield. Feddan = 4200 m²

Production and use of Artemisia annua (sweet wormwood) against bacterial diseases in poultry stocks and its effect on food quality

Necrotic enteritis (NE) in broilers is caused by Clostridium perfringens type A (CP) resulting in severe production losses and mortality. Present preventive treatments include the dietary addition of ionophores which may be banned in the EU before long. The plant Artemisia annua L. (AA) produces antimicrobial essential oil components (EOCs) [1] that could substitute the use of these antibiotics in poultry production. A first study focused on improving the production of bioactive EOCs in AA by applying physical and chemical stresses during cultivation. Jasmonic acid induced a significant increase in the content of EOCs such as germacrene D and γ-elemene [2]. Extracts of AA aerial parts were tested for antimicrobial activity in overnight cultures of CP strains isolated from diseased broilers. The hexane extract containing EOCs showed the strongest inhibition (MIC = 170 ppm) confirming the potential use of AA EOCs as antimicrobial agents. This extract was incorporated in the diet of broilers applying a NE disease model. The treatment reduced the population of CP and the severity of the associated small intestinal lesions (p < 0.05). Furthermore, CP infected broilers fed the diet supplemented with AA hexane extract gained more weight than the control animals (p < 0.05). Healthy broilers were fed diets supplemented with dried AA material to ascertain that the palatability of the meat is not affected. Breast filets evaluated by a descriptive sensory analysis did not show any effect of the treatment on meat flavour/taste nor texture or appearance. Hence, AA extracts show promising results as antimicrobial additives in poultry diets. Keywords: Artemisia annua, necrotic enteritis, Clostridium perfringens, essential oil components, sensory analysis References: 1. Si W et al. (2009) J App Microb 106: 213 – 220 2. Jarvisen L et al. (2010) Phcog Mag 6(22 Suppl.); 126

Artemisinin is a sesquiterpene lactone isolated from Artemisia annua having an unusual endoperoxyl moiety which is essential for the activity when activated by iron [1]. In addition to its well established antimalarial properties artemisinin has potent anticancer activities in a variety of human cancer cell types [2]. The cytotoxic effect of artemisinin is specific to cancer cells because transferrin-receptors are highly expressed on the surfaces of tumour cells and iron content is higher than in normal cells [3]. The aim of our work was to test the cytotoxicity of artemisinin and stealth liposomes for passive targeting and transferrin-conjugated liposomes for active targeting loaded with artemisinin. Multilamellar vesicles were prepared according to the film hydration method; in order to reduce the dimensions of the vesicles, an high pressure homogenizer EmulsiFlex C3® was used. Conventional, stealth and targeted liposomes were fully characterized by particle size, zeta potential, Pdl, drug entrapment efficiency and transmission electron microscopy. Coupling of transferrin to the targeted liposomes was obtained by amide bond between Tf and lipid linker and the average amount of transferrin conjugated to the liposome was quantified with bicinchonic acid (BCA). A preliminary study about the cellular uptake of conventional liposomes loaded with fluorescein sodium salt has been performed in K562 cells using flow cytometry analysis and fluorescence microscopy; the highest internalization of fluorescein sodium salt loaded liposomes was after 60 minutes of exposure. References: 1. Nakase I et al. (2008) Int J Pharm 354: 28 – 33. 2. Firestone GL et al. (2009) Expert Rev Mol Med 11: e32. 3. Effert H T et al. (2004) Free Radic Biol Med 37: 998 – 1009.

In the search for biologically active natural products from Indonesian marine sources two strains of marine endophytic fungi could be isolated from the marine red alga Kappaphycus alvarezii. One strain (KT 30) could be identified as Xylaria psidii, the other one (KT 31) remains sterile and could not be identified till now. Ethylacetate extracts from the culture medium displayed considerable cytotoxic activity against a urinary bladder carcinoma cell line with IC50 values of 4 and 1.5 µg/ml, respectively. Both strains were also obviously active to inhibit the growth of fish and human pathogenic microorganisms. Most remarkable is the strong antimicrobial activity of ethylacetate extracts against the gram-negative bacteria Pseudomonas aeruginosa, Escherichia coli, Vibrio anguillarum and Aeromonas salmonicida. A new cyano methoxy benzoic acid derivative was isolated from X. psidii KT30 and a new quinone derivative was isolated from X. psidii KT31. The sesquiterpenes were prepared from the algicolous fungus KT31. Besides, cytochalasin B was detected for the first time in X. psidii. Keywords: Indonesian marine fungi, algicolous, antifungal, cytotoxic Acknowledgement: We thank PD Dr. Marc Stadler (InterMedDiscovery, Dortmund, Germany) for identification of the Xylaria species.
Proanthocyanidins (PCs) are some of the most abundant polyphenolic substances in the plant kingdom. OPCs are an integral part of the human diet, found in high concentrations in fruits such as apples, pear, tea, hawthorn, grapes, and in chocolate. Due to potent antioxidant activity, PCs have been the subject of recent research, demonstrating anticarcinogenic, anti-inflammatory, antimicrobial, and vasodilatory properties, making them a potentially valuable therapeutic tool for the treatment of a variety of conditions. PCs are present in plants as complex mixtures of polymers with an average degree of polymerization between 4 and 11, usually in association with their composing flavan-3-ols. Structural diversity is possible by variation in hydroxylation pattern, stereochemistry at the three chiral centers, and the location and type of interflavan linkage. The most frequent basic units of proanthocyanidins are derivatives of flavan-3-ols: (+)-catechin, (-)-epicatechin, (+)-gallocatechin, (-)-epigallocatechin (EGC) and (-)-epigallocatechin gallate (EGCG). PCs, naturally occurring antioxidants widely available in fruits, vegetables, nuts, seeds, flowers and bark, have been reported to possess a broad spectrum of biological, pharmacological and therapeutic activities against free radicals and oxidative stress. Epicatechin, dimeric procyanidin B2 and B5, proanthocyanidin A2 and trimeric procyanidin C1 of *Crataegus sinesis* Boiss. and/or *Adansonia digitata* L. display potent antioxidant antiviral properties in vitro. References: 1. De Bruyne T, et al. (1999) Biochem Syst Ecol 27: 445. 2. Shahat FM, et al. (1996) Planta Med 62(1): 10 – 13. 3. Shahat A et al. (2002) Planta Med 68: 539 – 541. 4. Shahat A, Ahmed H, Hassan R, Hussein A (2008) Asian Pacific Journal of Tropical Medicine (Asian Pac J Trop Med) 1(3): 55 – 59

Over the past years, marine microorganisms have proven to be a prolific source of structurally interesting and biologically active natural products. Marine fungi in particular have attracted considerable interest due to the diversity in chemical structures and biological activities observed for their secondary metabolites. Chemical investigation of the crude extract obtained from the sponge-associated fungus *Aspergillus* sp., isolated from a specimen of the Mediterranean sponge *Tethya aurantium*, yielded new meroterpenoid metabolites of the austalide type, as well as new tryptoquivaline and fumiquinazoline alkaloids, in addition to several known compounds. The structures of the new compounds were unambiguously elucidated on the basis of extensive one- and two-dimensional NMR (1H, 13C, DEPT, COSY, HMQC, HMBC, and ROESY spectra) and mass spectral analysis. The absolute configurations of the new compounds were established by means of TDDFT ECD calculations. All compounds were evaluated for their cytotoxic activity by the MTT method against the murine cancer cell line L5178Y, as well as the human cancer cell lines K562, A2780, and A2780 cisR, where some of the isolated compounds exhibited moderate to pronounced cytotoxicity. Keywords: Aspergillus, marine fungi, Structure elucidation, absolute configuration, cytotoxicity.
The Corona Charged Aerosol Detector – A “Universal” Detector for the Measurement of Non-volatile Components in Food, Natural Products and Supplements  
**PA1**  
Acworth IN  
ESA – A Dionex Company, Applications Department, Chelmsford, USA

Phytosterols (PSs) are a group of naturally occurring steroid alcohols found in plants. There is considerable interest in PSs as dietary supplements as they are reported to lower cholesterol levels and also have a positive impact on cardiovascular diseases. However, recent research suggests that PSs supplementation may aggravate atherosclerotic lesions and lead to aortic valve stenosis. PSs are typically measured by gas chromatography (GC), but this approach is time-consuming since it requires saponification of the sample, several extractions, and derivatization. We developed a simplified method using reverse-phase, HPLC and Charged Aerosol Detection (CAD). CAD is sensitive, has a dynamic range of >4 orders of magnitude, can measure any non-volatile species, and analyzes shows similar response independent of their chemical structure. Samples were prepared by simple dilution prior to analysis. Five PSs, campesterol, cholesterol, stigmasterol, beta-sitosterol, and stigmas-
tol, were resolved in <35 min. Calibration curves showed linear correlation coefficients > 0.997. The LOD was ~5 ng (on column). Analysis of red palm oil is used as an example. The method is simple to use, has good linearity and sensitivity, and is capable of measuring numerous PSs in plant extracts. This approach can be used to examine product purity, supplement content, and adulteration.

Food plants in the Apiaceae (formerly Umbelliferae) family (e.g., carrots, parsley and celery) contain a group of bioactive C-17-polyacetylene compounds, sometimes referred to as the polyacetylenic oxypolsins. These compounds have been shown to be highly toxic towards bacteria and fungi and to exhibit a diverse range of biological activities in mammals, both beneficial (e.g., their cytotoxicity is proposed to reduce the risk of developing cancer) and detrimental (e.g., occupational allergic contact dermatitis). Three such compounds, falcarinol, falcarindiol and falcarindiol-3-acetate, natural pesticides produced by carrots in response to fungal diseases, have recently garnered a lot of media attention. Although falcarinols have a distinctive UV spectrum, the consequence of conjugated triple bonds, sensitivity tends to be poor due to the actual number of unsaturated bonds present in their structure. Measurement at 205 nm offers the best sensitivity; however, sample chromators tend to be very complicated due to the presence of many other compounds absorbing at this wavelength. Charged aerosol detection (CAD) is a “universal”, mass-based detector and offers excellent sensitivity, a wide dynamic range, and the advantage that all non-volatile analytes produce similar response, independent of chemical structure. Additionally, unlike UV detection, analytes need not possess a chromophore in order to be determined. We developed a simple reversed-phase HPLC-CAD method to rapidly screen for falcarinol, falcarindiol, and falcarindiol-3-acetate. The method was sensitive (LOQ ~5 ng on column) and reproducible, and the analysis was completed in 15 mins. Data from fresh, baby carrots and Queen Anne’s Lace (root, leaf, and flower) are presented.

Fat-soluble vitamins (FSVs) play essential roles in a wide spectrum of physiological processes. One FSV, Vitamin E (tocopherol), along with a suite of other fat-soluble antioxidants (FSAs) (e.g., carotenoids, CoQ 10) mitigate the potentially disastrous effects of oxidative stress linked to numerous diseases. These compounds are thought to exert their beneficial effects by acting as chain-breaking antioxidants, inhibiting lipid peroxidation of polyunsaturated fatty acids contained within biological membranes, thereby preventing the formation of potentially cytotoxic and highly reactive aldehydes (malondialdehyde and 4-hydroxynone-
mal), which are generated by the oxidation of polyunsaturated fatty acids. Although a number of FSVs and FSAs have been measured by HPLC-UV, this approach typically lacks the sensitivity and selectivity required to measure these compounds in biological samples. Electrochemical detection, however, is both sensitive and selective and makes use of the inherent redox activity of these compounds. The CoulArray® Coulometric Array Detector-the only HPLC electrochemical detector that is a “universal”, mass-based detector and offers excellent sensitivity, a wide dynamic range, and the advantage that all non-volatile analytes produce similar response, independent of chemical structure. Additionally, unlike UV detection, analytes need not possess a chromophore in order to be determined. We developed a simple reversed-phase HPLC-CAD method to rapidly screen for falcarinol, falcarindiol, and falcarindiol-3-acetate. The method was sensitive (LOQ ~5 ng on column) and reproducible, and the analysis was completed in 15 mins. Data from fresh, baby carrots and Queen Anne’s Lace (root, leaf, and flower) are presented.

Many of the recently commercialized sweeteners have increased potency, and therefore the amount of the active ingredient added to beverages and other food products is reduced. This is problematic. But, this has contributed to a need for sensitive analytical methods to quantify the active product and detect low levels of breakdown products and impurities. Such product characterization is required for quality and safety issues. Traditional HPLC-UV approaches are inappropriate as these compounds typically do not possess any chromophore. This work describes a number of HPLC-CAD methods that can be used to study common natural sugars (fructose, glucose, turanose, saccharose, trehalose, maltose, melezitose, and raffinose); artificial sweeteners (sucralose, aspartame, saccharin, and acesulfame K); and newly introduced products containing Stevia extracts (rebahodium A and steviolose). These methods provide sensitivity at low (ng) levels with good reproducibility and accuracy, and correlation to the component concentrations. Stevia products were analyzed by Charged Aerosol Detection and UV; the CAD showed a greater than fivefold improvement in sensitivity over UV for all major components. Finally, the UHPLC methods developed showed a decreased run-time and an increased sensitivity for glucose, lactose, and sucrose. Typical limits of detection were found to be < 500 pg (on column) for glucose and other mono- and disaccharides. HPLC-CAD is a very flexible approach to measuring sweeteners and overcomes many of the limitations of UV, RI, LC-MS, ELSD, and HPLC-pulsed amperometric approaches.

Fat-soluble vitamins (FSVs) play essential roles in a wide spectrum of physiological processes. One FSV, Vitamin E (tocopherol), along with a suite of other fat-soluble antioxidants (FSAs) (e.g., carotenoids, CoQ 10) mitigate the potentially disastrous effects of oxidative stress linked to numerous diseases. These compounds are thought to exert their beneficial effects by acting as chain-breaking antioxidants, inhibiting lipid peroxidation of polyunsaturated fatty acids contained within biological membranes, thereby preventing the formation of potentially cytotoxic and highly reactive aldehydes (malondialdehyde and 4-hydroxynone-
mal), which are generated by the oxidation of polyunsaturated fatty acids. Although a number of FSVs and FSAs have been measured by HPLC-UV, this approach typically lacks the sensitivity and selectivity required to measure these compounds in biological samples. Electrochemical detection, however, is both sensitive and selective and makes use of the inherent redox activity of these compounds. The CoulArray® Coulometric Array Detector-the only HPLC electrochemical detector that is a “universal”, mass-based detector and offers excellent sensitivity, a wide dynamic range, and the advantage that all non-volatile analytes produce similar response, independent of chemical structure. Additionally, unlike UV detection, analytes need not possess a chromophore in order to be determined. We developed a simple reversed-phase HPLC-CAD method to rapidly screen for falcarinol, falcarindiol, and falcarindiol-3-acetate. The method was sensitive (LOQ ~5 ng on column) and reproducible, and the analysis was completed in 15 mins. Data from fresh, baby carrots and Queen Anne’s Lace (root, leaf, and flower) are presented.

**PA2**  
Simple and Direct Analysis of Phytosterols in Red Palm Oil by Reverse Phase HPLC and Charged Aerosol Detection  
Acworth IN, Bailey B, Plante M, Gamache P  
ESA – a Dionex Company, Applications Department, Chelmsford, USA

**PA3**  
Simple and Direct Analysis of Falcarniol and other polyacetylene Oxyxilins in Carrots by Reverse Phase HPLC and Charged Aerosol Detection  
Acworth IN, Plante M, Bailey B, Crafts C, Waraska J  
ESA – a Dionex Company, Applications Department, Chelmsford, USA

**PA4**  
Sensitive Analysis of Commonly Used Artificial and Natural Sweeteners Including Stevia and Their Impurities and Degradation Products  
Acworth IN, Crafts C, Plante M, Gamache P  
ESA – a Dionex Company, Applications Department, Chelmsford, USA

**PA5**  
A Versatile Detector for the Sensitive and Selective Measurement of Numerous Fat Soluble Vitamins and Antioxidants in Human Plasma and Plant Extracts  
Acworth IN, Gamache P, Waraska J  
ESA – a Dionex Company, Applications Department, Chelmsford, USA

Although falcarinols have a distinctive UV spectrum, the consequence of conjugated triple bonds, sensitivity tends to be poor due to the actual number of unsaturated bonds present in their structure. Measurement at 205 nm offers the best sensitivity; however, sample chromatograms tend to be very complicated due to the presence of many other compounds absorbing at this wavelength. Charged aerosol detection (CAD) is a “universal”, mass-based detector and offers excellent sensitivity, a wide dynamic range, and the advantage that all non-volatile analytes produce similar response, independent of chemical structure. Additionally, unlike UV detection, analytes need not possess a chromophore in order to be determined. We developed a simple reversed-phase HPLC-CAD method to rapidly screen for falcarinol, falcarindiol, and falcarindiol-3-acetate. The method was sensitive (LOQ ~5 ng on column) and reproducible, and the analysis was completed in 15 mins. Data from fresh, baby carrots and Queen Anne’s Lace (root, leaf, and flower) are presented.
of this detector is illustrated using a variety of examples including; a global gradient method for determination of FSVs and FSAs in plasma; a gradient method for the analysis of carotenoid isomers in carrots; an isotopic method for the measurement of reactive nitrogen species damage to biomembranes measuring 5-nitro-ghma-tocopherol in rat astrocytes and human plasma; a gradient method for the measurement of tocopherol and tocotrienol isomers in palm oil; and an isotopic method for the determination reduced and oxidized CoQ9 and CoQ10 in human plasma.

Iridoid and flavonoid patterns of the genus Veronica sect. Alsinebe subsect. Agrestis (Benth.) Stroh (Lamiaceae) and their systematic significance
Saeed Mehrvarz S, Mahmoodi N
Department of Biology, Faculty of Science, University of Ganhat, Rasht, Iran


Seed and mucilage yield of isabgol (Plantago ovata Forsk.) under salinity stress
Ghasemi Golzai K, Chaudhoo Jadda A, Zafarani
Moattar P
Faculty of Agriculture, University of Tabriz, Tabriz, Iran

Salinity may adversely reduce the overall productivity of plants by inducing numerous abnormal morphological, physiological and biochemical changes. An experiment was conducted in 2010 in the Department of Biology of the University of Tabriz, to investigate isabgol (Plantago ovata Forsk.) performance under non-saline (control) and three saline conditions (4, 8, 12 dSm-1 NaCl). The experiment was arranged as completely randomized block design with three replications. Ten seeds were sown 1 cm deep in each pot filled with 800 g perite. Salinity treatments were applied immediately after sowing. Tap water and saline solutions were added to the pots in accordance with the treatments to achieve 100% FC. After emergence, seedlings were thinned to keep four plants in each pot. During the growth period, the pots were weighed and the losses were made up with Hoagland solution. At maturity, plants from each pot were harvested and seed yield per plant was determined. Means of seed and mucilage yields per plant decreased with increasing salinity. However, seed yield per plant under 0 and 4 dSm/salinity were statistically similar. Mucilage percentage was not significantly affected by salinity stress. Thus, reduction in mucilage yield was attributed to deductions in seed yield per plant under high salinity treatments.

Composition of polysaccharides from aqueous extracts of some wound healing plants
Agare C, Lechtenberg M, Hensel A
Department of Pharmaceutics, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana; 2Institute for Pharmaceutical Biology and Phytochemistry, University of Muenster, Germany

Plant polysaccharides represent ideal candidates for therapeutics with immunomodulatory and wound healing actions. Polysaccharides from several medicinal plants have been shown to exhibit immunomodulatory activities [1–2] and stimulate proliferation of keratinocytes and dermal fibroblasts [3–6]. In most cases, hot water extracts containing the water-soluble polysaccharides are used for the treatment of wounds [7] and also the isolated pure polysaccharides have been shown to exhibit immunomodulatory and wound healing activities [8]. AQim has been to determine the monosaccharide composition of polysaccharides from aqueous extracts of selected medicinal plants traditionally used in Western Africa as wound healing agents [7] and to identify compounds which possess skin cell-promoting activities under in vitro conditions. Raw polysaccharides (RPS) were isolated from aqueous extracts of the selected plants by ETOH precipitation and dialysis (MWCO 3.5 kDa). TFA 2N hydrolyzed samples were analyzed concerning monosaccharide composition by TLC and HPAEC-PAD (Carbo PacTM PA1 stationary phase). 3 of 11 plants contained substantial amounts (>3%) of cold-water soluble mucilages. RPS from 3 plants were characterized by high fucose contents, a monosaccharide normally not forming a big part of polysaccharides from higher plants. The high amount of galactose (32%) and arabinose (30%) in the hydrolyzed RPS of Porqueinia nigrescens (Azel) Bullock probably gives an indication of presence of arabinogalactans. Glucan structure is reasonable for polysaccharides from Alstonia boonei De Wild. Further in vitro studies on influence of polysaccharides on skin cell physiology have to be initiated to establish exact structure-activity relationship. Acknowledgement: The authors thank Deutscher Akademischer Austausch Dienst (DAAD) for the fellowship awarded to C. Agare. References: 1. Diallo et al. (2003) J Ethnopharmacol 84: 279–287. 2. Innjajerdingen et al. (2006) Biomacromolecules 7: 48–53. 3. Deters et al. (2010) J Ethnopharmacol 127: 62–69. 4. Deters et al. (2008) J Pharm Pharmacol 60(2): 197–204. 5. Deters et al. (2005) J Ethnopharmacol 102(3): 391–399. 6. Deters et al. (2005) J Cell Physiol 202(3): 717–722. 7. Agare et al. (2009) J Ethnopharmacol 125(3): 393–403.

Seasonal variation of kauren type diterpenes and cinnamic acid derivatives in leaves of Mikania laevigata and Mikania glomerata cultivated under different shading conditions
Bertolucci SK, Pinto JB, Pereira AD, Oliveira AB, Braga FC
1Department of Agriculture, UFLA, Caixa Postal 3037, 37200–000, Lavras, MG, Brazil; 2Faculty of Pharmacy, UFMG, Av Antônio Carlos, 6627, 31270–901, Belo Horizonte, MG, Brazil

Mikania glomerata Spreng. and Mikania laevigata Sch.Bip. ex Baker are medicinal plants popularly named ‘guaco’, whose leaves are used to treat respiratory diseases, with coumarin (1) and kauren-type diterpenes regarded as the bioactive constituents. The goal of the study was to undertake seasonal studies on the contents of chemical markers in leaves under different shading conditions. Species were cultivated under different levels of solar radiation and full sunlight. The leaves were collected in the middle of each season/year. The contents of 1, coumaric (2), benzyolgrandifloric (3), cinamoylgrandifloric (4) and kaurane (5) acids were quantified in dried leaves of both species by RP-HPLC [1].Significant differences were found in the contents of cinnamic acid derivatives (1 and 2) and kauren-type diterpenes (3, 4 and 5) for the evaluated harvesting periods and cultivation environments. Coumaric acid was solely detected in M. laevigata in concentrations below the limit of quantification (<0.045%), in plants under 80% shading, collected in the autumn. Both 1 and 2 were not detected in the analyzed samples of M. glomerata. The average concentration of coumarin reached its maximum (0.94%) in the summer, in plants growing under 80% shading. In general, both species presented higher amounts of the kauren-type diterpenes in plants cultivated under sunlight, except for 3 in M. glomerata. Altogether, the obtained results point out that the highest content of coumarin is reached in M. laevigata cultivated under 80% shading, preferably harvested in the summer, but with reduced levels

Mikania laevisgata Sch. Bip. ex Baker and Mikania glomerata Sprun., known in Brazil as guaco, are medicinal species widely used to treat respiratory affections. Stability analyses of vegetal drugs are crucial to assure the quality of derived products. The present study aimed at undertaking qualitative and quantitative analysis of chemical markers [coumarin (CO), o-coumaric (OC), kaurenico (KA), benzoylglendrinic (BA) and cinnamylglendrinic (CA) acids] in dried leaves of M. laevigata and M. glomerata submitted to long-term storage. The plant materials were stored in a dark room with controlled temperature and humidity, and had their fingerprints analyzed three-monthly up to 18 months. Changes in chemical markers were evaluated by UV spectral purity of the peaks and by quantitative analysis of their contents (% w/w in dried leaves), employing an HPLC method previously reported by us [1]. The concentrations of the chemical markers did not vary significantly within the evaluated storage period (p > 0.05) for both species. In contrast, changes in BA, CA and KA peaks were detected for three-months stored samples of both species and CO peak, found only in M. laevigata, was detected after six months of storage, suggesting compound degradation. The CO contents in M. laevisgata samples ranged from 0.10 ± 0.03% to 0.12 ± 0.03% and therefore fulfill the Brazilian pharmacopeial requirement established for the species (minimum of 0.1% w/w, except for the 12-month sample: 0.09 ± 0.03%)[2]. Therefore, the quality control of Mikania species should be based both on the quantification of the selected compounds and fingerprint analysis. Acknowledgement: FAPEMIG, CAPES and CNPq, for the financial support. References: 1. Bertolucci SKV et al. (2009) Planta Med 75: 280 – 285. 2. Farmacopéia Brasileira IV.

Development of a rapid isocratic reverse phase-ultra fast liquid chromatographic method for determination of phenolic acids in fruits

Gomes ED, Naruin R, Ramalho SA, Guaitbero NC, Miranda MD. Laboratory of Chromatographic Analysis and Flavor, Federal University of Sergipe, São Cristóvão, Brazil

Some low molecular weight phenolic acids namely gallic, chlorogenic, protocatechuic, p-coumaric, vanillic and ferulic, are well-known in their health-promoting properties. Isocratic Ultra Fast Liquid Chromatographic methods (UFLC-DAD) for detecting these compounds are advantageous, due to their simplicity and economy of time and solvent usage. This paper aimed at the development of a rapid and comprehensive isocratic UFLC-DAD method for analysis of phenolic acids in Brazilian fruits mangaba (Hancornia speciosa Gomes) and umbu (Spondias tuberosa Arruda). Mobile phase compositions (different solvents A – Dihydrogen potassium phosphate, trichloroacetic acid and trifluoroacetic acid and different percentage of solvent B – 8; 10 and 12% of acetonitrile) were combined with flow rates (0.4: 0.5 and 0.6 ml/min) in a statistical factorial design. Among the combinations tried, the trichloroacetic acid was found to be the best solvent “A” and B – 10% of acetonitrile as the best solvent B, and flow rate of about 0.6 ml/min as the best range of flow. Method presented limits on detection ranging from 0.014 to 0.094 μg and higher recovery percentages were observed to extraction with methanol-acetone (69.51 to 72.59 for protocatechuic acid and 69.58 to 126.31 for the chlorogenic acid). Chlorogenic acid concentrations in mangaba samples (62.93 μg/g) were higher than in umbu samples (8.49 μg/g). Linearity of detector responses (represented by the linear regression coefficient of plots), was higher than 0.999 for all phenolic acids. These results permitted to develop a rapid and practical method for phenolic acids determination in the tropical fruits of umbu and mangaba. Acknowledgement: We thank the INCT/CNPq (National Council for the Development of Science & Technology, Brazil) for the financial support received while the first and last co-authors thank CAPES for fellowships.

Bioavailability and pharmacokinetic of the Algerian propolis constituent naringenin in rats after oral administration

Juengwatanatrakul T1, Sritularak B2, Tassanawat P3, Puttalin W4, Tanaka H1

1Faculty of Pharmaceutical Sciences, Ubon Ratchathani University, Ubon Ratchathani, 34190, Thailand; 2Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok, 10330, Thailand.; 3Faculty of Pharmaceutical Sciences, Khon Kaen University, Khon Kaen, 40002, Thailand; 4Graduate School of Pharmaceutical Sciences, Kyushu University, Fukuoka, 812 – 8582, Japan

The monoclonal antibody (Mab) against asiaticoside (AS), the bioactive constituent of Centella asiatica (L.), urban was produced and characterized [1]. As immunogen, AS was conjugated to keyhole limpet haemocyanin (KLH) and injected into a rabbit to produce the monoclonal antibody (Mab) against AS. The isocratic reverse phase-ultra fast liquid chromatographic method was established and evaluated comparing with HPLC method. The assay was suitable for quantitative AS in the range of 0.78 to 50 mg ml⁻¹. The validation study showed that the method was precise, accurate and sensitive. The ELISA method described should prove useful as an analytical tool for quality control and standardization of medicinal plants and pharmaceutical products containing AS. Acknowledgement: The JSPS-NRCT Core University Program, Dr. Mayuree Tantisira from Chulalongkorn University. References: 1. Juengwatanatrakul T (2011) Analyst 136:1013. 2. Galfre G (1981) Methods Enzymol 73: 3.
The use of astringents in the cosmetic industry is widespread as tonic lotions, cleansers, deodorants and antiperspirants. Aluminium chlorohydrate, among others aluminium salts, is highly used as an astringent. Recently, the use of aluminium in cosmetics raised the concern about its safety to humans. Although the regulatory agencies worldwide assure the safety of this raw material, this issue has led the search for substitutes of aluminium salts to serve consumer needs. In the development of new raw materials it is wise to evaluate a priori the in vitro efficacy to address the extremely complex functional systems of living organisms. Colorimetric titration methods for evaluating tannins based on precipitation of hide powder are laborious, not specific to tannins (2). The objective of this study was to apply the isothermal titration calorimetry (ITC) method, as described elsewhere by others (3,4,5), to evaluate the interaction of bovine gelatin with 8 commercially available natural and semi-synthetic tannin containing extracts. The bovine gelatin is an especially good source of proline binding sites found in human skin. The tannins belong to the hydrolysable and condensed types present in 5 different plant species. ITC is a reliable and fast technique to evaluate important parameters like enthalpy, entropy, stoichiometry and association binding constant in an unique experiment for further decision support. All raw materials had the efficacy compared to aluminium chlorohydrate. The developed methodology has provided an useful tool for astringency evaluation of tannins and will illuminate the road toward better cosmetics. References: 1. Folk O, Ciocalteu VJ (1972) Bion Chem 73: 627. 2. Verza S G, Kreinecker MT, Reis V, Henriques A T, Ortega G (2007) Quimica Nova 30(4): 815 – 820. 3. Frazier R A, Papadopoulou A, Mueller-Harvey I, Kissoon D (2003), J Agric Food Chem 51(18): 5189 – 5195. 4. Frazier R A, Papadopoulou A, Green RJ (2006) Pharm Biomed Anal 41(5): 1602 – 1605 5. Frazier R A, Deaville ER, Green RJ (2010) Pharm Biomed Anal 51(2): 490 – 495.
undergoes multiple filtration and washing steps prior to sterile filtration and freeze drying. The resulting starting material is named “e volumine cellae (lyophil, steril.)” [1] and is raised to homeopathic potencies (D2, D4, D5). HPLC-ESI-MS data is presented to prove the absence of Penicillin-derivatives and precursors (Penicillin G, m/e 335 [M+H]+ and 6-Aminopenicillanic acid, m/e 217 [M+H]+) in used active substances. Whilst being found in the fermented culture broth of e.g. Penicillium chrysogenum, Penicillium G and 6-Aminopenicillanic acid are no longer present in the active substance after processing. During the manufacture of the active substances, unwanted antibiotic compounds are eliminated whilst preserving the products high quality. Acknowledgement: HPLC-ESI-MS analysis was taken out at Phyto GmbH & Co. KG in Neu-Ulm.

Research work carried out in Sudan on the weed Argemone mexicana L. pointed to the potential of the plant as a source of larvicides against mosquito. Mosquito, Anopheles arabiensis, is on focus in Sudan because of its role as a vector of several tropical diseases. The objectives of this study was evaluation or selection of the best solvent used for extraction of the alkaloids and identification of the alkaloids using spectroscopy as well as chromatographic methods. The result showed that ethanol was the best for extraction of Argemone leaf alkaloids both qualitatively and quantitatively; hexane the poorest while acetone and chloroform were in between. Ethanol was selected as the best organic solvent for extraction and different water dilutions were used (10%, 25%, 50%, 75%, and 100%). The three alkaloids were detected in all ethanol dilutions. The alkaloid quantity increased with the increase of ethanol %. In addition, pure ethanol extract was comparable to distilled water. The three alkaloids detected in different methanol water dilutions (100%, 75%, 50%, and 0%) and 75% & 50 extract the higher concentration of alkaloids. Pure methanol extract was comparable to the distilled water extract. Distilled water extraction recovery was comparable to acidic water and hot water at [60°C]. Fractionation of the extract from the aerial part of Argemone mexicana led to the isolation of 3 major alkaloids which were identified using preparative TLC, IR spectroscopy, HPLC, GC-MS and literature reports as berberine, protopine and benzophenathridine. The seed isolated compound contain Dihydrosanguinarine as a major alkaloid. 

Effect of exogenous silicon and salt stress on germination and seedling establishment in Borago officinalis

Torabi F
Tarbiat Moalem University, Tehran, Iran

Borage (Borago officinalis L.) is a valuable medicinal plant with a high content of gamma linolenic acid. Seed germination and early seedling growth are critical stage for plant establishment and production. One of the most problems in the field of plant cultivation and production of this plant, is low and uniform seed germination. In addition salt stress in the soil or water is the major factor especially in arid and semi-arid regions which greatly influence plant growth and yield. Thus, in this experiment the influences of exogenous silicon (Si) concentration and salinity tolerance during germination and early seedling growth was evaluated. Treatments were 5 levels of silicon (0, 0.2, 0.5, 1.1 mg Na2SiO3) and 5 levels of NaCl (0, 20, 40, 80, 100 mM NaCl) in 3 replicates. The results showed that different treatments of salinity and Si had considerable effect on the germination rate (GR), germination index (GI) and seedling growth of borage. In all of the Si level, seeds possessed more germination rate and germination index than control. The highest germination percentage were obtained at 1 mM Si. Significant differences in seed germination were also detected among the NaCl concentrations used. The total dry and fresh weight of seedling were increase in Si treatment but reduced by increasing NaCl concentration. Result of this experiment is consistent with the hypothesis that salinity and Si have a priming effect and can prepare a suitable metabolic reaction in seeds and improve seed germination performance and seedling establishment.

Chicoric acid content and antioxidant activity of commercially available Echinacea herbal medicinal products

Brlecic N1, Krsalac F2, Zovko Konavic M2
1Pharmacological Institute, Dept of Pharmaceutical Biology, University Kiel, Gartenstrasse 27, 24118 Kiel, Germany; 2Dept of Pharmaceutical Biology, School of Veterinary Medicine Hannover, Buentweg 17, 30559 Hannover, Germany

Echinacea spp. are very well known medicinal plants with immunomodulatory activity. The purpose of this study was to investigate the phenolic content (chicoric acid and total phenol) and the antioxidant activity of seven medicinal products with Echinacea purpurea (L.) Moench commercially available in Croatia. The content of total phenolics was determined spectrophotometrically with Folin-Ciocalteu reagent, while the content of chicoric acid was established by using isocratic RP-HPLC. Antioxidant activity was established using the following techniques: radical scavenging activity of 2,2-diphenyl-1-picrylhydrazyl (DPPH) free-radical, reducing power and β-carotene-linoleic acid assay. The chicoric acid content varied greatly in products with a maximum of 1.6% (w/w) as well as the content of total phenols (0.03 – 16 mg/mL). In all the in vitro analyses the extracts demonstrated marked antioxidant activities. The activity in the reducing power assay correlated very well with the content of total phenols (r2 = 0.995, P < 0.0001), while the activity in the other assays did not correlate neither with the amount of chicoric acid nor with that of total phenolics. It seems that some other substances might be responsible for the activity of the investigated preparations in those assays. The results suggest that chicoric acid, as unstable phenolic compound characteristic for E. purpurea products vary as expected. On the other hand, the total phenolic and antioxidant activity of investigated products are greatly influenced by other ingredients of products.
Effect of static magnetic field on seed germination early growth and activities of some enzymes in Melissa officinalis seeds

Poorkabar L, Sedghi H, Samani MA

Urmia university, IRAN

The objective of the present study was to investigate the effect of static magnetic field (0, 25, 50 and 75μT) and exposure time (15, 30 and 60 minutes) on Melissa officinalis L. seed germination. Treatment of Melissa officinalis seeds in these magnetic fields increased the germination rate (GR), germination index (GI), germination rate coefficient (GRC), seedling length and seedling dry and fresh weight under laboratory germination tests. In germinating seeds, enzyme activities of α-amylase, dehydrogenase and protease were significantly higher in treated seeds in contrast to controls. The higher enzyme activity in magnetic-field-treated Melissa officinalis seeds could be triggering the fast germination and early vigor of seedlings. Keywords: Melissa officinalis, seed germination test, α-amylase, dehydrogenase, protease and magnetic field


HPLC-S/M Quantitative Determination of Gallic acid and Cyanidin-3-Glucoside Content of Bilberry Fruit Extract from Turkey

Kärmer N1, Gager F2, Basar KHC1,2

1Anadolu University Faculty of Pharmacy Department of Pharmacognosy 26470 Eskisehir/Turkey; 2King Saud University, College of Science, Botany and Microbiology Dept. 2455 – Riyadh/Saudi Arabia

Genus Vaccinium is a widespread genus with over 200 species of evergreen and deciduous woody plants varying in size from dwarf shrubs to trees. There is a great interest worldwide in the fruits of bilberry because of their high anthocyanin content. Anthocyanins and flavonoids comprising flavonol glycosides, flavan-3-ol and proanthocyanidins, whereas hydroxycinnamic acids are classified as phenolic acids. Anthocyanins are valued as pigments but are also widely used in natural health products due to their suggested positive effects on night vision, even though firm evidence from clinical trials is still lacking [1]. In this study, quality control of Vaccinium myrtillus L. fruits have been carried out according to the European pharmacopoeia (2). Furthermore, gallic acid and cyanidin-3-glucoside contents of Vaccinium myrtillus water and EtOH (70%) extracts were investigated using with HPLC ES/M/MS MRMs method. The assay was performed with different concentrations of gallic acid and cyanidin-3-glucoside chloride as standard solutions. The diagnostic fragmentations of gallic acid 168.7/125 – 79 and fragmentations of cyanidin-3-glucoside 448.7/287 – 150 were used for MRMs quantitative determination. Total anthocyanin content of the fruits were shown to be not less than 0.30 per cent and other features were found to correspond Pharm Eur requirements. Furthermore, the content of cyanidin-3-glucoside was shown to be 0.0538 ± 0.001 per cent in the EtOH (70%) extract and 0.045 ± 0.002 per cent in the water extract, respectively. The gallic acid contents measured in the ethanolic and water extracts were 0.001 per cent and 0.036 ± 0.001 per cent, resp. References: 1) Kihinem K et al.(2008) Food Chem 110(1): 156 – 160. 2. European Pharmacopoeia 7.1 (2010) Bilberry Fruit Fresh, Myrtillus fructus recens, p 1070.

Investigation of the existence of five major flavonoids in Satureja sobirena Bornm. and optimization of their extraction conditions using experimental design, solid phase extraction and HPLC

Sharifi V1, Hadjimohammadi M1, Elyasi H2

1Department of chemistry, University of Mazandaran, Babolsar, Iran; 2Department of chemistry, Payam noor university of Bijar, Bijar, Iran

Satureja sobirena Bornm. (SSB) (Lamiaceae) is an endemic species of Iran [1] and in traditional medicine is used as a rapid antidote for food poisoning [2]. In this work, chemometrics, solid phase extraction (SPE) and HPLC methods were used to investigate the existence of five major flavonoids including; myricetin, quercetin, luteolin, apigenin and kaempferol in (SSB) and to optimize the extraction conditions of detected flavonoids. The effects of five experimental factors including; percentage of ethanol, volume of extraction solvent, concentration of HCl, extraction time and temperature on the extraction recovery were investigated using a rotatable, orthogonal central composite design (CCD). Grid search method was used to find the optimum extraction conditions. The SPE was used to preconcentrate the presumably available flavonoids. The SPE parameters including; pH of loading solution, type and volume of elution solvent and break-through volumes were optimized. The preconcentrated extracts were analyzed by HPLC using a C18 column and methanol:0.5% phosphoric acid (60:40 v/v) with flow rate of 1.0 mL/min as mobile phase. Among the investigated flavonoids only quercetin, luteolin and apigenin were found in this species which showed two different patterns for extraction. Quercetin and luteolin were extracted using 20 mL of 68% aqueous ethanol containing 2.0 M HCl, refluxed for 30 minutes at 90 °C while apigenin was extracted using 20 mL of 68% aqueous ethanol containing 2.0 M HCl, refluxed for 1 hour at 45 °C. Concentrations of quercetin, luteolin and apigenin were 10.20, 19.21, and 48.50 mgkg<sup>-1</sup>, respectively. References: [1] Recherger K (1982) Flora Iranica. Akademie-Verlag, Berlin. [2] Taherpour A, Maroofi H, Nasri F (2005) Int J Appl Chem 1(1):57 – 61.

Phytochemical analysis of Anthyllis hermanniae – Leguminosae, and development of a sensitive UHPLC-HRMS/M method for the rapid analysis of the phenolic content

Paschali A, Termentzi A, Hadjimaki M, Skalsounis A

Laboratory of Pharmacognosy and Natural Products Chemistry, Department of Pharmacy, University of Athens, Panepistimiopolis-Zografou, Athens, GR-15771, Greece

Anthyllis genus includes several species, very few of which are investigated from a phytochemical point of view. Previous works about the phytochemical analysis of the aerial parts of some species describe the isolation and structure elucidation of several glycosides of kaempferol, quercetin and other flavonoid aglycons [1 – 4]. In the present study, a detailed phytochemical analysis of the methanolic extract of the aerial parts of Anthyllis hermanniae L. is described, a species for which there are no previous data concerning its metabolic content. Applying several chromatographic techniques (VLC, LC, prep-TLC, HPLC, CPC), twenty-two secondary metabolites, belonging to categories of cinnamic and benzoic acid derivatives, steroids, coumarins, isoflavons and flavonols were isolated and their structures were fully elucidated by means of UV-Vis, MS, HR-MS and NMR (18D spectra). Moreover, triglicycolides of quercetin and kaempferol, which are new natural products, were isolated and unambiguously elucidated [5]. After the structure elucidation of the isolated metabolites compounds were used as references for the development of a fast and sensitive method for the simultaneous characterization of the phenolic content of A. hermanniae. The analyses were performed on a UHPLC system hypenated with a hybrid-LTQ-Orbitrap mass spectrometer using ESI & APCl ionization probes, in both positive and negative modes. The study of the ion scan spectra together with the accurate MS/MS data enabled the identification of additional phenolics, with high confidence. This newly developed analytical method could be applied for the rapid identification of phenolics in other Anthyllis species, as well as in other Leguminosae plants. References: [1] Pistelli L et al. (2007) Nat Prod Res 21: 418 – 425. [2] Marco J et al. (1989) Phytochem 28: 1513 – 1516. [3] Adell J et al. (1988) Phytochem 27: 2967 – 2970. [4] Barbera O et al. (1989) Phytochem 28: 1513 – 1516. [5] Halabalaki, M et al. (2011) J Nat Prod In press.

Accelerated Solvent Extraction: development of a representative extraction method from medicinal plants for cosmetic applications

Giboulot J, Portes B, Gilbert A, Labranco C, Robin J

Centre de Recherche Yves Rocher, 101 Quai Roosevelt, 92444 Issy les Moulineaux Cédex, France.

The first crucial step to discover new cosmetic plant active ingredients is the extraction before the analysis of plant materials. Accelerated Solvent Extraction (ASE) is a fast and automatic sample preparation technique which offers the ability to carry out sequentially multiple extracts (up to 24 samples). Then, cosmetic activities (enzymatic targets and skin cells) can be evaluated. For preliminary phytochemical investigations, an efficient and exhaustive method is traditionally used to extract polar plant
metabolites. The aim of our study was to define ASE conditions to obtain the most complete and representative extraction. In contrary, most of publications deal with the optimization of operational parameters to enhance the selectivity of extraction of only compounds of interest. The transposition of our conventional solvent-based extraction method (hydroethanolic reflux extraction, 1h, ratio plant/solvent 10%) to ASE technology was performed with respect of amount of extracted material (dry matter, yield) and chemical composition. Among herbs selected, ASE extracts of Lepidium sativum Mill. and Scutellaria baicalensis Georgi extracts were obtained and analyzed by Thin-Layer Chromatography. The flavonoids content of the extract was identified by HPTLC coupled with UV densitometric detection. The quantification of orientin/homorientin and baicalin/baicalenin was achieved to select the best ASE parameters (50°C, 10 min, ratio plant/solvent 20%). In addition, the antioxidant activity of extracts was evaluated. The results showed that optimized ASE extracts were equivalent to conventional ones concerning phytochemical composition and antiradical activity. To conclude, standardization of ASE extraction method is a powerful tool for rapid screening of new cosmetic plant active ingredients.

**PA28** Determination of vitamin E (-tocopherol) in canola oils by high performance liquid chromatography
Karasakal A1, Seren C2
1Department of Analytical Chemistry, Namik Kemal University, Tekirdag, Turkey; 2Department of Analytical Chemistry, Trakya University, Edirne, Turkey

Tocopherols (Vitamin E) are natural phenolic antioxidants present in vegetable oils and are responsible for many of the healthful properties of these foods. They are effective radical scavengers and defend the body against free radical attack by protecting polyunsaturated fatty acids [1]. Vitamin E plays an important role at the intracellular level since its deficiency increases membrane fragility and promotes the damage of membranes by oxygen-reactive species, oxygen, or other free radicals [2]. The tocopherols belong to a group of structurally related compounds called tocols. Foods such as nuts, seeds, some grains, and vegetable oils are good sources of natural tocopherol antioxidants. The various tocols may exist in a free or a esterified form. In seed oils, they are called tocols. Foods such as nuts, seeds, some grains, and vegetable oils could be used as a starting method for the evaluation of antioxidant parameters of polyphenolic compounds using voltammetric methods and extend our knowledge of their electrooxidation mechanisms. Acknowledgement: This work was supported by the Czech Science Foundation (Grants Projects No. P503/11/P312 and P301/11/0767), by the Ministry of Education, Youth and Sports (MSM 619895216).

**PA29** Study of electrooxidation mechanisms and antioxidant properties of flavonoids and flavonolignans
Ulrichova J1, Zatoloukalova M1, Kren V2, Vacek J3
1Institute of Medical Chemistry and Biochemistry, Faculty of Medicine and Dentistry, Palacky University, Hradec Kralove, Czech Republic; 2Institute of Microbiology, Academy of Sciences of the Czech Republic, Videniska 1083, 142 2 Prague

Flavonoids are a large group of naturally occurring polyphenolic compounds that are distributed in vascular plants. A wide range of the biological activities are attributed to flavonoids' antioxidant abilities. Flavonoids are benzo- pazyl derivatives that can be divided into several groups according to their structural differences. Here we focused on quercetin, silybin and their derivatives which belong to the group of flavonols and flavonolignans, respectively. We described basic aspects of electrooxidation of flavonols and flavonolignans at a pyrolytic graphite electrode using cyclic and square wave voltammetry. Flavonols (quercitin, rutin and isorutin) and flavonolignans (silybin, 2,3-dehydrosilybin, 7-O-methylsilybin, 20-O-methylsilybin and isosilybin), were studied in an adiabatic state using ex situ (adsorptive transfer – AdT) voltammetric methods. Under the given conditions, flavonols and flavonolignans are subject to a multistep oxidation. The potential of the electrooxidation of hydroxy groups and other substituents corresponds with antioxidant properties of studied polyphenols. Using ex situ voltammetry, the following order in antioxidant capacities was proposed: flavonols >> 2,3-dehydrosilybin > silybin and its derivatives. The results provide a solid basis for further study of both the antioxidant and prooxidant parameters of polyphenolic compounds using voltammetric methods and extend our knowledge of their electrooxidation mechanisms. Lime flower is used worldwide for its sedative and antispasmodic properties. Traditionally it is used for migraine, hysteria, feverish colds, and for raised arterial pressure associated with arteriosclerosis and nervous tension [1]. Besides flavonoids, little is known for the rest of the phenol content. In the present study extensive HPLC-DAD, HPLC-ESI-MS and HPLC-MS/MS analyses were undertaken in the aqueous preparations of *Tilia platyphyllos* Scop. flavonoids. An HPLC-DAD-ESI-MS method was developed and optimised for the quantitative determination of the constituents. Analyses of the ethanol extracts confirmed the predominance of flavonol glycosides and protocatechuic acid. In contrast, both decocition and infusion, which are nevertheless the traditional herbal preparations, were more complex, containing polar simple phenolics and low molecular weight procyanidins. The use of different HPLC col-
The genus *Stachys* L. comprises over 450 species [1] and is one of the largest genera of the Lamiaceae. Aerial parts of *Stachys* spp. are used traditionally to treat genitourinary, gastrointestinal, and cardiovascular diseases. The species of *Stachys* such as *Stachys officinalis* are used widely in folk medicines for various purposes (i.e.; abdomi- nal pain, stomachache, would healing, carminative) [2,3]. Undoubtedly, this genus has also gained a great interest. In this study, we report a sensitive HPTLC method in order to determine and compare the rosmarinic acid contents of seven *Salvia* species: *S. candidissima Guss.*, *S. dichroantha Stapf.*, *S. heldreichiana Boiss.*, *S. schellenbergii Boiss.*, *S. scelerata L.*, *S. tormentosum Mill.*, and *S. officinalis* L. The methanolic extracts of the aerial parts of the plants were migrated on silica gel 60 F254 HPTLC plates with toluene: ethyl acetate: formic acid (5:4:1) as mobile phase and densitometric detection of rosmarinic acid was carried out at 330 nm. By this study we calculated the rosmarinic acid contents of seven *Salvia* species as w/w % by using HPTLC densitometric method which is validated in terms of accuracy, precision, repeatability, reproducibility, linearity, limit of detection, limit of quantification, sensitivity and specificity. References: 1. Hedge I C (1982) Salvia L. In: Davis PH (ed.) Flora of Turkey and East Aegean Islands. Edinburgh University Press, Edinburgh. 2. Sezik E et al. (2001) Astaxanthin (AXT), a superpotent antioxidant, is a cancer-protective carotenoid occurring in orange and red fruits, vegetables and leaves, but is also produced by microalgae *Haematococcus pluvialis* by means of in situ Raman spectroscopy and rationalized based on DFT computations. Although no visual changes are observed in the *Haematococcus* cells upon heating from -150 °C, the distinct changes in Raman spectra occurs from -100 /C30C systematically up to 150 /C30C. The exponential increase of the Raman shift of the C=C band at ca. 1520 cm-1 along with the change of 1190:1160 cm-1 ratio is observed that correlates with changes observed in theoretical spectra of conformers ordered by decreasing energy. It is assumed that AXT molecules, initially in the form of H-aggregates with the TT conformations of the end-rings, interconvert toward more stable gauche forms upon thermal stress.

**Figure 1:** The molecular structure of astaxanthin

**Acknowledgement:** This research was supported by the Polish Ministry of Science and High Education (grants no. N204310367 and N204013635). Computational Chemistry: King Saud University, Riyadh, Kingdom of Saudi Arabia; 2. Duymus¸H G 1, G/C246ger F1, Bas¸ er KHC 1,2 1. Hedge I C (1982) Salvia L. In: Davis PH (ed.) Flora of Turkey and East Aegean Islands. Edinburgh University Press, Edinburgh. 2. Sezik E et al. (2001) Astaxanthin (AXT), a superpotent antioxidant, is a cancer-protective carotenoid occurring in orange and red fruits, vegetables and leaves, but is also produced by microalgae *Haematococcus pluvialis* by means of in situ Raman spectroscopy and rationalized based on DFT computations. Although no visual changes are observed in the *Haematococcus* cells upon heating from -150 °C, the distinct changes in Raman spectra occurs from -100 °C systematically up to 150 °C. The exponential increase of the Raman shift of the C=C band at ca. 1520 cm-1 along with the change of 1190:1160 cm-1 ratio is observed that correlates with changes observed in theoretical spectra of conformers ordered by decreasing energy. It is assumed that AXT molecules, initially in the form of H-aggregates with the TT conformations of the end-rings, interconvert toward more stable gauche forms upon thermal stress.

**Figure 1:** The molecular structure of astaxanthin

**Acknowledgement:** This research was supported by the Polish Ministry of Science and High Education (grants no. N204310367 and N204013635). Computational Chemistry: King Saud University, Riyadh, Kingdom of Saudi Arabia; 2. Duymus¸H G 1, G/C246ger F1, Bas¸ er KHC 1,2 1. Hedge I C (1982) Salvia L. In: Davis PH (ed.) Flora of Turkey and East Aegean Islands. Edinburgh University Press, Edinburgh. 2. Sezik E et al. (2001) Astaxanthin (AXT), a superpotent antioxidant, is a cancer-protective carotenoid occurring in orange and red fruits, vegetables and leaves, but is also produced by microalgae *Haematococcus pluvialis* by means of in situ Raman spectroscopy and rationalized based on DFT computations. Although no visual changes are observed in the *Haematococcus* cells upon heating from -150 °C, the distinct changes in Raman spectra occurs from -100 °C systematically up to 150 °C. The exponential increase of the Raman shift of the C=C band at ca. 1520 cm-1 along with the change of 1190:1160 cm-1 ratio is observed that correlates with changes observed in theoretical spectra of conformers ordered by decreasing energy. It is assumed that AXT molecules, initially in the form of H-aggregates with the TT conformations of the end-rings, interconvert toward more stable gauche forms upon thermal stress.

**Figure 1:** The molecular structure of astaxanthin

**Acknowledgement:** This research was supported by the Polish Ministry of Science and High Education (grants no. N204310367 and N204013635). Computational Chemistry: King Saud University, Riyadh, Kingdom of Saudi Arabia; 2. Duymus¸H G 1, G/C246ger F1, Bas¸ er KHC 1,2 1. Hedge I C (1982) Salvia L. In: Davis PH (ed.) Flora of Turkey and East Aegean Islands. Edinburgh University Press, Edinburgh. 2. Sezik E et al. (2001) Astraxanthin (AXT), a superpotent antioxidant, is a cancer-protective carotenoid occurring in orange and red fruits, vegetables and leaves, but is also produced by microalgae *Haematococcus pluvialis* by means of in situ Raman spectroscopy and rationalized based on DFT computations. Although no visual changes are observed in the *Haematococcus* cells upon heating from -150 °C, the distinct changes in Raman spectra occurs from -100 °C systematically up to 150 °C. The exponential increase of the Raman shift of the C=C band at ca. 1520 cm-1 along with the change of 1190:1160 cm-1 ratio is observed that correlates with changes observed in theoretical spectra of conformers ordered by decreasing energy. It is assumed that AXT molecules, initially in the form of H-aggregates with the TT conformations of the end-rings, interconvert toward more stable gauche forms upon thermal stress.

**Figure 1:** The molecular structure of astaxanthin

**Acknowledgement:** This research was supported by the Polish Ministry of Science and High Education (grants no. N204310367 and N204013635). Computational Chemistry: King Saud University, Riyadh, Kingdom of Saudi Arabia; 2. Duymus¸H G 1, G/C246ger F1, Bas¸ er KHC 1,2 1. Hedge I C (1982) Salvia L. In: Davis PH (ed.) Flora of Turkey and East Aegean Islands. Edinburgh University Press, Edinburgh. 2. Sezik E et al. (2001) Astraxanthin (AXT), a superpotent antioxidant, is a cancer-protective carotenoid occurring in orange and red fruits, vegetables and leaves, but is also produced by microalgae *Haematococcus pluvialis* by means of in situ Raman spectroscopy and rationalized based on DFT computations. Although no visual changes are observed in the *Haematococcus* cells upon heating from -150 °C, the distinct changes in Raman spectra occurs from -100 °C systematically up to 150 °C. The exponential increase of the Raman shift of the C=C band at ca. 1520 cm-1 along with the change of 1190:1160 cm-1 ratio is observed that correlates with changes observed in theoretical spectra of conformers ordered by decreasing energy. It is assumed that AXT molecules, initially in the form of H-aggregates with the TT conformations of the end-rings, interconvert toward more stable gauche forms upon thermal stress.

**Figure 1:** The molecular structure of astaxanthin

**Acknowledgement:** This research was supported by the Polish Ministry of Science and High Education (grants no. N204310367 and N204013635). Computational Chemistry: King Saud University, Riyadh, Kingdom of Saudi Arabia; 2. Duymus¸H G 1, G/C246ger F1, Bas¸ er KHC 1,2 1. Hedge I C (1982) Salvia L. In: Davis PH (ed.) Flora of Turkey and East Aegean Islands. Edinburgh University Press, Edinburgh. 2. Sezik E et al. (2001) Astraxanthin (AXT), a superpotent antioxidant, is a cancer-protective carotenoid occurring in orange and red fruits, vegetables and leaves, but is also produced by microalgae *Haematococcus pluvialis* by means of in situ Raman spectroscopy and rationalized based on DFT computations. Although no visual changes are observed in the *Haematococcus* cells upon heating from -150 °C, the distinct changes in Raman spectra occurs from -100 °C systematically up to 150 °C. The exponential increase of the Raman shift of the C=C band at ca. 1520 cm-1 along with the change of 1190:1160 cm-1 ratio is observed that correlates with changes observed in theoretical spectra of conformers ordered by decreasing energy. It is assumed that AXT molecules, initially in the form of H-aggregates with the TT conformations of the end-rings, interconvert toward more stable gauche forms upon thermal stress.

**Figure 1:** The molecular structure of astaxanthin

**Acknowledgement:** This research was supported by the Polish Ministry of Science and High Education (grants no. N204310367 and N204013635). Computational Chemistry: King Saud University, Riyadh, Kingdom of Saudi Arabia; 2. Duymus¸H G 1, G/C246ger F1, Bas¸ er KHC 1,2 1. Hedge I C (1982) Salvia L. In: Davis PH (ed.) Flora of Turkey and East Aegean Islands. Edinburgh University Press, Edinburgh. 2. Sezik E et al. (2001) Astraxanthin (AXT), a superpotent antioxidant, is a cancer-protective carotenoid occurring in orange and red fruits, vegetables and leaves, but is also produced by microalgae *Haematococcus pluvialis* by means of in situ Raman spectroscopy and rationalized based on DFT computations. Although no visual changes are observed in the *Haematococcus* cells upon heating from -150 °C, the distinct changes in Raman spectra occurs from -100 °C systematically up to 150 °C. The exponential increase of the Raman shift of the C=C band at ca. 1520 cm-1 along with the change of 1190:1160 cm-1 ratio is observed that correlates with changes observed in theoretical spectra of conformers ordered by decreasing energy. It is assumed that AXT molecules, initially in the form of H-aggregates with the TT conformations of the end-rings, interconvert toward more stable gauche forms upon thermal stress.
Ultra-performance liquid chromatographic (UPLC) determination of the rutin and chlorogenic acid in the Ribes anatolica and its antioxidative activity

Kendar C1, Güvenç A1, Dinç E2
1Ankara University, Faculty of Pharmacy, Department of Pharmaceutical Botany, Tandogan 06100 Ankara, Turkey; 2Ankara University, Faculty of Pharmacy, Department of Analytical Chemistry, Tandogan 06100 Ankara, Turkey

A new ultra performance liquid chromatographic (UPLC) method was developed for the determination of rutin and chlorogenic acid in Ribes anatolica Behcet (Grossulariaceae) which is an endemic species in Turkey [1]. Good chromatographic separation and determination were performed on an Acquity UPLCTM BEH phenyl column (100 mm x 1.0 mm, i.d., 1.7 µm) system by using a mobile phase containing acetonitrile and formic acid buffer solution (pH = 3.77) with 3% triethylamine (15:85) (v/v) (See Figure 1). Chromatographic separation is by an isotropic elution with the flow rate of 0.3 mL/min. Calibration graphs for both compounds in the linear concentration range of 5 – 40 µg/mL were obtained by using the relationship between the concentration and the peak area based on the detection at 340 nm. The validity of the UPLC method was done by analyzing the plant samples. The developed UPLC method was applied to the quantitative analysis of R. anatolica consisting of rutin and chlorogenic acid and a good agreement was reported. The estimation of the antioxidant activities of water and methanol extracts of the leaf is based on thiobarbituric acid (TBA) assay in order to detect their lipid peroxidation. In this investigation, the significant activities were obtained from the water (IC50 = 24.85 ± 6.33) and MeOH (IC50 = 27.03 ± 6.64) extracts of R. anatolica in the TBA test. Propyl gallate (IC50 = 0.04 ± 0.18) was used as a positive control. References: 1. Behçet L (2001) Turk J Botany 25: 103 – 105.

Rapid and efficient isolation of polymethoxylated flavonoids and artemisinin from Artemisia annua L. acetone extract

Timoteo P, Wessels C, Ros G, Righeschi C, Bilia A
Department of Pharmaceutical Sciences, University of Florence, Florence, Italy


Finding the most appropriate IR technique for plant species identification

Kokal M1, Kolar J2, Trafela P1, Kreft S1
1Faculty of Pharmacy, University of Ljubljana, Alščerkova 7, 1000 Ljubljana, Slovenia; 2Faculty of Chemistry and Chemical Technology, University of Ljubljana, Alščerkova 5, 1000 Ljubljana, Slovenia

Quality control of herbal medicinal products is of extreme importance. Procedures for identification of plant species, such as biochemical analysis or macroscopic and microscopic examination of morphological and anatomical properties are time consuming and expensive[1]. A good alternative is infrared spectroscopy since it is rapid, easy to use, non-destructive and low-cost. Identification of species from dried whole leaf samples of pharmacologically important Epilobium and Hypericum genera were investigated. To determine which infrared spectroscopy mode gives most informative spectra for plant species identification different modes of infrared spectroscopy were applied. These were diffuse reflectance, attenuated total reflectance (ATR), direct transmission of whole leaves, and KBr tablet transmission with comminuted leaves. First the informative wavenumbers were chosen by one-way analysis of variance. Afterwards the colinearity was reduced with principal component analysis. At last the species identification was determined with discriminant analysis. Best results were obtained with ATR and KBr tablet transmission. Still there were important differences between genera. ATR proved to be appropriate for discrimination among Epilobium species (accuracy of plant species identification was 98%), Epilobium species differ in distribution and morphology of trichomes on the surface of the leaves[2]. While for Hypericum species KBr tablet transmission proved to give best results (accuracy of plant species identification was 97%). Hypericum species differ in secondary metabolites that are accumulated in the interior of the leaves[3,4]. Results show that morphological properties of plant material should be taken into consideration when developing an infrared spectroscopy based method for identification of plant species. References: 1. European Pharmacopoeia 6th Edition, 2007. EDQM (European Directorate for the Quality of Medicines & Health Care), Council of Europe, Strasbourg Cedex, France 2. Strggulc Krajde S, Dermastia M, Jogan N (2006). Bot Helv 116: 169 – 178. 3. Umeå A, Kreft S, Kartnig T, Heydel B (1999). Planta Med 65: 553 – 556. 4. Maggi E, Poceschi M, Menghini L, Ricciuti F (2004) FITOPAT 75: 702 – 177.

Applicability of ultra- and nanofiltration for the concentration of medicinal plant extracts

Paun C1, Neagu E1, Ungureanu O1, Radu GL2
1National Institute for Research-Development of Biological Sciences, Centre of Bioanalysis, Bucharest, Bulgaria; 2Faculty of Applied Chemistry and Materials Science, Politehnica University of Bucharest, Bucharest, Bulgaria

The present investigation revealed the potential benefits of ultra- and nanofiltration application in herbal extracts processing. Helleborus purpurascens Waldst. & Kit., Geranium robertianum L. and Salvia officinalis L. were widely used in China for more than 3000 years for treating many disorders including malaria[1]. Polymethoxylated flavonoids and artemisinin, one of the main compounds present in Artemisia annua L., are among the most promising natural products for antimalarial and antancer purposes[2]. In this work, three different chromatographic methods, including a rapid and selective isolation method of the main polymethoxylated flavonoids (PMFs) – namely euptalan, the isomers casticin and chrysosplenatin, artemetin and 5-OH,3’,4’,5,7-tetramethoxylavone – and artemisinin from A. annua acetone dried extract, have been compared. Briefly, the method consists of a pretreatment of the original extract between organic and aqueous layers and further purification of the richest extract in PMFs and artemisinin with Sephadex LH-20, silica gel normal phase column chromatography and flash chromatography equipped with a packed normal phase silica column were performed. Quali-quantitative analyses of the main PMFs found in the extract were also reported. The best results in terms of efficiency of isolation were obtained by flash chromatography and to the best of our knowledge this is the first report on the separation of the pair of isomers casticin and chrysosplenatin from A. annua by flash chromatography. Acknowledgement: Acknowledgments: Hanze University Groningen, Institute for Life Sciences and Technology, Groningen (NE) in combination with Erasmus European Comission Education and Training for the fellowship to C. Wessels. References: 1. Bilia et al. (2006) Phytotherapy 33: 487 – 493 2. Ferreira et al. (2010) Molecules 15: 3135 – 3170
PA40 Profiling of African Mimosaceae for the rapid identification of new triterpenoid electrophiles

Noté O1, Urbain A1, Anteneh C2, Lobstein A1
1Pharmacy and Bioactive Natural Substances, UMR 7200, University of Strasbourg, Faculty of Pharmacy, 74 route du Rhin – BP 60024 – 64701 Illkirch, France; 2 Service Commun d’Analyse, University of Strasbourg, Faculty of Pharmacy, 74 route du Rhin – BP 60024 – 64701 Illkirch, France

Avicins are complex triterpenoid saponins isolated from an Australian Mimosaceae (Leguminosae), Acacia victoriae Bentham. They are based on an acyclic acid core, substituted by two glycosidic units and by a specific side chain at C-21 containing two monoterpen carbonylic acids and a quinovose moiety, conferring thereof particular electrophilic properties [1]. Avicins exhibit potent proapoptotic and anti-inflammatory activities, selectively inhibit the growth of tumor cells and thus appear as a new potential class of anticancer natural substances [2, 3]. In order to discover new avicins analogues and to identify the pharmacophore responsible for the activity, different African Mimosaceae species, including Acacia, Albizia, and Entada genera, were selected to screen their saponins content. Chemical profiling of saponin-enriched fractions, based on a LC-UV-MS/MS dereplication method and NMR experiments, has pointed out the presence of avicins analogues in the different studied species. Characteristic structural features of avicins consisting of an acyclic aglycone, a tri- or tetra-saccharide moiety at C-28, a sugar residue at C-3, and the acylation of C-21 were highlighted by the developed analytical techniques routinely used in the laboratory. The quantification of oleuropein in differently processed extracts of olive leaves was achieved by UV detection at 240 nm and excellent linear behaviors were statistically compared with the ones obtained from two well established analytical densitometric analysis technique [1 – 5]. In the present study, HPTLC methods were developed, validated and compared to analytical techniques routinely used in the laboratory. The quantification of oleuropein in differently processed extracts of olive leaves was carried out. A calibration curve was created with oleuropein as standard and their content was determined with HPTLC. Quantification was achieved by UV detection at 240 nm and excellent linear behaviors over the investigated concentrations were observed. The results were statistically compared with the ones obtained from two well validated techniques, namely HPLC and NMR, and proved to be precise and accurate. Furthermore, HPTLC was used for the documentation of the fingerprint of Genista halacsiy Heldr., in order to detect and quantify the major compounds. The chromatograms allowed the identification of seven main constituents. Moreover, the possibility of elaborating HPTLC for the determination of the partition coefficients used in counter-current chromatography was examined. The obtained results were successfull for the purification of the target compounds of the aforementioned plant, indicating that the partition coefficients could effectively be determined with HPTLC analysis and not necessarily with HPLC. HPTLC provided reliable results in all the methods which were developed. It was shown to be sensitive, selective, repeatable, easy to handle, requiring low analysis time and less cost per analysis. Overall, HPTLC could be efficiently employed instead of expensive and time-consuming techniques. References: 1. Vanhaelen-Fastre RJ et al. (2000). Chromatogr A 868: 269 – 276. 2. Yadav D et al. (2011) Sep Sci 34: 286 – 291. 3. Rashmi et al. (2011) Phcog J 3: 41 – 44. 4. Dhalwal K et al. (2010) J Med Plants Res 4: 1289 – 1296. 5. Plocharz P et al. (2010) Chromatogr A 1217: 4868 – 4872.

PA41 Exploitation of HPTLC for methodology development: quantification, fingerprinting and partition coefficient determination

Boka V, Amountzias V, Avgirropoulou A, Aliignannis N, Skaltsounis A
Department of Pharmaceutical and Natural Products Chemistry, Faculty of Pharmacy, University of Athens, Panepistimiopolis Zografou, 157 71, Athens, Greece

High Performance Thin Layer Chromatography (HPTLC) is a simple, modern analytical densitometric analysis technique [1 – 5]. In the present study, HPTLC methods were developed, validated and compared to analytical techniques routinely used in the laboratory. The quantification of oleuropein in differently processed extracts of olive leaves was carried out. A calibration curve was created with oleuropein as standard and their content was determined with HPTLC. Quantification was achieved by UV detection at 240 nm and excellent linear behaviors over the investigated concentrations were observed. The results were statistically compared with the ones obtained from two well validated techniques, namely HPLC and NMR, and proved to be precise and accurate. Furthermore, HPTLC was used for the documentation of the fingerprint of Genista halacsiy Heldr., in order to detect and quantify the major compounds. The chromatograms allowed the identification of seven main constituents. Moreover, the possibility of elaborating HPTLC for the determination of the partition coefficients used in counter-current chromatography was examined. The obtained results were successfully for the purification of the target compounds of the aforementioned plant, indicating that the partition coefficients could effectively be determined with HPTLC analysis and not necessarily with HPLC. HPTLC provided reliable results in all the methods which were developed. It was shown to be sensitive, selective, repeatable, easy to handle, requiring low analysis time and less cost per analysis. Overall, HPTLC could be efficiently employed instead of expensive and time-consuming techniques. References: 1. Vanhaelen-Fastre RJ et al. (2000). Chromatogr A 868: 269 – 276. 2. Yadav D et al. (2011) Sep Sci 34: 286 – 291. 3. Rashmi et al. (2011) Phcog J 3: 41 – 44. 4. Dhalwal K et al. (2010) J Med Plants Res 4: 1289 – 1296. 5. Plocharz P et al. (2010) Chromatogr A 1217: 4868 – 4872.

PA42 Phytochemical study of plants and plant cell cultures of three Salvia Species

Schulz S1, Haas C1, Berkov S2, Pavlova A1, Ulber R4, Neuhais E1, Bley T1, Steingroever J1
1Institute of Food Technology and Bioprocess Engineering, TU Dresden, Dresden, Germany; 2AgroBioInstitute, Sofia, Bulgaria; 3Laboratory of Applied Biotechnologies, The Stephan Angeloff Institute of Microbiology, Bulgarian Academy of Sciences, Plovdiv, Bulgaria; 4Institute of Bioprocess Engineering, University of Kaiserslautern, Kaiserslautern, Germany

The genus Salvia L. is widely distributed cultivated in various regions all over the world because of his numerous biological and pharmacological properties. These positive effects have its source in the high diversity of their secondary metabolites and enable its application on pharmaceutical, cosmetics and food industries. The main activities namely adstringent, antibacterial, anti-inflammatory and antioxidant are effected for instance by the essential oils as well as phenolic acids, sterols and higher terpenoids. Beside cultivation parameters, the composition of these secondary metabolites is mainly affected by the species. According to their interesting spectrum of secondary metabolites, S. officinalis L., S. triloba L. and S. virgata L. were selected for the induction of alternative source for the production of selected secondary metabolites by plant is influenced by various parameters like climate, geological conditions and infestation by parasites, the application of plant cell and tissue cultures in vitro reveal a potential alternative. In this case cultivation can be conducted under defined and optimized conditions in a bioreactor without the need of herbicides. Different phytochemical methods including extraction, isolation and chromatography techniques were applied on both the plants and their in vitro cultures in order to compare their secondary metabolite production. Therefore GC/MS analysis was performed for the identification and structure determination of present secondary metabolites. The observed relevant experiments will be presented. Acknowledgement: This work has been supported by European Social Funds and the Free State of Saxony, project number 080938406.

PA43 Raman spectroscopy analysis of tobacco alkaloids

Kaczor A1, Garz K1, Dobroowski JC2, Baranska M1
1Faculty of Chemistry, Jagiellonian University, Ingardena 3, 30 – 060 Krakow, Poland; 2Laboratory for Theoretical Methods and Calculations, National Medicines Institute, Chelmiska 30/34, 00 – 725 Warsaw, Poland; Spectroscopy and Molecular Modeling Group, Industrial Chemistry Research Institute, Rydygiera 8, 01 – 793 Warsaw, Poland

Tobacco plants and products contain alkaloids, mainly nicotine, beside e.g., nornicotine, cotinine, and anabasine. The latter are also metabolites of nicotine produced in the liver by cytochrome P450. The aim of our research was in situ investigation of tobacco alkaloids directly in the plant as well as in some pharmaceutical products. Two-dimensional Raman maps of nicotine distribution were obtained with 1064 nm excitation wavelength, the spatial resolution of 50 – 200 μm and analyzed with the aid of quantum-chemistry calculations (B3LYP/6–31+G(d,p) and B3LYP/ aug-cc-pVDZ). Additionally, calculations were performed for salts and protonated forms of nicotine. Distribution of nicotine was obtained by integration of characteristic bands of the compound over the measured surface. Acknowledgement: This research was supported by the Polish Ministry of Science and High Education (grant no. N204013635). Computational center “Cynofren” (Krakow, Poland) is acknowledged for CPU time.
Artemisia annua L. (Asteraceae) contains various phytochemical compounds such as monoterpeneoids, sesquiterpenoids, flavonoids, coumarins, sterols etc. Sideritis (7-hydroxy-6-methoxycoumarin) is coumarin which can be found in plants of family Asteraceae. In this study, using HPLC-ED system, quantitative analysis of scopoletin was carried out in water extracts of A. annua. The shade dried plant material (10 g) was powdered and sub-samples were prepared. The ethanol extraction was performed with 25 mL of 70% ethanol at room temperature for 24 h with a shaker. The total phenolic content was estimated by the Folin-Ciocalteu method (6). Ethanol extractions were centrifuged and 100 µl extracts were taken, nine hundred micro-litres of water were added. 1:10 diluted Folin-Ciocalteu reagent and 4 ml of sodium carbonate (75 g/l) added to extracts. After 2 h of incubation in the dark at room temperature, the absorbance at 765 nm was measured. The total amount of phenolic compounds varied in the range of 56.1 – 109.1 mg GAE/g at the level of location and 616.9 – 986.6 mg GAE/g at the level of species. The highest value at the level of location was obtained from Origanum vulgare L. subsp. hirtum (Link) Ietswa., in Kemer location. However, Origanum sylamicum P.H.Davis, local endemic species had high values in all three locations and the highest average at the level of species. Origanum vulgare L. had the lowest average at the level of species. References: 1. Ietswaart J H (1982) Origanum L. In: Davis, P.H. (Editor), Flora of Turkey and the East Aegean Islands, Vol. 7: 297 – 313, Edinburgh University Press, Edinburgh. 2. Davis PH, Mill R R and Tan K (1998) Flora of Turkey and the East Aegean Islands, Vol. 7: 297 – 313, Edinburgh University Press, Edinburgh. 3. Güner A, Özhatay N, Ekim T and Başer K H C (2000) Flora of Turkey and the East Aegean Islands (Suppl. II), Vol 11, Edinburgh University Press, Edinburgh. 4. Basir K H C (2002) The Turkish Origanum species. In: Kintzios S E (Editor), Oregano: the genera Origanum and Lippia. Taylor & Francis, pp.109 – 126, London.
Modern drug discovery is greatly based on the identification and structural characterization of new lead compounds, stemming from the huge diversity of natural plant chemicals. The process is tedious facing the complexity of plant metabolism, and the time-consuming steps of purification. In order to reduce the number of de novo purification and elucidations of chemical entities, an interesting strategy is to create a natural-product database. Using about two hundred natural compounds including alkaloids, steroids, terpenes and phenolic compounds from commercial and our in-house chemical library (UMR 7200), we developed a database named “Marie-Lise” through a combination of HPLC-DAD and LC-ToF-MS/MS analysis using Galaxie and Masslynx softwares. High detection sensitivity and selectivity of these methods permits us to characterize: retention time, UV spectra, mass spectra, MS/MS data and metabolite specific calibration curve of the two hundred validated pure substances. This data matrix allowed us to build programs that permit to achieve high-throughput screening (HTS) of chemical structures in order to accelerate the discovery of new compounds or to identify and quantify known compounds of specific biological interest or toxicity from complex mixtures such as plant extracts or preparations.

**References:**
Linear in the concentration range from 0.05 to 0.25 mg/ml. Analysis on different days showed that the method was precise with an average concentration of 4.73% and RSD of 1.39%. A recovery experiment was performed resulting in a 95% confidence interval between 97.5% and 100.4%, meaning that the method is also accurate. Specificity was confirmed by the investigation of the peak purity. Using this newly validated method the quality of the plant material of *P. hysterophorus*, used as an active principle in pharmaceutical preparations, can be guaranteed.


### PA52

#### Liquid chromatography techniques for separation of flavonoids from Droseraceae

Flavonoids which are present in insectivorous plants of the Droseraceae family have wide range of advantageous properties i.e. an antioxidant, anti-inflammatory and antimicrobial activities, antitumor activity was reported as well. The purpose of the research was to develop the most favourable conditions for liquid chromatographic separation and identification of myricetin and quercetin in extracts of insectivorous plants. In the research a methanol and chloroform extracts of the *Drosa binata* Labill., *Drosa capensis* L., *Drosa aliciae* Raym.-Hamet and *Dionea muscipula* Ellis cultivated in vitro were used. In the first stage of the research an optimal composition of the eluent for selective separation of the components included in the extracts was selected. The thin layer chromatography (TLC) in the reversed phase and hydrophilic interaction chromatography (HILIC) conditions was used for selection of optimal chromatographic systems. In the second stage of the research a high performance liquid chromatography (HPLC) in RP and HILIC optimal conditions was used for detailed characteristic of analysed mixtures. The results show that thin layer chromatography is helpful technique for pre selection components of the eluent to separation of the flavonoids from complex herbal matrix. In the paper there is reported that application gradient elution is more preferable than isocratic elution in HPLC techniques. Acknowledgement: State Committee for Scientific Research, Grant No N N405 3757 37. This research work was supported by the European Union in the framework of the European Social Fund.

#### PA53

#### Optimal conditions of naphthoquinones separation from carnivorous plants extracts using thin-layer chromatography and high performance liquid chromatography

*Jaszczołt M*, *Skrzypczak A*, *Krolicka A*, *Lewandowski A*, *Kaminski M*

1Gdansk University of Technology, Gdansk, Poland; 2University of Gdansk and Medical University of Gdansk, Gdansk, Poland

Droseraceae plants are owned by the family of carnivorous plants, which in an unusual way adapt to environments poor in nutrients. Droseraceae obtain essential nutrients catching insects and other small invertebrates. Plumbagin, chloroplatumbagin, ramataneacon and droseron are naphthoquinones, which could be found in leaves and shoots of plants from Droseraceae family. In the literature there are a lot of information about the pharmacological properties of these secondary metabolites. The purpose of this research was to develop optimal conditions for separation of naphthoquinones contained in extracts of insectivorous plants of the species *Dionaea muscipula* Ellis, *Drosa aliciae* Raym.-Hamet, *Drosa capensis* L. and *Drosa binata* Labill. The experimental studies have been divided into two stages, the first included study using thin layer chromatography (TLC) and the second using high performance liquid chromatography (HPLC) technique. Both the studies by thin layer chromatography and high performance liquid chromatography was performed in normal and reversed phase system. Optimal conditions of naphthoquinones separation using TLC and HPLC in normal and reversed phase system will be presented.

Acknowledgement: State Committee for Scientific Research, Grant No N N405 3757 37. This research work was supported by the European Union in the framework of the European Social Fund. The system project of the Pomorskie Voivodeship “InnoDoktorant – Scholarships for PhD students, II edition”.

### PA54

#### Application of HPTLC-MS for the on-line identification of oxypregnan glycosides in *Hoodia gordonii*

*Bauer R*, *Meier M*, *Pferschy Wenzig E*, *Wölkart K*, *Reich E*

1Institute of Pharmaceutical Sciences, Pharmacognosy, Karl-Franzens-University Graz, Universitätsplatz 4, 8010 Graz, Austria; 2CAMAG-Laboratory, 4132 Muttenz, Switzerland

*Hoodia gordonii* (Mass.) Sweet is a succulent plant from South Africa and Namibia which has been used by the indigenous people to suppress appetite. Oxypregnan glycosides (hoodigidosides) are considered as active principles [1]. HPTLC methods have been previously developed for the fingerprint analysis and identification of extracts from *Hoodia gordonii* [2,3]. Ion-trap tandem mass spectrometry and liquid chromatography coupled with electrospray ionization time-of-flight mass spectrometry have been used for the identification of isolated steroidal glycosides in *Hoodia gordonii* [4]. Recently, an HPTLC-MS Interface became available, which semi-automatically can extract zones of interest directly from a TLC/HPTLC plate and can direct them into a LC-MS system so that mass spectra can be obtained [5,6]. Previously we have tested the HPTLC-MS Interface to analysis containing herbal drugs [7]. We now have investigated the HPTLC-MS Interface for the identification of hoo-digosides in extracts of *Hoodia gordonii*. Extracts have been applied as bands onto HPTLC plates using an automatic TLC sampler. Separated zones were eluted from the plate by the HPTLC-MS interface using methanol as solvent delivered by an HPLC pump at 100 μl/min. The interface was hyphenated to a Finnigan LCQ Deca XP Plus ion trap mass spectrometer equipped with an electric spray ionization (ESI) source. Hoodigosides M, L, U, O, E, F, J, N, and C could be identified on the basis of the mass spectra obtained by HPTLC-MS. Therefore, the HPTLC-MS interface is a quick and powerful tool for the on-line identification of hoodigosides in TLC separations and can complement the classical TLC detection tools. References: 1. Vermaak I et al. (2011) Planta Med [Epub ahead of print]. 2. Widmer V et al. (2008) Planar Chromatogr (2008) 21(1): 21 – 26. 3. Rumulla Ch et al. (2008) J Sep Sci 31: 3959 – 3964. 4. Avula B et al. (2008) Rapid Commun Mass Spectrom 22(16): 2587 – 2596. 5. Luftmann H et al. (2007) Rapid Commum Mass Spectrom 21: 3772 – 3776. 6. Reich E, Widmer V (2009) Planta Med 75(7): 711 – 718. 7. Bauer R et al. (2010) Planta Med 76: 1334.

### PA55

#### Application of near-infrared spectroscopy (NIRS) as a tool for quality control in Traditional Chinese Medicine (TCM)

*Huck Pezzi VA, Pallua JD, Pezzi C, Schönbichler SA, Bitterer LK, Born GC, Huck CW*

Institute of Analytical Chemistry and Radiochemistry, Leopold-Franzens University, Innrain 52a, 6020 Innsbruck, Austria

Traditional Chinese Medicine is becoming more and more popular all over the world. Novel analytical tools for quality control are highly demanded enabling analysis starting at breeding and ending at biological fluids including urine or serum. Compared to analytical separation methods (chromatography, electrophoresis) near-infrared spectroscopy (NIRS) allows analyzing matter of interest non-invasively, fast and physical/chemical parameters simultaneously. It can be used for the quantitative control of certain ingredients. In many cases identification can only be achieved by pattern recognition. Therefore, NIRS combined with cluster analysis offers huge potential to identify e.g. species, geographic origin, special medicinal formula etc (Figure 1). In the present contribution the fundamentals, possibilities of NIRS applied in quality control of TCM are pointed out and its advantages and disadvantages are discussed in detail by several practical examples [1,2]. A Büchi FT-NIR spectrometer was used for recording. Cluster analyses and PLs calibration models were generated with NiciCal 4.2.1 and/or The Unscrambler. A Perkin Elmer 400 spectrometer in combination with a microscope with a nitrogen cooled MCT detector-array was used to acquire the hyperspectral images. NIR imaging is highly useful to judge the botany and morphology of the sample and allows visualizing the distribution of active plant...
ingredients. Stable PLS calibration models can be applied for quantitative determination of APIs, judgment of raw materials, during the production and the preparation of medicinal formulations. Cluster analyses are highly suitable for identifying falsification, species and geographic regions. Both methods in combination are applied to monitor the quality of patented formulations.

Figure 1: Flow diagram of NIRS application fields in TCM


Study and comparison of the Pistacia atlantica Desf. oleoresins from Iran

Savordoroudi P1, Mirzajani F1, Memar A2, Ghasempour A3
1Department of Phytochemistry, Medicinal Plants and Drugs Research Institute, Shahid Beheshti University, G.C. Evin, Tehran, Iran; 2No.278, third floor, between Hafez and Aban, Karimkhani Zand, Tehran, Iran

Pistacia atlantica Desf. is one of the species which is valued because it is the source of mastic gum, exudates which strengthens gums, deodorizes breath, fights coughs, chills and stomach diseases [1, 2]. Furthermore, the extracted oleoresin from P. atlantica is used in the chewing gum industries. One of the difficulties in chewing gum industries is the influence of the preliminary processing of the raw materials on the quality of the final products. It may caused by the variation of the different geographical area where the raw materials were collected or the process of preliminary preparation. These factors were monitored according to the chemical and physical examinations. The preparation process was monitored over the final physical properties of samples using thermal gravimetric method, (TG) and differential scanning calorimetry, (DSC). Moreover, in order to evaluate these geographical influence, the fragrance and the structural compounds of the raw samples collected from different areas were studied. The essential oil from the oleoresin, collected from six different locations in Iran, was obtained and its chemical composition was determined with GC and GC-MS. The yield range of essential oil was 17 – 22% (w/w), and the major compound is -pinene, (92%). Because of the versatile usage in food industries and high concentration of -pinene, it subjected for the antimicrobial activity against variety of bacteria [8], as spices and tea due to different types of coffee and tea. Acknowledgement: CNPq, FAPERP, CAPES. References: 1. Schripsema J (2010) Phytochem Anal 21: 14 – 21.


1H NMR Metabolic analysis of coffee and tea samples for the quantitative determination of the main constituents

Schripsema J, Vianna MD, Lemos MA, Dagnino D
Grupo Metabolómica, Universidade Estadual do Norte Fluminense, Campos dos Goytacazes, Brazil.

In metabolomics the aim is to detect and quantify all metabolites present in a certain organism, tissue or cell at a specific moment. One of the major problems in the analysis is the incomplete extraction [1]. In NMR based metabolomics generally aqueous solutions are used. In studies with humans or animals, where the samples are generally derived from body fluids this seems logical, but in plants a much larger variety of metabolites is encountered and the metabolites are obtained through the extraction of tissues. For the analysis of samples of tea and coffee the extraction procedure was investigated. Direct extraction with commonly used NMR solvents, did show that no single solvent provided a complete profile, but the best results were obtained with an extraction with a two-phase system consisting of water and chloroform. Water was found to be essential to obtain a good extraction of the apolar constituents with chloroform. The direct use of chloroform on dried plant material yielded incomplete extractions. The application of the developed protocol on the coffee and tea samples permitted an accurate quantification of the caffeine content and the detection of specific metabolites in different types of coffee and tea. Acknowledgement: CNPq, FAPERJ, CAPES. References: 1. Schripsema J (2010) Phytochem Anal 21: 14 – 21.

Quantitative and qualitative analyses of rosmarinic acid in South African Salvia species using two chromatographic techniques

Kamatou G, Chen W, Viljoen A
Department of Pharmaceutical Sciences, Faculty of Science, Tshwane University of Technology, Private Bag X680, 0001, Pretoria, South Africa


Expression of amorphoph-4,11-diene synthase (ADS) gene in Iranian Artemisia annua L. genotypes

Naghavi M, Reshidi Monfared S, Alizadeh H, Yazidisamadi B
Department of Biotechnology, Agricultural & Natural Resources College, University of Tehran, Tehran, Iran

Malaria disease is caused by unicide of Plasmodium falciparum. Nowadays, malaria has been reported in more than 100 countries. The artemisinin is a sesquiterpene that is produced by two pathways of isoprenoid and mevalonate in Artemisia annua L. Artemisinin as new and effective drug is widelyused. Artemisia annua is annual plant and native to Asia and most probably China. In this study, six different genotypes Artemisia annua was collected from the province of Golestan. Amorpha-4, 11-diene synthase (ADS) promoter was analyzed by using different

ACKNOWLEDGEMENTS

Planta Med 2011; 77: 1229–1472 Georg Thieme Verlag KG Stuttgart · New York · ISSN 0032-0943

Synthesis of a Novel 3,3-dialkyl-2-propyl-3H-pyrrole-2,5-dione Based Nitroxide Mediator.

Table 1: 3,3-Dialkyl-2-propyl-3H-pyrrole-2,5-dione Based Nitroxide Mediators

<table>
<thead>
<tr>
<th>Compound</th>
<th>Structure</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaN</td>
<td>Na</td>
<td>A typical nitroxide mediator</td>
</tr>
<tr>
<td>BBN</td>
<td>B</td>
<td>A benzylated nitroxide mediator</td>
</tr>
<tr>
<td>TBBN</td>
<td>T</td>
<td>A tert-butyldimethylsilylated nitroxide mediator</td>
</tr>
</tbody>
</table>

ACKNOWLEDGEMENTS

This work was supported by the National Science Foundation (Grant DMR-1006021) and the Air Force Office of Scientific Research (Grant FA9550-08-1-0271).
cis-elements database of PLATcare, TRANSFAC and PLACE. The result showed that there are different cis-elements responding to plant hormones and abiotic stress in ADS promoter. We also identified two new putative transcription factors in EST library of Artemisia annua then studied the expression of ADS gene and three transcription factors by using real time PCR technique. The result showed that WRKY transcription factor had more important role than other transcription factors.

PB2 Development of NaCl-tolerant line in Tanacetum cinerariaefolium through shoot organogenesis of selected callus line
Abdi G, Persian Gulf Research and Studies Center, Persian Gulfs University, Boushehr, Iran.

Plants were regenerated successfully through shoot organogenesis of a NaCl-selected callus line of Tanacetum cinerariaefolium (Trevir.) Schultz-Bip developed through stepwise increase in NaCl concentration in MS medium. Increasing NaCl level concentration (0, 5, 10, 15, 20, 25, 30, 35, 40, 45mM) from low level to high level was found to be a better way to isolate NaCl-tolerant callus line, since direct transfer of callus to high saline medium was detrimental to callus survival and growth. Among different media and growth regulator treatments, MS medium containing 1 mgl-1 BA and 1 mg l-1 NAA or 1 mg l-1 BA, 2 mg l-1 NAA and 0.5 mg l-1 GA3 for shoot organogenesis in selected callus line and B5 medium supplemented with 2 mg l-1 NAA showed best response for root regeneration. As increasing NaCl concentrations (From 0 to 45 mM) the ability of shoot and root regeneration were decreased. The selected callus line showed significance increase in proline content and decrease in pyrrole content. Based on growth performance and proline content (20 mg in callus line and 35 mM in shoot culture) could be considered as NaCl-tolerant line showing all positive adaptive features towards the salinity stress. Further studies about agronomic performance of obtained plants under saline soil condition are necessary for understanding to check the genetic stability of the induced salt-tolerance plants.

PB3 Stable Over expression of codeinone reductase gene in transgenic Papaver somniferum plant
Hosseini B1, Hashemi H2, Shahraki F3
1Department of Horticulture, Faculty of Agriculture, Urmia University, P.O.Box 165, Urmia, Iran; 2National Institute for Genetic Engineering and Biotechnology (NIGEB), P.O.Box: 14155 – 6344, Tehran, Iran; 3Department of Biotechnology and Plant Breeding, Faculty of Agriculture, Ferdowsi University of Mashhad, P.O.Box 91775 – 1163, Mashhad, Iran.

Papaver somniferum today is the commercial source of the narcotic analgesics morphine and codeine. By conversion of codeine and morphine to morphine, codeine reductase is a key gene in metabolic engineering of isoquinoline alkaloids pathway. In this project, at first we optimised expression of gene in P. somniferum via Agrobacterium tumefaciens containing pBI121 plasmid. Encoding gene of COR enzyme was isolated using primers which designed on the base of gene sequence available on data banks (NCBI) for P. somniferum. This gene then cloned in expression vectors under controlled of CaMV35 promoter and transferred to plants by agro transformation. The result of evaluation showed the qualitative and quantitative changes in metabolite production of transgenic and control plants.

PB4 Accumulation of phytoalexins in potato tuber treated with plant extract
Rachida VZ, Farid Z, Loubna A, Saida M, Ali B
University of Bejaia, Department of Biology, Physics and Chemistry, Faculty of Sciences, Béjaia, Algeria

One of the best and longest-studied defense responses of plants to infection is the induced accumulation of antimicrobial, low-molecular-weight secondary metabolites known as phytoalexins. A role for these compounds in defense has been revealed through several experimental approaches. Samples of Olea europeae L. leaves were collected from Bejaia in January 2009. The whole samples were dried in shade and crushed to fine powder. 20 g of dried powder of olive leaves were submitted to extractions which were carried out twice for 24 h with 400 mL of ethanol according to Ranall et al (2006). The total phenolic contents of the samples were determined with the Folin Ciocalteu reagent. The half tuber cultivars Desiree and Spunta, was treated by depositing 100 μl of one of the previously prepared phenolic extracts in the hole drilled with a cork as described by val et al. (2006). They were then inoculated with an inoculum of Pectobacterium atrosepticum (106 cfu/ml). The tubers were assessed after five days for the development of disease symptoms, and were used to evaluate production of phytoalexin. The rate of phytoalexins in relation to cessation of pathogen development, quantification of phytoalexins at the infection site, of potato tubers treated with plant extract was studied. The results of the half-tuber inoculation treated by various plant extracts showed a remarkable reduction in the amount of rotted tissue. Evidence in support of phytoalexins in resistance as well some recent advances in phytoalexin biosynthesis are reviewed. Criteria for evaluating a role for phytoalexins in disease resistance are also discussed. Keywords: Alkaloids, codeinone reductase expression, plant extract.

PB5 Effect of Plant Growth Promoting Rhizobacteria (PGPR) on agronomic characteristic and root colonization in fennel
Mirzaei A1, Naseri R2, Soleymanniafar A1, Vaziri S1
1Islamic Azad University, Karaj Branch, Iran; 2The University of Ilam, Ilam, Iran.

In order to study the effect of Plant Growth Promoting Rhizobacteria (PGPR) on agronomic characteristic and root colonization in fennel (Foeniculum vulgare Mill.), an experiment was conducted in western of Iran in 2008 and 2009 growing seasons. The factors were Plant Growth Promoting Rhizobacteria (Azotobacter inoculation with and non-inoculated) and nitrogen application (0, 40, 80 Kg/ha-1). The treatments were arranged as factorial in a randomized complete blocks design with three replications. Results showed that the highest grain yield, umbrella per plant, biological yield and root colonization percent were obtained with Azotobacter treatment. Nitrogen application was significant affected on studies traits. The highest grain yield, biological yield and root colonization percent obtain by 80 Kg/ha-1. Interaction effect PGPR x nitrogen application was affected on grain yield and colonization percent. The highest grain yield and root colonization percent obtained Azotobacter x 80 Kg/ha-1 nitrogen.

PB6 Effect of Mycorriza on root characteristic and concentration of phosphate, iron and zinc in cumin (Cuminum cuminum L.)
Naseri R1, Mirzaei A2, Soleymanniafar A1
1The University of Ilam, Ilam, Iran; 2Islamic Azad University, Karaj Branch, Karaj, Iran; 3Islamic Azad University, Dezful Branch, Dezful, Iran.

Symbiosis between plants and mycorrhizal fungus are very important for agriculture system and natural resource. In soil whit, less fertility and nutrient, mycorrhiza can lead to improvement in growth that results increase in mycorrhizal plant resistance in comparison whit non-mycorrhizal plants in stress condition. In order to test the influence of the strains of Mycorriza on root characteristic and nutrients on Cuminum cuminum L. an experiment was conducted based on completed design with three replications in western of Iran in 2009 – 2010 growing season. Strains of mycorriza including of Glomus fasciculatum, Glomus etunicatum, Glomus mosseae and Glomus intraradices. In these research traits as: root length, root length, root length/root dry weight ratio, total dry weight of colonization and concentration of phosphate, iron and zinc. Results of variance of analysis showed that strains of mycorriza had significant affected on studies characteristic, as Glomus fasciculatum had the highest root colonization, root dry weight, root length/root dry weight ratio. Strains of Mycorriza had significant affect on absorption of nutrients. Glomus fasciculatum had a more ability in absorption of phosphate, iron and zinc other then strains of mycorriza.
Bioactive metabolites isolated from the endophytic fungus *Penicillium chrysogenum* isolated from Red Sea algae

Hawas UW, Ahmed EJ, Lantsch H

*Phytochemistry and Plant Systematic Department, National Research Centre, Dokki, Cairo, Egypt; *Chemistry of Natural and Microbial Products Dept., National Research Centre, Egypt, *Institut für Organische und Biomolekulare Chemie, der Universität Göttingen, Tammannstr. 2, 37077 Göttingen, Germany

Endophytic fungi constitute one of the most interesting sources of bioactive natural products. They are synergistic to their respective host and at least some of them are thought to play an important role in the host’s defence by producing secondary metabolites that protect the host from being attacked by pathogenic fungi [12]. Ten known compounds alatinone, emodin, w-hydroxyemodin, 2-Acetylquinazolin-4(3 H)-one, chrysonophan, cyclo-L-Ala-L-Leu, cis-cyclo (pro, val), 2,3-Dihydroboricillin, meлагenezin, and uracil were identified from the EtOAc-extract of a Crass-p-spermol culture of the endophytic fungus *Penicillium chrysogenum* isolated from red algae (*Liagora viscida* (Forsskal) C. Agardh) collected from the Egyptian Red Sea. The structures of the compounds were elucidated on the basis of comprehensive NMR spectral analysis (1 H- and 13C-NMR, HRICOSY, HOCO, HMBC) as well as mass spectrometry. The crude organic extract and some of the pure compounds showed moderate to strong antimicrobial activity. References: [1] Stierle A et al. (1993) Science 260: 214 – 216, [2] Strobel G et al. (1997) Aust J Bot 45: 1037 – 1062.

Microbial Transformation of β-Phellandrene

Iscan G, Kırımer N, Demirci F, Başer KHC

*Anadolu University, Faculty of Pharmacy, Department of Pharmacognosy, 26470, Eskisehir, Turkey; *Botany and Microbiology Dept, College of Science, King Saud University, Riyadh 11451, Saudi Arabia

Derivatives of natural and aromatic compounds obtained by biotransformation constitute an important resource for natural fragrances and aroma ingredients or active compounds. Monoterpenes and their new metabolites are very important resources in such applications. At the present study the monoterpene β-phellandrene was biotransformed by fungal whole cell cultures for the first time to best of our knowledge. The substrate β-phellandrene was primarily isolated and purified from its natural source *Angelica archangelica* seed essential oil (the main compound of essential oil 78%) and then subject to biotransformations by *Corynespora cassicola, Fusarium heterosporum, Aspergillus aliusces*, *Yarrowia lipolytica, Alternaria alternata* fungal cultures. As a result cis-p-Menth-2-en-7-ol and 4-isopropylcyclohexene-2-on (syn. cryptone) were determined as metabolites by using chromato-spectral methods. Key words: β-phellandrene, biotransformation, monoterpens.

Transformation of h6 h gene from *Atropa belladonna* to *Hyoscyamus kurdicus* in order to enhance scopolaamine production

Mirzadeh S, Sanjarian F, Salimi A, Haghebn S

*Pharmaceutical Biotechnology Department, National Institute of Genetic Engineering and Biotechnology (NIGEB), Tehran, Iran; *Biology Department, Tarbiat Moallem University of Tehran, Tehran, Iran

Hyoscyamine and scopolaamine are medicinally important tropane alkaloids, which possess anticholinergic and central nervous system activities. They have well established therapeutic uses (in ophthalmology, cardiology, gastroenterology, etc). For medicinal purpose, scopolamine is much more useful and valuable because of its higher physiological activity and fewer side effects. These natural substances are exclusively extracted from plants [14]. Several species from the family Solanaceae like the genus *Atropa, Datura, Duboisia, Hyoscyamus, Scopolia* produce these alkaloids. *Atropa belladonna* L. is a perennial herbaceous plant and most importantly commercial source of pharmaceutical tropane alkaloids in the family Solanaceae. *Hyoscyamus* species in Iran; one of them is *Hyoscyamus kurdicus* Bornm. from Kermanshah province [2]. Hyoscyamine 68 hydroxylase (h6h) is a bifunctional enzyme which catalyzes the last two oxidative reactions of tropane alkaloid biosynthetic pathway, converting hyoscyamine to scopolaamine [3]. We expressed the h6h gene from *A. belladonna* in *H. kurdicus*, which caused over accumulation of scopolaamine. For this purpose RNA was extracted from leaf disk of *A. belladonna* plants. cDNA was synthesized and amplified by specific h6 h primers. PCR products were sequenced and constructed to pbH121 shuttle vector. *H. kurdicus* seeds were collected from its natural habitats in Kurdistan-Iran and cultured. Keywords: Hyoscyamus kurdicus, Atropa belladonna, scopolaamine, h6h gene transformation


Mushroom polysaccharides offer a lot of hope for cancer patients and sufferers of many devastating diseases. A variety of polysaccharides from a number of mushroom varieties have been demonstrated to enhance the immune system. Yield and functionality of polysaccharides by fermentation are highly dependent on their culture conditions, such as different culture compositions and environmental parameter (1). In this study the effects of different Nitrogen sources including: yeast extract, Mycelicopeptone, polyeptone, ammonium nitrate, ammonium sulfate and ammonium oxalate in two different media (Complex and synthetic liquid culture) were investigated. For the determination of polysaccharides produced by *Agaricus blazei* Murrill the total polysaccharides which were precipitated by absolute alcohol were weighed. The experiments showed that the highest growth and polysaccharide production were obtained when yeast extract used as nitrogen source. The concentration of polysaccharide in both complex and synthetic media when yeast extract was used were similar. The lowest growth and productivity were also seen in medium containing ammonium sulfate. References: 1) Shu CH, Lin K-J, and Wen B-J (2004) J Chem Technol Biotechnol 79: 998 – 1002.

In the present study, we evaluated genetic diversity between seventy one samples of olive (*Olea europaea* L.) in Kermanshah province. Sixty four accessions were collected from Kermanshah province by Morphological, RAPD and ISSR markers. Morphological characters were compared to the molecular data obtained using RAPD and ISSR markers. Thirty-four RAPD primers and 8 ISSR primers amplified 412 loci from the total molecular data obtained using RAPD and ISSR markers and combined both markers include 7, 5 and 7 groups, respectively. The results of mantel’s test indicated significant correlation between grouping obtained by RAPD and ISSR markers respectively. The dendrograms based on UPGMA cluster analysis using Jaccard’s similarity index for RAPD, ISSR markers and combined both markers include 7, 5 and 7 groups, respectively. The results of mantel’s test indicated significant correlation between grouping obtained by RAPD and ISSR markers (r = 0.493) and also between morphological and molecular markers. The morphological and molecular data led to similar representations of the cultivar relationships. The results indicated not a relationship between genetic diversity and different geographical regions in Kermanshah Province. This shows that cultivar selection has occurred in different genetic pools and in different areas. The results of these analyses showed the existence of a genetic divergence between accessions and this diversity can be used in olive breeding programs. This study allowed us to analyze genetic diversity for further prospecting, to provide additional genetic information on the agronomic and quality characteristics of the olive varieties, and for introducing new olive accessions. Keywords: Genetic diversity, *Olea europaea* L., RAPD, ISSR, Kermanshah province.
Production of phytohormone auxin by rhizospheric cyanobacterium Leptolyngbya sp. MMG-1

PB12

Ahmed M1, Stol LF2, Hasanin S2
1Department of Microbiology and Molecular Genetics, University of the Punjab, Lahore-54590, Pakistan; 2Department of Marine Microbiology, Netherlands Institute of Ecology – KNAW, PO Box 140, 4400 AC Yerseke, The Netherlands

The genus Leptolyngbya is one of the most flourishing filamentous cyanobacterium in rice fields. Leptolyngbya sp. MMG-1 was isolated from the rhizosphere of rice plants. The strain was characterized morphologically by light microscopy and confocal laser scanning microscopy and later identified by ITS rDNA sequence analysis. The ability of this strain to synthesize the auxin like bioactive compound was demonstrated under various culturing conditions. Auxin was extracted from the culture of Leptolyngbya strain MMG-1 and its identity was confirmed as IAA (indoole-3-acetic acid) by thin layer chromatography (TLC) as well as by high performance liquid chromatography (HPLC) for flavonoid l-tryptophan was required for IAA biosynthesis. Highly significant correlation was recorded between the IAA secreted by the strain and the initial concentration of l-tryptophan in the medium as well as the incubation time. Leptolyngbya strain MMG-1 tends to accumulate more IAA than it released into the medium. The bioactivity of the secreted IAA was established by its effect on the formation of roots by Pisum sativum. There was a significant positive effect of the supernatant of cultures of MMG-1 on the number of lateral roots of G. inflata. hairy roots were induced by infecting stems and leaves of G. inflata with Agrobacterium rhizogenes ATCC 15834. Transformed roots were grown in half MS liquid medium without hormones. The optimization of growth and glycyrrhizin accumulation of G. inflata hairy root was studied. The maximum biomass of the hairy roots culture occurred on nine weeks of culture 0.29 ± 0.05 g/flask dry wt. Glycyrrhizin production reached the maximum level in the fourth week of culture (34.79 ± 4.11 g/g dry wt). Sucrose (6%, w/v) was optimum for growth and glycyrrhizin accumulation in G. inflata hairy roots (76.07 ± 6.82 g/g dry wt). Effects of elicitors like chitosan, methyl Jasmonate, and yeast extract on glycyrrhizin production in hairy root of G. inflata were studied. Methyl Jasmonate (100 μM) was the most efficient for enhancing the glycyrrhizin production up to 10.89 ± 1.15 g/g dry wt on day 5 of elicitation. The results from this investigation indicate that application of elicitors can enhance the capacity of G. inflata hairy roots to produce glycyrrhizin. Acknowledgement: Khon Kaen University, The Japan Society for the Promotion of Science (JSPS) References: 1. Rauchenstein R et al. (2005) J Pharmaceut. Biomed 38: 594 – 600. 2. Wongsiw, W et al. (2008) Z. Naturforsch 63C: 413 – 417.

In vitro regeneration and analysis of total phenolics in Ocimum basilicum L. (sweet basil)

PB13

Abant Izzet Baysal University, Department of Biology, 14280 Bolu, Turkey

An efficient in vitro regeneration system via direct and indirect shoot organogenesis was developed from cotyledonal leaf and hypocotyl explants of Ocimum basilicum L., commonly known as a sweet basil, belonging to the family Lamiaceae. Sweet basil is used in traditional medicine as a culinary herb and as a well known source of flavouring properties. Various types and concentrations (ranging from 0.1 to 3.0 mg/ml) of plant growth regulators in different combinations (TDZ+IAA, BAP+IAA, KIN+IAA, TDZ+NA) were tested using Murashige and Skoog medium. The highest number of shoots (1.3 shoots per explant) was obtained from hypocotyl explants on medium supplemented with the growth regulators 6-benzylaminopurine (BAP) and indole-3-acetic acid (IAA). For rooting, regenerated shoots were transferred to auxin-containing media. After the rooting stage, healthy regenerants were transferred to pots for acclimatization process, through which almost all of them grew vigorously. Attained maturity and produced fertile seeds. We also analyzed variations in patterns of total phenolics in the in vitro cultured callus, regenerated plantlets from callus as well as in leaves of ex vitro plants by UV-spectrophotometer. The phenolic contents of regenerated plantlets and leaves of ex vitro plants were found very similar but considerably higher than the callus. However, the greatest difference of phenolics content between callus and regenerated plantlets was observed when they were cultured on media containing combinations of BAP and IAA; callus produced 492.75 mg/g dry weight (dw) while regenerated plantlets yielded 1258.81 mg/g dw.

Enhanced glycyrrhizin production in Glycyrrhiza inflata hairy roots cultures using elicitation

PB14

Puttan W, Wongwicha W, Tanaka H, Shoyama Y
1Faculty of Pharmaceutical Sciences, Kohn Kaen University, Kohn Kaen, 40002, Thailand; 2Graduate School of Pharmaceutical Sciences, Kyushu University, Fukuoka 812 – 8582, Japan; 3Faculty of Pharmaceutical Sciences, Nagasaki International University, Nagasaki 859 – 3298, Japan

Glycyrrhiza inflata Batal (Leguminosae) has been used as a source of licorice. Licorice has a high market demand due to its high medicinal value, whereas the licorice resources in the world regions are limited [1]. In vitro culture of plant is an alternative source for the production of valuable secondary metabolites [2]. In the present study, we report the condition for optimizing cell growth and glycyrrhizin production and the influence of elicitors on glycyrrhizin accumulation in hairy roots cultures of G. inflata. hairy roots were induced by infecting stems and leaves of G. inflata with Agrobacterium rhizogenes ATCC 15834. Transformed roots were grown in half MS liquid medium without hormones. The optimization of growth and glycyrrhizin accumulation of G. inflata hairy root was studied. The maximum biomass of the hairy roots culture occurred on nine weeks of culture 0.29 ± 0.05 g/flask dry wt. Glycyrrhizin production reached the maximum level in the fourth week of culture (34.79 ± 4.11 g/g dry wt). Sucrose (6%, w/v) was optimum for growth and glycyrrhizin accumulation in G. inflata hairy roots (76.07 ± 6.82 g/g dry wt). Effects of elicitors like chitosan, methyl Jasmonate, and yeast extract on glycyrrhizin production in hairy root of G. inflata were studied. Methyl Jasmonate (100 μM) was the most efficient for enhancing the glycyrrhizin production up to 10.89 ± 1.15 g/g dry wt on day 5 of elicitation. The results from this investigation indicate that application of elicitors can enhance the capacity of G. inflata hairy roots to produce glycyrrhizin. Acknowledgement: Khon Kaen University, The Japan Society for the Promotion of Science (JSPS) References: 1. Rauchenstein R et al. (2005) J Pharmaceut. Biomed 38: 594 – 600. 2. Wongsiw, W et al. (2008) Z. Naturforsch 63C: 413 – 417.

Production of phytohormone auxin by rhizospheric cyanobacterium Leptolyngbya sp. MMG-1

PB12

In vitro regeneration and analysis of total phenolics in Ocimum basilicum L. (sweet basil)

PB13

Enhanced glycyrrhizin production in Glycyrrhiza inflata hairy roots cultures using elicitation

PB14

The challenges of Podophyllum tissue culture

PB16

Søhoel H1, Simonsen HR1, Steiner F, Verma SK, Sahbaz N, Sahin G, Yucesan B, Garel E
1Department of Plant Biology and Biotechnology, VKR Research Centre Pro-Active Plants, Faculty of Life Sciences, University of Copenhagen, Denmark; 2Dept. of Biological Sciences, University of Calgary, Canada

Terpenoids is the biggest group of secondary metabolites among plants. Their accumulation in species belonging to the Apiaceae and Asteraceae family is the reason why several of these plants possess biological activities that are used in the treatment of various diseases [1,2]. Thapsigargin's ability to induce apoptosis by inhibiting the endosomal calcium ATPase (SERCA) makes it a promising agent for the therapy of cancer. The development of a pro-drug targeted to prostate cancer cells allows its selective use [3]. Comparison with related sesquiterpenes for which biosynthetic enzymes have been identified enabled us to propose an enzymatic pathway by which thapsigargin could be generated from farnesyl diphosphate via several intermediates [1]. Large scale High Throughput Screening of expressed mRNAs from Thapsia species was undertaken to provide contig database of gene fragments, to date we have identified and cloned several genes of interest, which are undergoing characterization. Our first targets are two sesquiterpene synthases. Secondly, we have cloned 12 P450's in the CYP71 clade, which is believed to be involved in secondary metabolism. These are currently undergoing characterization in yeast. To optimize Physcomitrella as a production host for thapsigargin we aim at constitutively upregulate the expression of enzymes involved in the isopentenyl diphosphate (IPP) biosynthesis [4]. These metabolic modifications will increase the pool of the terpenoid precursor IPP that is available for sesquiterpene biosynthesis. We aim at establishing the moss Physcomitrella patents as the system of choice for the production of all kinds of terpenoids. Acknowledgements: We would like to thank The Danish Council for Strategic Research for their financial support. References: 1. Drew DP et al. (2009) Phytochem Rev 8: 581 – 599 2. Drew DP et al. (2011) Phytochem Anal In press 3. Saheol H et al. (2006) Biogro Med Chem 14: 2810 – 2815 4. Simonsen HR et al. (2009) Perspect Med Chem 3: 1 – 6

The challenges of Podophyllum tissue culture

PB16

Silve G, Davey MK, Power J
1School of Biosciences, University of Nottingham, Sutton Bonington Campus, Loughborough LE12 5RD, UK; Division of Ciências Farmacêuticas, Fundação Ezequiel Dias, Belo Horizonte, CEP 30510 – 010, MG, Brazil; 2School of Biosciences, University of Nottingham, Sutton Bonington Campus, Loughborough LE12 5RD, UK

Podophytoxins is obtained from the rhizomes and roots of wild populations of Podophyllum hexandrum Royce. This is a low-growing plant with a long juvenile phase, making the availability of this natural pro-

Spotlight: Sustainable Production of Thapsigargin using Light – turning moss into a terpenoid producer

PB15

Manczak T, Wetzel C, Klem AHF, Pan X, Ro D, Lunde C, Simonsen HR
1School of Biosciences, University of Nottingham, Sutton Bonington Campus, Loughborough LE12 5RD, UK; Division of Ciências Farmacêuticas, Fundação Ezequiel Dias, Belo Horizonte, CEP 30510 – 010, MG, Brazil; 2School of Biosciences, University of Nottingham, Sutton Bonington Campus, Loughborough LE12 5RD, UK

The challenges of Podophyllum tissue culture

PB16

The challenges of Podophyllum tissue culture

PB16
duct limited (1). Demand for podophyllotoxin was created with the introduction of its semi-synthetic derivatives in cancer chemotherapy (2). The species is endangered in the Himalayan region (3) through over collecting and lack of organized cultivation (4). Efforts remain to facilitate the in vitro propagation (5, 6) of Podophyllum. In the present investigation on the tissue culture of *P. hexandrum*, seeds germinated within 35 to 40 days in moist, dark conditions, with in vitro grown seedlings being obtained either on 0.2 normal strength semi-solid B5 medium (7) or full-strength MS medium both lacking growth regulators. Although callus induction from root explants cultured on 0.5 normal strength B5 medium containing 1.0 mg l⁻¹ 2,4-D, 1.0 mg l⁻¹ BAP and 1.0 mg l⁻¹ GA₃ was slow, tissue became embryogenic after successive subcultures. Embryogenic cell suspensions were established in the dark from root-derived callus, cultured in liquid MS medium containing 2,4-D and kinetin, at 2.0 mg l⁻¹ and 0.25 mg l⁻¹, respectively. Differentiation of somatic embryos and subsequent shoot formation occurred on either full-strength or half-strength MS medium with 0.45 mg l⁻¹ BAP. Rooting of somatic embryo-derived plants was stimulated by the inclusion of 10⁻⁵ M lipo-oligosaccharide in the culture medium. A robust explant-to-plant micropropagation system was able to reduce pressure on wild resources and may offer an alternative source of podophyllotoxin production. Acknowledgement: To RHAE/CNPq and Funded for their financial support to CGS, which is greatly appreciated. References: 1. Choudhary DK et al. (1990) Med Aromat Plant Sci 20: 1071 – 1073. 2. Farukya S et al. (2004) Appl Microbiol Biotechnol 65: 504 – 519. 3. Aini S et al. (1997) Plant Genet Resour News 110: 20 – 34. 4. Nadeem M et al. (2000) Biol Conserv 92: 121 – 129. 5. Chalraborty A et al. (2010) Indian J Biotechnol 9: 217 – 220. 6. Silva GC (2000) Ph. D. Thesis. University of Nottingham, Nottingham, UK. 7. Heyenga AG et al. (1990) Plant Cell Rep 9: 382 – 385.
and composition of phenolics compounds as well as their antioxidant activity of chickpea (Cicer arietinum L.) were examined. The chickpea seed were milled and the plant extracts were prepared by using 80% (v/v) ethanol. The total phenolics compounds content was determined based on a standard curve with chlorogenic acid concentrations covering the range from 50 to 1000 μmol and the antioxidant activity was determined by using DPPH radical scavenging capacity [3]. The composition of phenolics compounds was determined by HPLC analyses on an Agilent 1100 Series HPLC system. Agilent Eclipse XDB-C18 column and spectrophotometric detection in the UV region at 350 nm was used [4]. The phenolics compounds content was 8.33 μmol of chlorogenic acid per g of dried extract residue i.e. 0.33 μmol of chlorogenic acid per g of milled chickpea seed. Results of antioxidant capacity of investigated phenolic extracts showed the maximum DPPH radical scavenging capacity was 3% at extract's concentration of 10.9 mg/ml and the extract's concentration sufficient to obtain 50% of maximum scavenging capacity was 2.9 mg/ml. By using the HPLC analysis, chlorogenic acid (4.26%), hydroxycinnamic acid “C 1” (10.86%), 5-O-cafeolylshikimic acid (5.21%), kaempferol 3-O-7-O-diglucoside (7.7%), kaempferol 3-O-rhamnoside (33.52%), kaempferol 7-O-rhamnose (7.66%) and genkwanin 4-O-glucoside (4.59%) of phenolics compounds were found. In total, the content of kaempferol glucosides was the biggest (48.96%). Acknowledgement: This work was supported under the projects No.01I 172/047 by the Ministry of Science of the Republic of Serbia.

References:

Effects of cAMP modulators on flavonoid accumulation in cell cultures of Hypericum androsaemum L. Paranhos A Faculdade de Farmácia and Centro de Estudos Farmacêuticos, Universidade de Coimbra, Azenhaga de Santa Comba, 3000 – 548 Coimbra, Portugal

Hypericum androsaemum L. has been used in traditional medicine for its diuretic and hepatoprotective properties [1], which are attributed to the diverse flavonoids and phenolic acids found in this species. Cell suspension cultures established from hypocotyl-derived calli of H. androsaemum were reported [2] to accumulate low amounts of flavonoids, with maximum levels occurring on the 14th day of the growth cycle. More recently [3], it was shown that treatment of 11-day-old cultures for 72 h with 15 mM CaCl2 or 5 mM calcium ionophore A23187 increased considerably the accumulation of flavonoids and the activity of phenylalanine ammonia-lyase (PAL, a key regulatory enzyme of phenylpropanoid metabolism). Since adenyl cyclases can be regulated by Ca2+ [4], similar experiments were carried out in this study using three different modulators of intracellular cAMP: dibutyryl-cAMP (100 μM), a membrane permeable cAMP analogue, IBMX (100 μM, a cAMP phosphodiesterase inhibitor) and forskolin (20 μM, an adenyl cyclase activator). The first two treatments induced a marked increase in both PAL activity and flavonoid content of cells, as compared to control cultures. Increased levels of flavonoids were also found in forskolin-treated cells, but in this case accompanied by an insignificant rise in PAL activity. Considered together, these findings are in agreement with the involvement of cAMP signaling in flavonoid metabolism of H. androsaemum cell cultures. Acknowledgement: FCT and POCTI-FEDER for financial support. References: 1. Novais M et al. (2004) J Ethnopharmacol 93: 183 – 195. 2. Paranhos A (2006) Planta Med 72: 1060 – 1061. 3. Paranhos A (2007) Planta Med 73: 1017.

Towards the sustainable and continuous in-vitro production of active pharmaceutical ingredients from medicinal plants

Michoux F, Nixon PJ


Medicinal plants have been used for the past millennium to treat various conditions, especially in the oncology market. Despite an increasing interest in the translation of traditional knowledge of medicinal plants into clinical drugs, progress has been quite slow since the discovery of Paclitaxel and Camptothecin in the 1970’s. One aspect which could explain the limited number of complex molecules entering clinical trials and reaching the patient is the restricted supply chain of the plant raw material, thus limiting the availability of the Active Pharmaceutical Ingredients (API). Still today, most of the raw materials needed for the extraction of the active ingredients are harvested from cultivated or wild plant populations, posing a threat to the bioavailability of certain medicinal plants and strong variability in the yield of API. We have developed a new in-vitro propagation method based on the use of temporary immersion bioreactors that allows for the rapid and abundant generation of a leafy-biomass from transgenic plants (Michoux et al., 2010). This technology is now being applied to medicinal plants and the results will be discussed. This technology provides a unique opportunity for the sustainable production of complex APIs which require plant cell differentiation. References: Michoux F, Ahmad N, McCarthy J and Nixon PJ (2010) Plant Biotechnology Journal Online Nov 24.

Hairy roots induction in Nepeta crispa (Lamiaceae) from Iran by using Agrobacterium rhizogenes

Habibi P, Piri K, Ostadahmadi P, Ahmadi Moghadam Y Department of Faculty of Agriculture Bu-Ali Sina University, Hamedan, Iran

Nepeta crispa Wild. (Lamiaceae) is an aromatic endemc plant of Iran. This plant with the common local name Mofarah has been of great interest to Iranian traditional medicine. Infusion and beverage obtained from the aerial parts of N. crispa were used traditionally as sedative, relaxing, carminative, restorative tonic for nervous and respiratory tract disorders. N. crispa has antimicrobial activity against bacteria and fungi. The main constituents were 18-cineole (47.9%), 4-α-cis,7α-cis-epi-nepetalactone (20.3%), α-pinene (5%) and β-terpinene (4.1%). Agrobacterium rhizogenes causes hairy root disease in plants. The neoplastic (cancerous) roots produced by A. rhizogenes infection are characterized by high growth rate, genetic stability and growth in hormone free media. Hairy root cultures offer promise for high production and productivity of valuable secondary metabolites (used as pharmaceuticals, pigments and flavors) in many plants. Hairy roots were induced from cotyledon explants excised prototrophic aseptically grown seedlings of N.crisspa using Agrobacterium rhizogenes 15834. The cefotaxime concentration of 250 mg/l was found to be most suitable for hairy root induction in N.crispa. To confirm transformation, PCR analysis was performed by using of specific primers for rol B gene amplification. Results of PCR analysis showed the presence of diagnostic 780 bp rol B product amplification and thus confirmed the transformation of hairy roots. This is the first report on the induction of hairy roots in N.crispa. References: 1. Sonboli A, Salehi P, Yousefzadi M (2004) J Naturforsch 59: 653 – 656. 2. Jamzad Z, Grayor R-J, Kite G-C, Simmonds M-S-J., Ingrouille M, and Jalili A (2003) Biotechnol Biofuels Syst Ecol 31: 807 – 800. 3. Mojab F, Nickavav A, Tehrani H H (2009) JIPS S(1): 43 – 46.

Hairy roots induction in purslane (Portulaca oleracea Linn.) using Agrobacterium rhizogenes

Ahmadi Moghadam Y, Piri K, Bahrammnejad B, Habibi P

1Department of Biotechnology – Faculty of Agriculture – Bu-Ali Sina University, Hamedan, Iran; 2Department of Biotechnology Faculty of Agriculture – Kurdistan University, Sanandaj, Iran

Portulaca oleracea Linn. is a medicinal plant found in Europe and Asia. P. oleracea has a variety of pharmacological activity, including analgesic, anti-inflammatory, anti-fungal, wound healing, and hypoglycemic. This plant contains a variety of bioconstituents, including catecholamine, 1-noradrenaline, dopamine, 1-dopa, α-aminergic, β-aminergic, and portculoside A. The development of genetically transformed by Agrobacterium rhizogenes (hairy roots), is a key step in the use of in vitro culture for the production of secondary metabolites. In our research hairy roots system were induced from cotyledon explants excised in seven day old aseptically grown seedling of P. oleracea using Agrobacterium rhizogenes 15834 strain. The cotyledon segment was soak in the bacterial suspension for infection. After 2 day of co-cultivation at 24 °C in the dark, the explants were transferred onto 1/2 MS medium containing 300 mg/l cefotaxime to remove Agrobacterium rhizogenes. Hairy roots for further proliferation were transferred to liquid 1/2 MS medium and maintained in 16 h light/ 8 h dark photo period at 24 °C on orbital shaker (110 rpm). To confirm transformation, PCR analysis was performed by using of specific primers for rol B gene amplification. Results of PCR analysis showed the presence

Planta Med 2011; 77: 1229–1472 Georg Thieme Verlag KG Stuttgart · New York · ISSN 0032-0943
The production and accumulation of secondary metabolites in plants is always regulated by the expression of genes involved in their biosynthesis. There are very few reports about the regulation of the biosynthesis of the anticycnic agent taxol and other related taxanes and the rate-limiting steps involved, especially during the development of Taxus plants. Using Taxus baccata L. plantlets grown in vitro for 1 year, our group has studied the relationship between the profile and production of taxanes and the expression of genes codifying for enzymes that participate in early and late steps of taxane biosynthesis ([TXS, DBAT, BAPT and DRTX]). At higher taxane concentration and in the aerial part of the plantlets than in the roots, 10-deacetylbaccatin III being the most abundant taxane, with very low conversion to baccatin III and taxol. The RNA accumulation of the studied genes was also higher in the aerial part than in the corresponding roots. Our results indicate that the low taxane levels in the roots could reflect the low transcript accumulation of the aforementioned genes in this part of the plant, although an active metabolism or translocation of taxanes to the aerial part could also be responsible. The high content of 10-deacetylbaccatin III and very low levels of baccatin III, together with the low mRNA accumulation of DBAT in the aerial part, suggest that this gene could control a limiting step in the taxane biosynthetic pathway in T. baccata plantlets grown for 1 year in in vitro conditions.

**PB27**

In vitro propagation and total alkaloid evaluation of Catharanthus roseus L.

Malekzadeh M1, Mirmazlooom F1, Babaei A1, Omidbaigi R2

1Department of Horticulture, College of Agriculture, Tarbiat Modares University, Tehran, Iran; 2Cytomix Lab, Department of Budapest, Department of Medicinal and Aromatic Plants, Budapest, Hungary

The Madagascar periwinkle (Catharanthus roseus L.) is an important medicinal plant from the family Apocynaceae produces over ninety terpenoid indole alkaloids. Among its alkaloids, ajmalicine and serpentine are used in the treatment of hypertension and vircrinidine and vinblatinine applied in cancer chemotherapy. The aim of this study was to evaluate the effect of various hormone treatments on callus growth and regeneration during tissue culture and to study the total alkaloid content of callus. The explants were sterilized and cultured in the MS media consisting of different concentrations and combinations of hormones. Traits such as fresh callus weight, color, vitrification and the quality of tissue callus were considered. The results indicated that comparisons were significantly different at 1% probability level. The comparison of callus weight in various hormones levels indicated that the 14.42 mg/l concentration of 6-Benzyladenine (BAP) combined with 3 mg/l concentration of 1-Naphthalene Acetic Acid (NAA) resulted highest callus weight. The combination of 1 mg/l of NAA and 1 ml/l of BAP in foliar callus and NAA (2 mg/l) with BAP (8 mg/l) in single lateral buds, gave the highest number of plantlet regeneration. The highest amount of alkaloids in foliar callus was obtained when 1.5 mg/l of BAP and 0.25 mg/l of 2, 4-Dichlorophenoxy acetic acid (2, 4-D) were employed. The highest amount of alkaloids was produced using NAA (3 mg/l) and BAP (14.42 mg/l). The results of the present study emphasized the potential of production of periwinkle active compounds through in vitro cultivation.

**PB28**

Characterization and in vitro evaluation of a new chitosan-based propolis tooth varnish

Santos VB1, De Luca MP1, Macedo PA2, Cortés ME2, Moreira AN3, Franca JR4, Funaco AA1

1Department of Clinical Pathology and Surgery, Faculty of Dentistry, Universidade Federal de Minas Gerais (UFMG), Belo Horizonte, Minas Gerais, Brazil; 2Department of Restauratrice Dentistry, Faculty of Dentistry, Universidade Federal de Minas Gerais; 3Department of Pharmaceutical Technology, Faculty of Pharmacy, Universidade Federal de Minas Gerais

Propolis has been recently studied due to its cariostatic activity. Although its activity has been exhaustively demonstrated, there is no formulation commercially available using this agent in tooth care. Chitosan has been extensively studied due its film-forming properties. Recent studies had also shown that chitosan can adhere on tooth surface and has an effective inhibition effect on the initial adherence of oral bacteria onto human tooth surface. Chitosan-based propolis varnish was successfully developed and characterized by ATR-FTIR spectroscopy, SEM, hydration potential, casting time and mucoadhesive properties. The formulation presented good tooth surface adherence and ability to form films very fast on tooth surface when compressed air was used as casting agent. Also, the varnish presented interesting hydration potential (Figure 2), which suggests that the formulation will not be easily removed from tooth surface by saliva. Propolis varnish has, also, shown antimicrobial activity against Streptococcus mutans (MD 8.67±0.52) e Streptococcus sanguinis (MD 11.70±2.11). Its cytotoxicity was made by direct contact with osteoblasts and evaluated by the MTT method. After 24 hours, the varnish reduced 20% of the cells, showing low toxicity (ISO 10993 – 5). The results were analyzed statistically by ANOVA in a significance of p < 0.05. Acknowledgement: The authors thank for the financial support to Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Coordenação de Aperfeiçoamento de Pessoal de Nível
Cis-jasmone, well known as a component of plant volatiles, is produced also by damaged plant vegetative tissues [1]. This natural ketone is considered to be the final product in the jasmonic acid biosynthetic pathway from linolenic acid [2]. This pale yellow, viscous liquid compound possesses strong jasmine fragrance and interesting biological activities. It is an activator of chemical defence in plants, causing the release of volatile semiochemicals e.g. bean plants, Vicia faba, treated with cis-jasmone showed a significant increase in the production of (E)-ocimene [3]. Cis-jasmone is also responsible for plant-insect interactions. The population of grain aphid Sitobion avenae (Fabricius) is reduced by cis-jasmone which plays a role of repellent [4, 5] while members of two families of insect parasitoids (Braconidae and Sarcophagidae) in hop (Humulus lupulus L.) cultivation are attracted by cis-jasmone [6]. In our study we focused on the biotransformations of cis-jasmone by fungal cultures: Penicillium, Absidia, Syncephalastrum, Botrytis, Aspergillus, Cun

cultures: 1


Fig. 1


PLANT BIOTRANSFORMATION OF CIS-JASMONE BY FUNGAL CULTURES

Giszczyńska AM, Górecka M

Department of Chemistry, Wrocław University of Environment and Life Sciences, Wrocław, Poland

Biotransformation of cis-jasmone by fungal strains

Fig. 1

Fig. 2


PLANT BIOTRANSFORMATION OF CIS-JASMONE BY FUNGAL CULTURES

Giszczyńska AM, Górecka M

Department of Chemistry, Wrocław University of Environment and Life Sciences, Wrocław, Poland

Biotransformation of cis-jasmone by fungal strains

Fig. 1

Fig. 2


PLANT BIOTRANSFORMATION OF CIS-JASMONE BY FUNGAL CULTURES

Giszczyńska AM, Górecka M

Department of Chemistry, Wrocław University of Environment and Life Sciences, Wrocław, Poland

Biotransformation of cis-jasmone by fungal strains

Fig. 1

Fig. 2


PLANT BIOTRANSFORMATION OF CIS-JASMONE BY FUNGAL CULTURES

Giszczyńska AM, Górecka M

Department of Chemistry, Wrocław University of Environment and Life Sciences, Wrocław, Poland

Biotransformation of cis-jasmone by fungal strains

Fig. 1

Fig. 2


PLANT BIOTRANSFORMATION OF CIS-JASMONE BY FUNGAL CULTURES

Giszczyńska AM, Górecka M

Department of Chemistry, Wrocław University of Environment and Life Sciences, Wrocław, Poland

Biotransformation of cis-jasmone by fungal strains

Fig. 1

Fig. 2


PLANT BIOTRANSFORMATION OF CIS-JASMONE BY FUNGAL CULTURES

Giszczyńska AM, Górecka M

Department of Chemistry, Wrocław University of Environment and Life Sciences, Wrocław, Poland

Biotransformation of cis-jasmone by fungal strains

Fig. 1

Fig. 2


PLANT BIOTRANSFORMATION OF CIS-JASMONE BY FUNGAL CULTURES

Giszczyńska AM, Górecka M

Department of Chemistry, Wrocław University of Environment and Life Sciences, Wrocław, Poland

Biotransformation of cis-jasmone by fungal strains

Fig. 1
Soybean (*Glycine max* (L.) Merr.) is an important source of vegetable oil and high protein. Use of soybean meal by the food industry is increasing, but severely limiting dietary choices and the quality of life of food-allergic individuals. Gly m Bd 30K (P34) is known as the main seed allergen in soybean-sensitive patients. The objective of this work was to determine the molecular basis of the low mutation of soybean P34 and to design molecular marker for the selection of the causative mutations for wild homozygous, heterozygous and mutant homozygous. We developed a co-dominant marker based on the sequence of *Glycma08g12270* containing a four-base pair insertion at the P34 start codon. Also, we made a polyclonal antibody for investigation of P34 protein levels. Using a co-dominant marker and a polyclonal antibody, polymorphism and amount of protein for Glycma08g12270 were analyzed in F2 and F3 generation crossing PI 567476 and Hwanggumkong, Korean cultivar. To investigate the association of the P34 genotype with the P34 protein phenotype, segregating populations in F2 and F3 generation crossing PI 567476 and Hwanggumkong, Korean cultivar. To investigate the association of the P34 genotype with the P34 protein phenotype, segregating populations in F2 were developed from crossed Hwanggum and PI567476. For the 258 samples analyzed, the ratio of homozygous wild-type: heterozygous: mutant homozygous P34 genotypes was 34:94:30 = 1:2:1 (test for goodness of fit by X2 analysis). As results, the polymorphism analysis was accustomed to a difference of protein level of wild homozygous, heterozygous and mutant homozygous. References: Bilyeu K et al. (2009) The plant Genome 2: 141 – 148 Joseph LM et al. (2006) Crop Sci 46: 1755 – 1763 Herman E M et al. (2005) Plant Physiology 132: 36 – 43

**Effect of taxine B feeding on taxol production and cell viability in Taxus suspension cultures**

**PB33**

Kwang Ho 1, Man Soo C 1, Suk Ki L 1, Min Jung S 1, Yul Ho K 1, Hong Sig K 1

1National Institute of Crop Science, Rural development Administration, Suwon 411 – 857, Korea; 2Dept. of Crop Science, Chungbuk National University, Cheongju 361 – 763, Korea

After several years, still the affair of paclitaxel (Taxol) and its analogs is an evergreen tree with very large simple leaves, attains a height of about 30 m, belonging to the family Anacardiaceae is a critically endangered swamp tree and consists of major chemical compounds like phenols, biflavonoids and traditionally having high medicinal importance being used as an antimicrobial, antioxidant and anticancer. Hence considering its population, it is an endangered species and endemic to western ghats region of shimoga district in India. The tissue culture of different plant parts was carried out on MS medium using different concentration of plant growth regulators in the culture tubes and the explants were incubated at 25 ± 2 C under 48 hrs photoperiod. Due its high phenolic content callus initiation was not occurred further isolation and identification of endophytic fungi from Semecarpus kathalekanensis plant was performed. References: 1. Dasappa & Swaminath MH (2000) Indian For: 126: 78 – 82 2.Vasudeva R, Raghub HR, Dasappa, Uma Shaanker R & Ganeshiah KN (2001) Population structure, reproductive biology and conservation of Semecarpus kathalekanensis: A critically endangered freshwater swamp tree species of the Western Ghats. In Forest Genetic Resources: Status, Threats and conservation Strategies (Eds Uma Shaanker, R., Ganeshiah, K.N., and Bawa, K.S.) 211 – 223 (Oxford & IBH, New Delhi.), 3.Murashige T, Skoog F (1962) Physiol plant 15:473 – 9.

**PB34**

Tissue culture studies on *Semecarpus kathalekanensis* an endangered medicinal plant

Harikode PJ 1, Parvathiti MK 1, put SR 1

1Pramod H J, Department of Pharmaceutical Biotechnology, KLE University College of Pharmacy, Nehru Nagar, Bengaluru-590 010, Karnataka, India; 2Manjunath K P, Department of Pharmaceutical Biotechnology, KLE University College of Pharmacy, Nehru Nagar, Bengaluru-590 010, Karnataka, India; 3Sandeep R P, Regional Medical Research Centre, ICMMR, Bengaluru-Belgaum-590 010, Karnataka, India

*Semecarpus kathalekanensis* Dasappa & M.H. Swaminath is an evergreen tree with very large simple leaves, attains a height of about 30 m, belonging to the family Anacardiaceae is a critically endangered swamp tree and consists of major chemical compounds like phenols, biflavonoids and traditionally having high medicinal importance being used as an antimicrobial, antioxidant and anticancer. Hence considering its population, it is an endangered species and endemic to western ghat region of shimoga district in India. The tissue culture of different plant parts was carried out on MS medium using different concentration of plant growth regulators in the culture tubes and the explants were incubated at 25 ± 2 C under 48 hrs photoperiod. Due its high phenolic content callus initiation was not occurred further isolation and identification of endophytic fungi from *Semecarpus kathalekanensis* plant was performed. References: 1. Dasappa & Swaminath MH (2000) Indian For: 126: 78 – 82 2.Vasudeva R, Raghub HR, Dasappa, Uma Shaanker R & Ganeshiah KN (2001) Population structure, reproductive biology and conservation of *Semecarpus kathalekanensis*: A critically endangered freshwater swamp tree species of the Western Ghats. In Forest Genetic Resources: Status, Threats and conservation Strategies (Eds Uma Shaanker, R., Ganeshiah, K.N., and Bawa, K.S.) 211 – 223 (Oxford & IBH, New Delhi.), 3.Murashige T, Skoog F (1962) Physiol plant 15:473 – 9.

**PB35**

Metabolic engineering: an effective approach for optimal production of secondary metabolite compounds

Ahadi Dolatshara E 1, Salami S 1, Shoerpoour M 1, Naghavi M 2

1Department of Plant Sciences, Faculty of Agriculture, University of Tehran, Karaj, Iran; 2Department of Agronomy and Plant Breeding, Faculty of Agriculture, University of Tehran, Karaj, Iran

Plants have a limited capacity to produce secondary metabolites in natural environmental conditions; however, recent developments in genetic engineering and recombinant DNA technology have had a great impact on their production. Metabolic engineering is considered as an efficient tool towards achieving higher level of the secondary metabolites. Globally, metabolite engineering is widely used for increasing the content of secondary metabolites or even producing novel medicinal compounds. Iran has a high level of genetic and phytochemical variability for different types of medicinal plants. Knowing the biochemical pathways and manipulating these native plants improve their commercial production. Consequently, the research group has started large-scale experiments on native medicinal plants such as different species of genus Artemisia, Cannabis, Papaver, and Salvia. We aim to increase and/or decrease and change metabolites by using anti-sense and RNAi technologies, isolation and transformation of related genes, promoter analysis, and changes in regulatory gene expression. Such studies would definitely provide a great chance to improve the production of secondary metabolites in these plants and to better understand the novel genes involve in bio-synthetic pathways.

**PB36**

Biotransformation of trans-farnesol by *Ceriporiopsis subvermispora* in solid-state fermentation

Lee S, Kim S, Choi I

Department of Forest Sciences, College of Agriculture & Life Sciences, Seoul National University, Seoul, South Korea

Terpenoids which are included in various plants are important for the food, animal feed, cosmetics and pharmaceuticals industries greatly. However they are expensive due to low concentration and difficult isolation. Therefore biotransformation of terpenoids could be an alternative way to produce them. In this study, whole cell of *Ceriporiopsis subvermispora* was used in biotransformation as biocatalyst. *C. subvermispora* is a naturally occurring fungus able to remove phenolic compounds than other compounds. Farnesol is a sesquiterpene which is an acyclic ses-
quiterpene alcohol derivative of farnesol Pyrophosphate and the building block of most acyclic sesquiterpenoids is an important starting compound for organic synthesis. Therefore it has been chosen as starting material for biotransformation. The object of this study was to investigate the microbial biotransformation of trans-farnesol by C. subvermispora at different culture condition in order to obtain the more valuable sesqui-terpenoid structures. Biotransformation was started after inoculation by added 0.005 g M-1 of homogenized strains into the 100 ml SSC medium culture flasks. After 48 hours, substrate 200ul was added in the 250 ml flask with 0.1% Tween 80. Per 24h, one flask were stopped for cultivation and extracted with solvents at 250 rpm, 3 times using the shaker. The final solutions were subjected to gas chromatography FID spectrometry (GC-FID) and GC-MS. After 10 days, when used the C. subvermispora as the bio-catalyst, novel compounds produced in SSC medium. These products were nerolidol, farnesol, β-farnesol, bisabolene, eudesm-7(11)-en-4α-ol. References: 1. De Carvalho C and da Fonseca M (2006) Biotechnology Advances 24(2): 134 – 142. 2. Asther M (2006) Biotechnology Advances 24(2): 134 – 142. 2. Ashter M, Fonseca M, and Glombitza C. subvermispora and its metabolic interactions with their hosts. The APEF-12,13 dimethyl triterpenic acid for organic synthesis. Therefore it has been chosen as start-

development material for biotransformation. The object of this study was to investigate the microbial biotransformation of trans-farnesol by C. subvermispora at different culture condition in order to obtain the more valuable sesqui-terpenoid structures. Biotransformation was started after inoculation by added 0.005 g M-1 of homogenized strains into the 100 ml SSC medium culture flasks. After 48 hours, substrate 200ul was added in the 250 ml flask with 0.1% Tween 80. Per 24h, one flask were stopped for cultivation and extracted with solvents at 250 rpm, 3 times using the shaker. The final solutions were subjected to gas chromatography FID spectrometry (GC-FID) and GC-MS. After 10 days, when used the C. subvermispora as the bio-catalyst, novel compounds produced in SSC medium. These products were nerolidol, farnesol, β-farnesol, bisabolene, eudesm-7(11)-en-4α-ol. References: 1. De Carvalho C and da Fonseca M (2006) Biotechnology Advances 24(2): 134 – 142. 2. Asther M, Fonseca M, and Glombitza.

Hydrocucurbitus pantendra Buch-Ham (Flacourticeae) an endangered spe-
cies from Western Ghats region of India has been exploited traditionally against leprosy, rheumatic pain and inflammation. The callus cultures were established from leaf explants on MS media supplemented with sucrose and varying amounts of auxins and cytokinins. The callus initiation was observed with 2,4-D and NAA followed by incubation at 25 °C under photoperiod of 16 hrs.

Hydrocucurbitus pantendra Buch-Ham (Flacourticeae) an endangered spe-
cies from Western Ghats region of India has been exploited traditionally against leprosy, rheumatic pain and inflammation. The callus cultures were established from leaf explants on MS media supplemented with sucrose and varying amounts of auxins and cytokinins. The callus initiation was observed with 2,4-D and NAA followed by incubation at 25 °C under photoperiod of 16 hrs.

Hydrocucurbitus pantendra Buch-Ham (Flacourticeae) an endangered spe-
cies from Western Ghats region of India has been exploited traditionally against leprosy, rheumatic pain and inflammation. The callus cultures were established from leaf explants on MS media supplemented with sucrose and varying amounts of auxins and cytokinins. The callus initiation was observed with 2,4-D and NAA followed by incubation at 25 °C under photoperiod of 16 hrs.

In vitro regeneration studies on Hydrocucurbitus pantendra

Hydrocucurbitus pantendra Buch-Ham (Flacourticeae) an endangered spe-
cies from Western Ghats region of India has been exploited traditionally against leprosy, rheumatic pain and inflammation. The callus cultures were established from leaf explants on MS media supplemented with sucrose and varying amounts of auxins and cytokinins. The callus initiation was observed with 2,4-D and NAA followed by incubation at 25 °C under photoperiod of 16 hrs.

Hydrocucurbitus pantendra Buch-Ham (Flacourticeae) an endangered spe-
cies from Western Ghats region of India has been exploited traditionally against leprosy, rheumatic pain and inflammation. The callus cultures were established from leaf explants on MS media supplemented with sucrose and varying amounts of auxins and cytokinins. The callus initiation was observed with 2,4-D and NAA followed by incubation at 25 °C under photoperiod of 16 hrs.

Hydrocucurbitus pantendra Buch-Ham (Flacourticeae) an endangered spe-
cies from Western Ghats region of India has been exploited traditionally against leprosy, rheumatic pain and inflammation. The callus cultures were established from leaf explants on MS media supplemented with sucrose and varying amounts of auxins and cytokinins. The callus initiation was observed with 2,4-D and NAA followed by incubation at 25 °C under photoperiod of 16 hrs.
In this study, effect of Methyl jasmonate as a chemical elicitor on the secondary metabolites and essential oils of Calendula officinalis L. shoot were evaluated. The plants were grown in hydroponic conditions in Hogland nutrient solution and were treated with 50 and 100 𝜇M methyl jasmonate. The results showed that lignin content were increased significantly in treated plants. In comparison, the content of total wall-associated phenolic compounds and anthocyanins and flavonoid of shoots of treated plants decreased. However, there was no significant difference in membrane lipid peroxidation rate of treated plants and control plants. In the essential oils of C. officinalis shoots, α-Cadinol was the major constituent. A sesquiterpene compound, α-Murolene, with anti fungal properties was induced in those plants treated with methyl jasmonate, therefore, this chemical elicitor can be suggested for inducing changes in isoprenoid biosynthesis pathway and special phytoalexin production. References: 1. Yukinumas T, Tabata H, Higashi Y, Hara Y (1996) Nat Biotechnol 14: 1129 – 1132. 2. Kim DG, Kim YJ, Lee SH, Lee al. (2010) Org Lett 12: 4252 – 4255. 3. Gazim ZC, Rezende CM, Fraga SR, et al. (2008) Planta Med 2011; 77: 1229–1472 Georg Thieme Verlag KG Stuttgart · New York · ISSN 0032-0943

**PB42**

Induction of changes in secondary metabolites and essential oils of Calendula officinalis L. shoot were evaluated. The plants were grown in hydroponic conditions in Hogland nutrient solution and were treated with 50 and 100 𝜇M methyl jasmonate. The results showed that lignin content were increased significantly in treated plants. In comparison, the content of total wall-associated phenolic compounds and anthocyanins and flavonoid of shoots of treated plants decreased. However, there was no significant difference in membrane lipid peroxidation rate of treated plants and control plants. In the essential oils of C. officinalis shoots, α-Cadinol was the major constituent. A sesquiterpene compound, α-Murolene, with anti fungal properties was induced in those plants treated with methyl jasmonate, therefore, this chemical elicitor can be suggested for inducing changes in isoprenoid biosynthesis pathway and special phytoalexin production. References: 1. Yukinumas T, Tabata H, Higashi Y, Hara Y (1996) Nat Biotechnol 14: 1129 – 1132. 2. Kim DG, Kim YJ, Lee SH, Lee al. (2010) Org Lett 12: 4252 – 4255. 3. Gazim ZC, Rezende CM, Fraga SR, et al. (2008) Planta Med 2011; 77: 1229–1472 Georg Thieme Verlag KG Stuttgart · New York · ISSN 0032-0943

**PB43**

Polyphenols and their antioxidant activity in callus-cultured Malva neglecta cells under UV-B and UV-C irradiation

**PB44**

Antioxidant capacity of phenolic phytochemicals from peel of apples, pears, plums, red and white grapes

**PB45**

Secondary Metabolites from Phomopsis amygdali, an Endophytic Fungus Isolated from Hazelnut (Corylus avellana)

Endophytes are microbial entities that live within living tissues of plants. In most cases their relationship with the host plant is symbiotic and probably mutualistic. Many are capable of synthesizing bioactive compounds that have been proven useful for novel drug discovery. The early literature reports that species of Phomopsis isolated from plants produce different bioactive metabolites. The main aim of the study was to isolate endophytes from different parts of hazelnut, to extract bioactive secondary metabolites and then to elucidate their structures. Different plant materials including the roots, branches and leaves were collected from BlackSea region of Turkey and surface sterilized with 3% sodium hypochlorite (NaOCl). The outer layers removed with a sharp, sterilized blade and cut into pieces. Small pieces of the inner tissue were placed on the surface of potato/dextrose/agar (PDA) medium and incubated at 28°C. Subsequently 7 fungal species was isolated and grown in 1 L flask containing 250 ml of Multi Extract Broth medium and cultured at 150 rpm at 28°C for 21 days in a rotary shaker. Then the fermentation broths were extracted with chloroform. The chloroform extracts were screened for their cytotoxic activities by MTT method. Based on the activity results, the isolate L1, identified as Phomopsis amygdali, was
selected for further studies. After large scale fermentation and purification studies, a new metabolite (L1F3) together with a known compound, named (-)pestalotin (L1F2) were obtained. The structure of the new metabolite was elucidated as (R)-4-butoxy-6-((S)-1-hydroxybutyl)-5,6-dihydro-2H-pyran-2-one by the extensive use of 1D- and 2D NMR.

Figure 1


PB46

Biotechnological process for obtaining bioaromas from leaves of cashew apple trees De Araújo YL, Narain N, Aquino LC, Da Silva MA, Galvão MS, Leal AJ

Laboratory of Chromatographic Analysis and Flavor, Federal University of Sergipe, São Cristóvão Brazil

The cashew tree (Anacardium occidentale L.) is native to mainland Central and South America belonging to the family Anacardiaceae. The cashew apple fruit and nut are widely used and appreciated for its flavor quality. The aim of this work was to obtain bioaromas produced from the fermentation of the leaves of the cashew tree. Fermentations were performed at room temperature for 48 hours in three conditions: natural (leaves only), leaves in sterile distilled water and leaves in a solution containing 10% glucose. Every 24 hours of processing, sensorial (by a panel of five trained judges), microbiological (total mesophilic bacteria and fungi) and chromatographic (extraction by dynamic headspace method) analysis was performed on fermented products. Sensorial analysis revealed the presence of sweet fruity aromas characterizing citrus and green odor notes in all samples. The citric note of aroma was more prominent in all three types of fermentation without significant difference between them. However, the aroma was less intense in the fermentations realized with green leaf alone and it was significantly different (p < 0.05) between the other fermentations. Furthermore the production of bioaromas generated after 24 hours led to an increase of total bacteria and fungi to the order of 102 CFU/mL. A large number of volatile compounds belonging to esters, terpenes, ketones and aldehyde classes were identified in the fermented products. Sensorial analysis did not reveal a significant difference between them. However, the aroma was less intense in the fermentations realized with green leaf alone and it was significantly different (p < 0.05) between the other fermentations. Furthermore the production of bioaromas generated after 24 hours led to an increase of total bacteria and fungi to the order of 102 CFU/mL. A large number of volatile compounds belonging to esters, terpenes, ketones and aldehyde classes were identified in the fermented products. Sensorial analysis did not reveal a significant difference between them. However, the aroma was less intense in the fermentations realized with green leaf alone and it was significantly different (p < 0.05) between the other fermentations. Furthermore the production of bioaromas generated after 24 hours led to an increase of total bacteria and fungi to the order of 102 CFU/mL. A large number of volatile compounds belonging to esters, terpenes, ketones and aldehyde classes were identified in the fermented products.

Acknowledgement: We thank the INCIT/CNPq (National Council for the Development of Science & Technology, Brazil for the financial support received for the research on cashew leaves). The work was supported by funds received from the Institute of Medicinal Plants, Karaj, Iran.

PB47

Assessment of somaclonal variation in Durocenia anethifolia plants regenerated from long-term callus cultures using AFLP Shoshtari L1, Omidi M2, Majidi E3, Mohamadreza N2, Shamsali R4, Etminan A3, Omidbaigi R5

1Department of Horticulture, Tarbiat Modares University, Tehran, Iran; 2Department of Plant Biotechnology, Tarbiat Modares University, Tehran, Iran; 3Department of Biology, Islamic Azad University, Kermanshah Branch, Plant Breeding Department, Kermanshah, Iran; 4Department of Biological Sciences and Chemistry, College of Arts & Sciences, University of Nizwa, Nizwa, Oman

Somasclonal variation may be defined as tissue-culture-induced variation that has relevance in the micropropagation of endangered germplasm. It has been studied in some plant species, but only a few studies have reported on the assessment somaclonal variation in medicinal plants using molecular markers. Durocenia anethifolia Boiss. is an endangered medicinal herb belonging to the Apiaceae family. In this study somaclonal variation in plants regenerated from long-term cultured calluses of Durocenia anethifolia was characterized using amplified length polymorphism profile. Genomic DNA was double-digested with two Bgl II and Msel and the digested fragments were ligated to double stranded adaptors appropriate with the Bgl II and Msel restriction sequences. Results revealed that banding patterns were different between various explants in different subcultures. A total of 112 polymorphic fragments were scored, with an average of 22.4 fragments per primer combination. Results also showed that this method is reliable and effective way of assessing somaclonal variation in tissue culture-derived plants. Acknowledgement: The research was supported by funds received from the Institute of Medicinal Plants, Karaj, Iran.

PB48

Responses of zygotic embryos of galbanum in vitro conditions Hadi N1, Moeni A2, Omidbaigi R3

1Department of Horticulture, Tarbiat Modares University, Tehran, Iran; 2Department of Plant Biotechnology, Tarbiat Modares University, Tehran, Iran

Galbanum (Ferula gummosa Boiss.) is a valuable medicinal-industrial plant native to Iran which is at risk of extinction due to irregular and overharvesting from natural habitats. The objective of the study was to investigate the growth response of zygotic embryos of galbanum, originated from center of Iran, in in vitro conditions. The results however, showed that zygotic embryos of galbanum had not a suitable germination in in vitro conditions. However, the best treatment for in vitro germination of embryos was 1/4 MS (MS with 1/4 macro elements) medium supplemented with 0.3 mg l-1 GA3. The results also showed the embryos had good callus production in 1/4 MS medium (MS with 1/4 macro elements) supplemented with 2 mg l-1 BA and 10 mg l-1 NAA. In this study, the results showed that although applied treatments did not lead to normal germination of zygotic embryos of galbanum in in vitro conditions, but these treatments were able to force zygotic embryos into the callus production phase with good quality and quantity. The pH of primary media for zygotic embryo germination which was adjusted before adding plant growth regulators, can be effective on good callus production. Nevertheless, more experiments are needed to reveal the effect of pH on callogenesis in galbanum plant. References: 1. Bernard F et al. (2007) Pakistan Journal of Biological Sciences 10: 1977 – 1983 2. Irvan N et al. (2009) Plant Cell, Tissue and Organ Culture 100: 293 – 299 3. Tafreshi RS et al. (2008) Iranian Journal of Medicinal Plants 27: 71 – 81

PB49

Conservation and multiplication of an endangered medicinal plant – Caralluma arabica using tissue culture Bouhouche N

Department of Biological Sciences and Chemistry, College of Arts & Sciences, University of Nizwa, Nizwa, Oman

Caralluma arabica N.E.Br. (Asclepiadaceae) is a succulent, perennial herb that grows in arid regions, in West Asia and in the Middle East, including Oman and the United Arab Emirates. This plant is highly valued for its medicinal properties, and is commonly used in the preparation of traditional medicine for the treatment of diabetes, liver ailments, and painful and inflammatory conditions. Pharmacological studies revealed that C. arabica extract has anti-nociceptive, anti-gastric ulcer, cytoprotective, and anti-inflammatory properties (1, 2). Unfortunately, this plant is facing considerable pressures which threaten its survival. Therefore, the development of a protocol for the propagation of C. arabica in vitro is of high importance for the conservation of this species and its commercial cultivation. Plant regeneration via organogenesis was initiated for C. arabica using stem segments excised from young shoots and used as explants for in vitro culture. Stem explants were cultured on Murashige and Skoog (MS) (3) medium containing different concentrations of kinetin and indol-acetic acid (IAA). Preliminary results showed that differentiation of adventitious shoots was initiated within 5 weeks of culture on a medium containing 1 mM Kinetin and 3 mM IAA. Root induction was obtained on half-strength MS medium containing Indol-3-butyric acid. Further investigation is underway to establish optimal culture conditions for the regeneration of this important medicinal plant. References: 1. Zakaria et al. (2001) J. Ethnopharmacol 76(2): 155 – 158 2. Zakaria et al. (2002) Pharmaceutical Biology 40(3): 225 – 230 3. Murashige, Skoog (1962) Physiol Plant 15: 473 – 497
Abstracts | 59th International Congress of the GA | 4th–9th September 2011, Antalya, Turkey

PB50

In vitro study of callus induction and regeneration in Iranian Cichorium intybus
Zebardasti A, Kohrtzi D, Yazdani S
Dept. of Agronomy and Plant Breeding, Faculty of Agriculture, Dept. of Biotechnology for Drought Tolerance Research, Razi University, Kermanshah, Iran

Cichorium intybus L. belongs to the Asteraceae family and is one of the medicinally important plant that contains some useful secondary metabolites such as bitter sesquiterpene lactones, coumarins and flavonoids. This plant is conventionally propagated through seeds. In this research, in vitro culture of Iranian Cichorium intybus was studied, thus an experiment was laid out as a completely randomized design (CRD) in a factorial arrangement with there replications that investigated factors were different concentrations of plant growth regulators (NAA and BAP) and explants (hypocotyls and cotyledon). The results indicated that significant and non-significant differences among levels of explants and other factors for callus induction and plant regeneration, as we observed the highest percentage of callus formation from MS medium supplemented with 1.5 mg/l NAA and 2 mg/l BAP. Morphometric showed that the best explant was cotyledon versus hypocotyls for the traits and maximum of regeneration recorded for MS medium with 0.4 mg/l NAA and 5 mg/l BAP.

PC1

Clinical Evaluation of Cissus quadrangularis, Moringa oleifera and combination of two as Osteoagentics agents in Mandibular fractures
Singh V, Singh N
Department of Maxillofacial Surgery, Faculty of Dental Sciences, CS. M. Medical University, Lucknow, India

Fractures of the jaw bones renders not only physical trauma but also makes the person miss out on work productivity and other social obligations for a period ranging from 4–8 weeks on an average. Ayurveda the ancient science system of the medicine describes various herbs preparation that achieves the hardening of bone healing. Hadjor (Cissus quadrangularis L.) and Moringa (Moringa oleifera Lam.) showed clinical efficacy in treatment of fractures. Our study also showed reduction in time of inter maxillary fixation time from 6 weeks to 3–4 weeks with Ostseoal (combination of Cissus quadrangularis, Moringa oleifera) lesser with Hadjor, least with Moringa and in the case of Placebo there was no reduction in time of inter maxillary fixation. Serum Ca level both ionic and total and serum phosphorous level was significantly increased in other three groups but decreased in placebo group.

PC2

Gemmotherapy – adjuvant treatment in juvenile spondyloarthropathy
Miliutin AS, Pop G, Peev C, Dehelean C, Alexa E
1First Pediatric Clinic, University of Medicine and Pharmacy Victor Babes, Timisoara, Romania; 2Pharmacology Department, University of Medicine and Pharmacy Victor Babes, Timisoara, Romania; 3University of Agronomy and Veterinary Medicine Banatul, Timisoara, Romania

The spondyloarthropathies (SpA) are a group of rheumatic diseases that predominantly affect the axial skeleton’s joints accompanied by enthesitis. SpA are strongly associated with HLA-B27, a haplotype associated with the disease. Gemmotherapy is a form of herbal medicine that uses remedies made from the embryonic tissue of various trees and shrubs, the reproductive parts and from newly-grown tissues. Monitoring the response to gemmotherapy of a selected pediatric cohort of spondyloarthropaty. The assessment of the 21 children included complete clinical and functional evaluation (disease activity score, visual analogue scale, disability index), lab tests, x-ray and genetic study. Reevaluation was performed at 3 and 6 months. According to the European Spondyloarthropathy Study Group criteria, we diagnosed 4 children with juvenile anklosing spondylitis (AS), 7 cases of undifferentiated spondyloarthropathy, 6 patients with reactive arthritis (RA), Treatment of AS and of arthritis with biologics included nonsteroidal antiinflammatory drugs (NSAID), Sulfalazine, biologics and glucocorticoids. In US and RA, NSAID and gemmotherapy was used. Cases with mild or no inflammation in lab test was selected for exclusive herbal therapy. Administration of extracts of Ribes nigrum L., Abies pectinata Poir., Pinus montana Schur, Vaccinium vitis-idaea L., Amelopsis veitchii Hort. was daily, during 6 months, in adapted dose and was accompanied by physical therapy. Reevaluation (3 and 6 months) noted an amelioration of clinical signs and functional scores in all 13 cases and remission in 6 cases. Ggemmotherapy associated with exercises minimized symptoms, ameliorated joint mobility and allowed a well tolerated, natural therapy. References: Pitera F (2000) Compendio di gemmoterapia clinica (Meristemoterapia) con indice clinico, Genova, Cassidy JF, Petty R E, Laser RM, Lindsey CB (2006) Textbook of Pediatric Rheumatology, fifth edition, Elsevier Saunders.

PC3

The effects of a combination of silymarin and selenium on prostate health
Simaneck V1, Ulrichova J1, Vidar A1, Vrbkova J1, Student J1, Vostalova J1
1Department of Medical Chemistry and Biochemistry, Faculty of Medicine and Dentistry, Palacky University, Hradec Králové 3, 775 15 Olomouc, Czech Republic; 2Department of Urology, University Hospital, I.P.Pavlova 5, 775 00 Olomouc, Czech Republic; 3Department of Mathematical Analysis and Application of Mathematics, Faculty of Science, Palacky University,17. Listopadu 192/12, 771 46 Olomouc, 77146 Czech Republic

Complementary and alternative medicine increasingly being used by men who wish to decrease their susceptibility to prostate cancer. The aim of our intervention study was to assess the safety of a research preparation based on a combination of silymarin and selenium on healthy men with a prostate specific antigen (PSA) level lower than 2.0μg/L. In this double-blind, placebo-controlled pilot study, a total of 55 participants were randomized to either treatment with 570mg silymarin, and 240μg selenium as selenomethionine per day (n=26) or placebo (n=29). Baseline clinical and demographic characteristics were comparable. Outcome measures were changes in the International Prostate Symptom Score (IPSS), quality of life score, safety clinical chemistry and hematology parameters, serum selenium, PSA and testosterone levels, antioxidant status, transrectal ultrasound prostate volume, urinary flow rate, ultrasound estimated postvoid residual urine volume at baseline and 180 days. The results showed statistically significant differences between treatment and control groups for the following parameters: decreased PSA:tot value, improved selenium level, IPSS, quality of life score, urination parameters including voiding parameters-rate of urine flow (Qmax), average flow (Qave), total volume V and postvoid residual urine volume (RV). There was no effect on blood testosterone level. Overall the treatment was well-tolerated with no adverse effects. In conclusion, the chosen combination of silymarin and selenium proved effective and may be beneficial for the maintenance of prostate health in men. Acknowledgement: Financial support from the Czech Ministry of Education, Youth and Sport (Grant No. MSM 6198952916) is gratefully acknowledged. References: 1.Vidar A et al. (2010) Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub 154(3): 239 – 244

PC4

Molluscidical Activity of Some Solanum Species Extracts against the Snail Biomphalaria alexandrina
El Sherbini CT1, Zayed RA2, El Sherbini ET3
1Department of Parasitology, October 6 University Cairo, Egypt; 2Department of Pharmacogonys, Zagazig University, Zagazig, Egypt; 3Department of Zoology, El Naha University, Beni Swef, Egypt

Snails’ species are associated with transmission parasitic disease as intermediate host. Biological control stands to be a better alternative to the chemical controls aimed against snails. The search of herbal preparations that do not produce any adverse effects in the non-target organisms and are easily biodegradable remains a top research issue for scientists associated with alternative molluscicides control. Solvent extracts of fresh mature leaves of Solanum nigrum L., S. villosum Mill., and S. villosum Boiss. were tested against Biomphalaria alexandrina, a common intermediate host of Schistosoma mansoni. A phytochemical analysis of chloroform: ethanol extract was performed to search for active toxic ingredient. The lethal concentration was determined. Extracts isolated from mature leaves of Solanum species were found to be having molluscidial properties. S. nigrum extract was recorded as...
the highest mortality rate. When the mortality of different solvent extracts was compared, the maximum (P<0.05) mortality was recorded at a concentration of 90 ppm of ethanol extract of S. nigrum. Extract of mature leaves of S. nigrum exhibited molluscidal activity followed by S. sinalcum and the least one was S. vulgum. The study provides considerable scope in exploiting local indigenous resources for snails’ molluscidal agents. Acknowledgement: The authors thank all the participants who shared their time for working on this study. References: Ahmed AH, Kamal IH, and Ramzy RM (2001) Journal of the Egyptian Society of Parasitology, 31(3): 843 – 852. Massoud AM and Habib FS (2003) Journal of the Egyptian Society of Parasitology, 33(2): 585 – 596.

Efficacy of Punica granatum extract on in vitro and in vivo control of Trichomonas vaginalis

El Sherbini GT1, Ibrahim KMF2, El Sherbini ET3, Abdel Hady NM4, Morsy TA5
1Department of Parasitology, October 6 university Cairo, Egypt.; 2Department of Zoology, Al-Azahar university, Cairo, Egypt.; 3Department of Zoology, El Nahda university, Beni Sweif, Egypt.; 4Department of Pharmacognosy, Al-Azahar university, Cairo, Egypt.; 5Department of Parasitology, Ain-Shams, Cairo, Egypt.

Trichomoniasis vaginalis is now an important worldwide health problem. Metronidazole has so far been used in treatment, but the metronidazole resistant strains and unpleasant adverse effects have been developed. Treatment of patients with metronidazole refractory vaginal trichomoniasis constitutes a major therapeutic challenge and treatment options are extremely limited. The last 7 years have seen over seven times as many publication indexed by Medline dealing with pomegranate (Punica granatum L.) than in all the years preceding them, because of this, and the virtual explosion of interest in pomegranate (Roman) was in vitro investigated for its efficacy against T. vaginalis on Diamond media. Besides, infected women (18/20) who accepted to be treated with P. granatum juice were completely cured and followed- up for two months. The anti-trichomoniasis vaginalis activity of P. granatum extract (in vitro and in vivo) gave very promising results. Acknowledgement: The authors thank all the participants who shared their time for working on this study. References: 1. Abdel Hady NM, El- Sherbini GT, Morst TA (2008) Egypt Soc Parasitol 38(3): 1024 – 5. 2. Adams LS, Zhang Y (2010) Cancer Prev Res (Phila Pa) 3(1):108

Evaluation of the protective effect of Urtica dioica leaf extract on Beta cell islet langerhans of diabetic rats

Keshavarz M1, Minaii B2, Monsaf H2, Charoatyz M2
1Department of Physiology, Tehran University of Medical Science, Tehran, Iran; 2Department of Histology, Tehran University of Medical Science, Tehran, Iran; 3Department of Pharmacognosy, Tehran University of Medical Science, Tehran, Iran; 4Tehran University of Medical Science, Tehran, Iran

Herbal medicine is a complementary way to improve the health. The traditional Iranian Medicine introduces many plants for treatment. This study investigates the anti-diabetic effects of Urtica dioica L. leaves that were introduced as anti-diabetic plant in the traditional Iranian medicine. The animal was made diabetic with intra tail vein injection of 50 mg/kg STZ (streptosotocine). Animals with fasting blood sugar > 250 mg/kg were considered as diabetic. One group of diabetics were treated with Urtica dioica leaf extract (1 ml/kg/day intra peritoneal). After one month, animals were decapitated to take the blood sample and pancreas tissue. Tissue was observed by histologist. The tissue parameters were studied in both diabetic and experimental group. Blood glucose in treated group decreased from 400 ± 54.2 mg/kg to 87.9 ± 11.9 mg/kg whereas no change was observed in diabetic group. In diabetic group, necrosis and infiltration of mononuclear cells were produced in plenty. Capillaries, islet cells, Beta cells and secretary vacuoles were damaged while in treated group the necrotic tissues was repaired and infiltration of mononuclear cells were a bit. Beta cells increased and secretory vacuoles were appeared. The number of capillaries and undifferentiated cells also increased. Urtica dioica repairs pancreas tissue and improves its function. This may lead to increase insulin secretion and Urtica Dioica direct influence in decreasing blood sugar. References: 1. Kavalati G et al. (2003) J Ethnopharmacol 84(2 – 3): 241 – 245 2. Farzami B et al. (2003). J Ethnopharmacol 89: 47 – 53 3. Kumar V et al. (2003) Basic Pathology, the Pancreas 7 ed, Saunders; pp: 635 – 657

Antibacterial activity of some medicinal plants against antibiotics

Jamshidi M1, Charee Fathabod E2, Eslamifar M3
1Young Researchers Club, Islamic Azad University, Sari, Iran.; 2Department of Pharmacognosy and Biotechnology, Faculty of Pharmacy, Mazandaran University of Medical Sciences, Sari, Iran; 3Department of Environmental Health, faculty of Hygiene, Mazandaran University of Medical Sciences, Sari, Iran.

In this study, the antibacterial activity of 6 species of Lamiaceae family: Mentha spicata L., Mentha aquatica L., Stachys byzantina K.Koch, Marrubium vulgare L., Rosmarinus officinalis L. and Melissa officinalis L. were investigated. The methanolic extracts of aerial parts of plants were evaluated in different concentrations according to the disk diffusion method by using Bacillus subtilis, Staphylococcus aureus, Escherichia coli, Salmonella typhimurium, Streptococcus fecalis and Klebsiella pneumoniae and all the extracts were compared with standard antibiotic discs like vancomycin, ampicillin and chloramphenicol. The methanolic extracts of Stachys byzantina and Rosmarinus officinalis were shown to inhibit, to different degrees, the growth of microorganisms (15 – 20 mm). Furthermore, Stachys byzantina showed significant antimicrobial activity against Staphylococcus aureus resistant to vancomycin. The other plant extracts had shown lower antibacterial activity in comparison with standard antibiotics. This study showed that some medicinal plants that were used in folk medicine significantly Stachys byzantina could be comparable with antibiotics and potential sources of new antimicrobial agents.

Plasma oligoelements levels in pediatric cohort of spondyloarthropathy

Militaru AS1, Alexa E2, Pop G3, Radulov I4, Bas M5
1First Pediatric Clinic, University of Medicine and Pharmacy “Victor Babes” Timisoara; 2Banat’s University of Agricultural Sciences, Timisoara, Romania; 3Tismana Timis College; 4Department of Histology, Tehran University of Medical Sciences, Tehran, Iran; 5Department of Histology, University of Medicine and Pharmacy, Sinaic University, Alexandria, Egypt.

The importance of trace elements in chronic inflammatory diseases is related to their cofactor role in immune system functions and in different metabolic processes in articular tissues. Spondyloarthropathies (SpA) are a group of rheumatic diseases linked by common pathology, including inflammatory back pain and radiographic changes. To investigate the status of plasma trace metals in a pediatric cohort of spondyloarthropathy, to establish the relationship between these trace metals and the main biological and clinical parameters of the disease. We studied plasma concentrations of zinc (Zn), copper (Cu), iron (Fe), cadmium (Cd), nickel (Ni), plumb (Pb), manganese (Mn), calcium (Ca), magnesium (Mg) in 11 patients with juvenile spondyloarthropathy and compared them with 14 sex- and age-matched healthy subjects. Disease activity was measured by lab tests. Oligoelements concentrations were determined by atomic absorption spectrophotometry. There were no significant differences in plasma concentrations of Cd, Ni, Pb, Mn among the two groups (p > 0.05). Plasma zinc was significantly lower in cases with SpA than control group (p < 0.05) and was correlated with numerous of the biochemical as well as clinical markers of SpA. Plasma zinc was found to be lower in SpA patients taking anti-inflammatory drugs. Cu concentrations were higher, but not significantly, in patients with SpA than those of healthy subjects. Ca and Fe plasma levels was significantly lower in children with SpA (p < 0.05). Administration of supplements with the proper quantity of oligoelements could balance the plasma concentrations of these trace elements in juvenile SpA.

How does long term exposure to base stations and mobile phones affect human hormones profile?

Fawzy Eskander E
Medical Division, Hormones Department, National Research Center, Dokki, Giza, Egypt.

This study is concerned with assessing the role of long-term exposure to high frequency non-ionizing electromagnetic radiation (RFR) emitted either from mobile phones or from base stations. One hundred volunteers from different areas in Egypt exposed to radio frequency non-io-
nizing electromagnetic radiation emitted from mobile phones or from base stations for period extended to six years suffered tangible effects on the pituitary – adrenal axis. Volunteers were divided into three subgroups according to non-ionizing radiation exposure; according to the time of exposure to RF per day. In addition to negative control subjects of compatible age ranges, sex and socioeconomic status. Volunteers’ plasma ACTH and serum cortisol levels were measured. Also thyroid hormones were detected for all individuals. In addition, each of their serum prolactin, progestosterone and testosterone level were measured to detect the different relations between all these seromarkers in all volunteers who expose to non-ionizing electromagnetic radiation emitted either from mobile phones or from base stations. Authors have previously found no association between self-reported illness symptoms and the exposures to microwave radiation emitted by mobile phones or electromagnetic field induced by other major sources [1]. The thyroid gland is one of the most exposed vital organs and may be a target for electromagnetic radiation. It has been established that even a small change in circulating thyroid hormone levels is sufficient to alter the brain functions [2].

Keywords: base stations, mobile phones, long-term exposure, electromagnetic radiation, ACTH, cortisol, thyroid, prolactin, progestosterone, testosterone

**Topic D: Cultivation and Breeding**

**PD1** Response of seed priming on seed germination and seedling growth in basil
Mirzaei A1, Naseri R2, Vazan S3
1Department of Agronomy, Islamic Azad University, Karaj Branch, Karaj, Iran.; 2The University of Ilam, Ilam, Iran.

In order to evaluate the effect of priming on seed germination and seedling growth in basil (Ocimum basilicum L.), an experiment was conducted based factorial in randomized complete block design with three replications in western of Iran. Priming factor including: witness, KCL 2% and priming time including: 0, 3, 5, 7 and 9 hours. Results showed that Priming was affected on radicle length, radicle dry weight, stem let dry weight, germination percentage and speed germination. KCL 2% had better results due to negative osmotic adjustment. Priming had significant affected on radicle length, radicle dry weight, stem let length and stem let dry weight. Seed priming in 7 hours had positive affected on all studied traits. The highest radicle length, radicle dry weight and stem let length obtained from 7 hours. According to the results Priming and priming time played in plant germination. Among treatments KCL 2% and 7 hours had an important role in germination. Keywords: Seed priming, Basil, Germination, Ocimum basilicum

**PD2** Effect of plant growth promoting rhizobacteria (PGPR) on the healthy and productivity of soy bean plant
Salama AR, Hamed EK, Shehata HS
Medicinal and Aromatic plants Dept. National Research Centre, Cairo, Egypt.

A pot and field experiment were conducted to evaluation some rhizobacteria namely Pseudomonas fluorescens, and Bacillus subtilis. The pot experiment was executed to evaluate probable suppressive effect of rhizobacteria as bioagents against Macrophoma phaseolina, Fusarium solani and Scerortium rolfsii under artifically infested soil. Results showed that co-inoculation of soy bean with rhizobacteria led to a significant decrease in pre- and post-emergence damping-off caused by all pathogens under investigation. In addition to enhance the nodulation status, growth, N-content and pod yield plant under uninfested or infested soil. Field experiment were carried out in El-Sharqia governorate to evaluate the promotive and suppressive disease effects of rhizobacteria on nodulation, plant growth and yield of soy bean. Results showed that the inoculation with rhizobacteria led to a significant increase in the nodulation status, shoot dry matter and N-content after 15, 45, 75 days of planting. Moreover, the co-inoculation of Bacillus subtilis with Rhizobium sp. Salient superiority in suppressive disease. The obtained results explained that the synergy between rhizobacteria (Bacillus subtilis, Pseudomonas fluorescens and Rhizobium sp.) considered the efficient microorganisms to save the protection against the phytopathogenes and promote the nodulation and symbiotic nitrogen fixation leading to a high quality yield of soy bean.

**PD3** Effect of plant density and application rates of vermicompost on essential oil content and composition of Balm (Melisaa officinalis L.)
Tohghraei A1, Daneshian F2, Shiri Rad A2, Zarei Kooshki M2, Toghraei A1
1Academic Center Of Education And Cultural Researchs (ACECR), Qazvin Unit, 34138 – 63694, Qazvin, Iran; 2Department of Agriculture, Azad Islamic University, Takestan Unit, 34819 – 49479, Takestan, Iran

In order to investigate plant density and application rates of vermicompost on essential oil content and composition of Balm, the experiment was conducted during 6 months in Dineh phytomedic company in 2010. This experiment was carried out in complete randomized block design with 3 replications at three plant densities (8, 10 and 10 plant/m²) and four application rates of vermicompost (0, 5, 10 and 15 ton/ha). In floral imitation, plants harvested and essential oil were extracted by water distillation. The essential oils were analyzed by GC and GC/MS. The results showed a significant difference (P<0.05) among plant densities and application rates of vermicompost on essential oil yield, maximum amount of essential oil obtained from 10 plant/m² and 10 ton/ha vermicompost consumption. Identification of essential oil components showed that plant density had no effect on essential oil composition but some compounds of the oil decreased with more application of vermicompost, whereas some other compounds increased with most application of vermicompost.

**PD4** Study the effect of different levels of phosphobiofertilizer’s inoculation on some traits of Anethum graveolens L. in Rudhen
Tavakoli Dinani E1, Masoumi A2, Darzi M3
1Young Researchers Club of Islamic Azad University, Roudehen Branch, Roudehen, Iran; 2Shahrood University of Technology, Shahrood, Iran; 3Islamic Azad University, Roudehen Branch, Roudehen, Iran

It is well known that In nature, a considerable number of microorganism (e.g. bacterial species), mostly those associated with the plant rhizosphere, are able to exert a beneficial effect upon plant growth. Therefore, their use as biofertilizers or control agents for agriculture improvement has been a focus of numerous researchers for a number of years. The use of phosphobio-solubilizing bacteria as inoculants simultaneously increases P uptake by the plant and crop yield. To inspect the influence of phosphobiofertilizer, we used 6 shapes of phosphobiofertilizers inoculation, included: (B seeds inoculated, B top dressing, E seeds inoculated, E top dressing, E-B & control) of Iranian (B) and non-Iranian (E) microorganisms, on hight of plant, yield of seed and essential oil production of the plant namely Anethum graveolens L. in the form of factorial on the basis of Randomized Complete Block Design in three replication was conducted in Rudhen university (Kolyak state). Results showed that in all traits, There was a significant difference between utilization of both types phosphobiofertilizers and other treatments. Acknowledgement: The authors are grateful to Dr. Baghi and Dr. Seyed Hadi for their scientific support to the present project.

**PD5** Effect of different nitrogen and phosphorus application on qualitative and quantitative characteristic of beebalm
Naseri R1, Mirzaei A2, Nazaraliadze Kh1, Soleymaniard A1, Abrash A2
1The University of Ilam, Ilam, Iran.; 2Islamic Azad University, Karaj Branch, Karaj, Iran.; 3Agriculture and Natural Resource Research Center, Ilam, Iran.

In order to test the effect of nitrogen and phosphorus fertilizers on qualitative and quantitative characteristic of beebalm, an experimental was carried out using factorial with randomized complete block design with three replication in Ilam, Iran in 2009 – 2010 growing season. Experimental factor including different of nitrogen fertilizer (70, 100 and 130 kg/ha) and phosphorus fertilizer (50, 70 and 90 kg/ha). Results showed that nitrogen fertilizer was affected on essential oil content, essential oil yield, plant height, number of tillering, stem diameter, root length, root weight and shoot ratio. The highest essential oil yield, plant height, number of tillering, stem diameter, root length was obtained 130 kg/ha nitrogen application. Phosphorus fertilizer affected essential
Effects of salt concentration on salt-sensitive weed plants.

**PD6**

Effect of salinity on essential oil content and composition of *Agastache foeniculum* Kuntze

Soleymanifard A1, Mirzaei A2, Naseri R3, Naserirad H4

1Payamnoor University Ilam, Iran; 2Islamic Azad University, Karaj Branch, Karaj, Iran; 3Ahvaz University, Khuzestan, Iran; 4The University of Ilam, Ilam, Iran.

Water and soil salinity on the environmental agents limit plant growth and its productivity in Iran. Anise Hyssop (*Agastache foeniculum* Kuntze) is a perennial aromatic plant, belonging to the family Lamiaceae. The essential oil of Anise Hyssop is used in many industries, pharmacy, perfumery and making soda. This experiment was conducted in a randomized complete blocks design with four salt treatments including 0 (control), 50, 100, and 150 mM NaCl and four replications in green house. Some parameters such as content and composition of essential oil were evaluated. The results showed that salt stress had significant effects on estimated parameters. Salinity decreased the fresh and dry weight of leaves and shoots, herbage yield and the amount of essential oil. In the composition of essential oil β-pinene, myrcene, anisaldehyde and β-bourbonene increased and the content of linalool and methyl chavicol decreased. High salinity (100 and 150mM) destroyed the plants.

**PD7**

Allelopathic effect of cumin (*Cuminum cyminum* L.) on seed germination of three weeds

Soleymanifard A1, Naseri R2, Mirzaei A2, Naserirad H4

1Islamic Azad University, Desful Branch, Desful, Iran; 2The University of Ilam, Ilam, Iran; 3Islamic Azad University, Karaj Branch, Karaj, Iran; 4Payamnoor University Ilam, Ilam, Iran.

The allelopathic effects of cumin (*Cuminum cyminum* L.) were evaluated on seed germination of velvet flower (*Amaranthus retroflexus* L.), flixweed (*Descurainia sophia* L.) and wild oat (*Avena fatua* L.) in laboratory using the aqueous extracts of dried powdered cumin leaves. The treatments were 1, 2, 5, 10 and 15% extract of cumin and distilled water control. According to the results, extracts significantly inhibited seed germination of weed species and the degree of inhibition increased with increasing concentration of extracts. Germination of *Amaranthus retroflexus* seeds was inhibited at concentrations greater than 5%. In addition, radicle and plumule lengths of *Amaranthus retroflexus* were significantly reduced at 1% compared to the distilled water. Results indicated germination percentage, germination rate and radicle and plumule lengths of *Avena fatua* were significantly reduced by the extracts compared to the distilled water. Results confirm germination of *Descurainia sophia* seeds was inhibited at concentrations greater than 2%. Accordingly germination rate and radicle lengths of *Descurainia sophia* were significantly reduced by the extracts compared to the distilled water. Therefore, extract of cumin might be useful as natural herbicides and might also contain numerous growth inhibitors that could be used for the development of biological herbicides.

**PD8**

Growth, yield and essential oil content of *Marrubium vulgare* as affected by three levels of nitrogen fertilizer

Sabry R1, Salama AB2, Mahmoud S2

1Medicinal and Aromatic Plants Dept., National Research Centre, Cairo, Egypt; 2Alkhair University, College of Sciences and Humanitarian Studies, Alkhair, Saudi Arabia

*Marrubium vulgare* L. (Lamiaceae) is a perennial herb commonly known as “White horehound” and grows wild in the Egyptian desert and commonly distributed in Europe, North and South America, the Mediterra­nean district and Western Asia. The plant is used in the folk medicine of several countries for the treatment of a variety of diseases, including inflammatory, gastroenterical and respiratory disorders. For the first time in Egypt, the plant was cultivated under systematic agriculture regime to estimate the nitrogen doses for the best plant growth. Three nitrogen doses were applied (N1 (33.5), N2 (50.25) or N3 (67) kg N/ fedden = 4200m2). Nitrogen fertilization had significant effects on most of agronomic parameters studied. Plant height (cm), number of branches per plant, plant fresh and dry weight (g) increased with the increase in nitrogen fertilization. Compared to the control (N2) extract, the best growth attributes, although the differences were not significant in most harvests (cuttings) between the rates of N2 and N3. On the other hand, oil content was not influenced by nitrogen fertilization in all harvests. References: 1. Boulos L (2002) Flora of Egypt. Al Hadara Publishing, Egypt. 2- Studler HK, Tagliapi MP, Zampirolo JA, Cechinil-Filho V, Schlemper V (2006) J Ethnopharmacol 108: 379 – 384. 3- Sabpaz S, Gar­backi N, Tits M, Baileu F (2002) J Ethnopharmacol 79: 389 – 392.

**PD9**

Changes in essential oil content and composition of *Agastache foeniculum* under water stress at second harvest

Mahmoodi Sourestani M, Malekshahi F

University of Shahid Chamran, Ahvaz, Iran.

To study the effect of different levels of water stress on essential oil content and composition of *Agastache foeniculum* Kuntze at second harvest, a field experiment in randomized complete block design with three replications was conducted. Water stress treatments were: 100% of field capacity (FC), 85% of FC, 55% of FC, 100 – 85% of FC (100% at vegetative stage and 85% at reproductive phases), 100 – 70% of FC (100% at vegetative and 70% at reproductive phases), 85 – 100% of FC (85% at vegetative and 100% at reproductive phases). The highest of essential oil content was observed at 70% of FC (1.69) and 100 – 85% of FC (1.67). Also the treatment 85% of FC (1.33) caused the lowest of essential oil content. There was not significant different between other treatments. The main essential oil constituent was methyl chavicol which showed a increasing with progress water stress till 70% of FC and then decreased at 55% of FC treatment. Limonene, another main component was decreased with increasing water stress levels from 100% of FC to 70% of FC. The highest amount of methyl chavicol (98.3%) and limonene (2.4%) were found at 70% of FC and 85 – 100% of FC, respectively.

**PD10**

Influence of biofertilizers on the essential oil content and constituents of *Dracocephalum moldavica* L.

Mafakheri S

Department of Agriculture, College of Horticulture, Tarbiat Modares University, Tehran, Iran.

In this study the effect of three levels of vermicompost (0, 15 and 30% V/Pot), two levels of biophosphate (application and not application) and two levels of azotobacter (application and not application) on content and constituents of essential oil of *Dracocephalum moldavica* L. was investigated. The results showed that the essential oil content of dragonhead (*Dracocephalum moldavica*) and its constituents were significantly affected by biofertilizer treatments. The highest essential oil content (0.74%) was obtained when 30% pot volume was vermicompost. Fifteen components were identified from the oil of plants which were fertilized by biofertilizers. The highest geranyl acetate content (61.1%) of essential oil were obtained when 30% pot volume was vermicompost, while the highest geraniol content in essential oil (24.2%) was obtained when 15% pot volume was vermicompost with application of biophosphate. Also the treatment 85% of FC (1.33) caused the lowest of essential oil content. There was not significant different between other treatments. The main essential oil constituent was methyl chavicol which showed a increasing with progress water stress till 70% of FC and then decreased at 55% of FC treatment. Limonene, another main component was decreased with increasing water stress levels from 100% of FC to 70% of FC. The highest amount of methyl chavicol (98.3%) and limonene (2.4%) were found at 70% of FC and 85 – 100% of FC, respectively.

**References:**

In order to study the effect of sowing date and seeding levels on quantitative and qualitative yield of chamomile (*Matricaria recutita*), an experiment was conducted. The experimental design was split-plot in the basic of randomized complete block design with three replications. Main plots consisted of three sowing dates (6 Nov, 5 Mar, and 4 Apr) and sub-plots included three seeding levels (0.2, 0.4 and 0.8 g/m²). On the basis of the results, highest plant (47.4 cm), the most number of plants in plot (135.4 plants), the most yield of fresh and dry flower yield (749.1 and 175.1 g/m²) was obtained from the plants were sown on 6 of Nov but the most percentage of essential oil and chamazulene content (0.63 and 5.9 w/w percent respectively) and essential oil yield (0.79 g/m²) was obtained from the plants were sown on 5 of Mar. On the basis of the results of their interaction, highest plant (49.7 cm), the most fresh and dry flower yield (749.1 and 175.1 g/m²) was obtained from the plants were sown on 6 of Nov with 0.8 g/m² but the most essential oil and chamazulene (0.59 and 5,9 w/w percent respectively) and essential oil yield (0.79 g/m²) was obtained from the plots were sown on 5 of Mar with 0.4 g/m².

References:

Atractylodes lancea (Thunb.) DC., which has been highly appreciated in medical literature of past dynasties, is a kind of authentic herb, and has a long history of medicinal in China. The selection of superior variety is an important measure to cultivate the Atractylodes lancea Wild Atractylodes lancea grown in Maoshan mountainous area, Jiangsu province, China, was divided into four types according to leaf shapes: incised leaf-type, ovate leaf-type, long lanceolate leaf-type, and short lanceolate leaf-type. The photosynthetic activity and chlorophyll fluorescence parameter of four types of Atractylodes lancea were measured. There were significant differences in photosynthetic activity among the four types of Atractylodes lancea. The net photosynthetic rate of incised leaf-type was much higher than that of other types. Substantial differences in the overall performance of the photosynthetic apparatus also existed among the four types of Atractylodes lancea. The capacity to regenerate the photosynthetic apparatus, photochemical quenching capacity and PS II electron transport activity of the incised leaf-type Atractylodes lancea were greater than those of other types, and the capture efficiency of light energy of the short lanceolate leaf-type was the lowest among the four types of Atractylodes lancea. This suggests that the growth rate of the incised leaf-type Atractylodes lancea was greater than that of other types in growing environment. It was consistent with the measurers to the growth of stem and leaves in the field [1]. The incised leaf-type Atractylodes lancea can be selected as the superior variety. Acknowledgement: The authors gratefully acknowledge the financial support from the High-tech Agriculture Research Program of Jiangsu Province, China (No. BG2006322).

between the biomass obtained from the image analysis and those from the manual measurement ($Y = 0.9497X + 0.0018$, $R^2 = 0.9861$, $n = 29$, $P < 0.01$). The difference of effect on the growth rates by various growth regulators assembly is significant. The high level of 6-BA and NAA is disadvantageous for the growth of Orthophragmus violaceus plantlets in vitro. MS medium supplemented with 0.02 mg l$^{-1}$ NAA and 2.00 $m$-BA was the optimal for the growth of Orthophragmus violaceus plantlets in vitro. Acknowledgement: The authors gratefully acknowledge the financial support from National Basic Research Program of China (973 Program) (No. 2006CB403206). References: 1. Wu YY (1997) Comprehensive studies on plant of adaptability to karst-Orthophragmus violaceus. Guizhou Sci. Publ. House. Guiyang. 2. Wu YY et al. (2004) The studies on Orthophragmus violaceus' adaptability to karst. Guizhou Sci. Publ. House. Guiyang.

**PD16**

Assessment diversity and cultivation potential of Coridothymus capitatus (L.) Reichenb. fil growing wild in Jordan

Safjan SM$^1$, Al Duwayri MA$^2$, Alali FQ$^1$
$^1$National Center for Agricultural Research and Extensin, 19381, Amman, Jordan.; $^2$University of Jordan, Amman, Jordan;


**PD17**

Saffran variations in saffron (Crocus sativus L.) under irrigation regimes in Iran

Alahdadi Farahani H Young Researchers Club, Islamic Azad University, Shahre-e-Qods Branch, 37515 – 374, Tehran, Iran

Saffron is a spice derived from the flower of the saffron crocus (Crocus sativus L.). Together with the styles stalks that connect the stigmas to their host plant the dried stigmas are used in cooking as a seasoning and colouring agent is native to Southwest Asia. Saffron also contains a carotenoid dye, crocin, which imparts a rich golden yellow hue to dishes and textiles. In order to the saffran variations in saffron under irrigation regimes, an experiment was carried out using a randomized complete blocks design with three replicates at Iran in 2010. The factors including irrigation regimes (control, irrigation interrupted from stem elongation stage, irrigation interrupted from flowering stage) were studied. The flower yield in saffron increased under control irrigation into interrupted irrigation but saffran variations was increased under interrupted irrigation into control irrigation. The findings may give applicable advice to medicinal and aromatic plants researchers for management and concern on water strategy and estimate of irrigation carefully. The increase of quantity and quality yields in medicinal and aromatic plants farming.

**PD18**

The study on the effect of irrigation levels and mulch application on growth indices and essential oil content of peppermint (Mentha piperita L.)

Shataani S Agricultural College, Ferdowsi University, Mashhad, Iran

In order to study the effects of different irrigation regime and mulch types on growth indices and, essential oil content of peppermint (Mentha piperita L.) in 2010 in research field of Agricultural college of Ferdowsi University of Mashhad as factorial randomized complete block design was performed in four replicates. Irrigation treatments included three levels (100, 80 and 60 percent of water requirements calculated by evaporation pan class A and two types of mulch (black plastic and wood chips) and the uncoated control. Traits include inter-node distance, number of flowers/plant, number of leaves/plant, plant height, fresh weight, dry weight, chlorophyll content, leaf relative water content, leaf area, essential oil percentage and yield were evaluated during full flowering. The results showed that the effect of irrigation on fresh weight, dry weight, leaf relative water content and leaf area was significant (at 0.05 level). The effect of mulch on fresh weight, dry weight, leaf area, inter-node distance, number of branch and leaf relative water content was significant (at 0.05 level). Interaction between irrigation and mulch on inter-node distance, number of branches, fresh weight, dry weight, relative water content of leaves and leaf area and height was significant (at a 0.05 level). The effect of irrigation, mulch and interaction effects on traits such as flower number, chlorophyll content, percentage essential oil (at 0.05 level) was not significant.

**PE1**

The effect of harvesting time on total phenolic content and antioxidant activity of five plants of the family Labiatae

Alizadeh O Islamic Azad University Firooz Abad Branch, Shiraz, Iran

Regarding the effects of the harvesting stage on the amount of phenolic content and antioxidant activity of medicinal herbs Satureja hortensis L., Origanum majorana L., Salvia virgata Ait., Melissa officinalis L. and Hys- sopus officinalis L. an experiment has been tested three S.D in form of randomized complete block design. The results of this survey in herbal medicine showed the effects of the harvesting stage on the amount of phenolic content and antioxidant activity in the pre-flowering had the highest and flowering time had the most phenolic content and antioxidant activity. The most amount of phenolic content and antioxidant activity in Satureja hortensis herbal medicine are (25.15mgGAE/gdw) in flowering and (8.38mg/g/ml) in pre-flowering stage in sequence. The most amount of phenolic content and antioxidant activity in Origanum majorana herbal medicine are (46.73 mg GAE/gdw) in pre-flowering and (6.53 mg/g/ml) in flowering stage in sequence. The most amount of phenolic content and antioxidant activity in Salvia virgata herbal medicine are (47.27 mg GAE/gdw) in flowering and (7.77 mg/g/ml) in pre-flowering stage in sequence. The most amount of phenolic content and antioxidant activity in Melissa officinalis herbal medicine are (42.60 mg GAE/gdw) and (7.32 mg/g/ml) in flowering stage in sequence. The most amount of phenolic content and antioxidant activity in Melissa officinalis herbal medicine are (21.88 mg GAE/gdw) and (9.53 mg/g/ml) in flowering stage in sequence. Keywords: Phenolics content, Antioxidant activity, Harvesting time, Satureja hortensis, Origanum majorana, Salvia virgata, Melissa officinalis, Hysopus officinalis

**PE2**

Antinociceptive mechanisms of Bunium persicum essential oil in the mouse writhing test

Zendehdel M, Torabi Z, Pourrahimi M Division of Pathology, Department of Basic Sciences, Faculty of Veterinary Medicine, University of Tehran, Tehran, P.O.Box:14155 – 6453- Iran

Antinociceptive profiles of Bunium persicum B.Fedtsch. were examined in NMRI mice. Essential oil of Bunium persicum administered intraperitoneally (0.001, 0.01, 0.05, 0.1, 0.5 and 1%; 10 ml/kg) in Tween-80(0.5%)
showed an antinociceptive effect in a dose-dependent manner as measured by writhing test as a model of visceral pain. Furthermore, to reveal the antinociceptive mechanisms of *Bunium persicum*, we examined the effect of opioidergic, serotonergic, and histamine receptor antagonists on *Bunium persicum*-induced antinociception. Intraperitoneal (i.p.) pretreatment with naloxone, chlorpromazine and cimetidine attenuated inhibition of the pain response induced by *Bunium persicum*. However, cyproheptadine did not affect inhibition of the pain response induced by *Bunium persicum*. Our results suggest that *Bunium persicum* shows an antinociceptive property in writhing test. Furthermore, antinociception of *Bunium persicum* may be mediated by opioidergic and histaminergic H1 and H2 receptors.

**PE3**

**Essential oils composition and antioxidant properties of three Thymus species**

Amini H1, Lari Yazdi H2, Dehshiri M3, Eghbali D3, Mohammadi A1, Zarei A1

1Department of Biology, Lorestan University, Khoramabad, Iran, 2Department of Biology, Isfahan Azad University, Branch of Broujerd, Broujerd, Iran.

The essential oils of three wild-growing Thymus species (*Thymus kotschyanus* Boiss. & Hohen., *Thymus eriocalyx* (Ronner) Jalas) and *Thymus daenensis* Celak subsp lancifolius (Celak) Jalas. collected from west of Iran during the flowering stage, were obtained by hydrodistillation and analyzed by gas chromatography (GC) and gas chromatography/mass spectrometry (GC-MS). Under the optimum extraction and analysis conditions, 44, 38 and 38 constituents (mainly monoterpens) were identified in *T. kotschyanus*, *T. eriocalyx* and *T. daenensis* respectively (89.9%, 99.7% and 95.8% of the oils, respectively). The main constituents were thymol (16.4–42.6%), carvacrol (7.6–52.3%) and γ-terpineol (3.0–11.4%). Antioxidant activity was employed by two complementary test systems namely 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging and β-carotene/linoleic acid systems. Antioxidant activity of polar sub-fraction of *T. daenensis* subsp lancifolius was found to be higher than those of the oils in DPPH assay while non-polar sub-fraction of *T. eriocalyx* has most antioxidant activity in β-carotene/linoleic acid test (19.1 ± 0.1 µg/ml and 96.1 ± 0.8% inhibition rate, respectively).

**PE4**

**Effect of heating on Zataria multiflora and Cinnamomum zeylanicum essential oils for the evaluation of their antiradical activities by using 2,2-diphenyl-1-picrylhydrazyl (DPPH)**

Kordbaroudi H1, Barzag M2, Safari MA1, Shahnaz M3

Food Technology Department, Agricultural Engineering, Tarbiat Modares University, Tehran, Iran

Oxidation of lipids, which occurs during raw material storage, processing, heat treatment and further storage of final products, is one of the basic processes causing rancidity in food products, leading to their deterioration. Since application of natural antioxidants may be one of the most scientifically simplest ways of reducing fat oxidation, The present study was designed to survey the effect of heating on antiradical property of *Cinnamomum zeylanicum* Breyne and *Zataria multiflora* (Za- shirazi) essential oils. The essential oils were heated in three temperatures (100, 140, 180 °C) for 1, 2, 3 hours and the antiradical property was compared with samples before heating. The antiradical property was evaluated by using DPPH assay. All the data were analysed by SPSS software (version 11.5). In the ambient conditions, *EC50* of *Zataria multiflora* and *Cinnamomum zeylanicum* essential oils were 4026.7 ± 2.2 and 2605.01 ± 3.7 ppm, respectively. According to the results, different behavior of essential oils, based on different heat conditions, were observed. In conclusion, the essential oils under study exhibited good antiradical properties and might be efficiently used to control lipid oxidation during food processing. Keywords: essential oil, *Cinnamomum zeylanicum*, *Zataria multiflora*, DPPH assay References: 1. El-Baroty GS et al. (2010) African Journal of Nutrition Research 4: 167 – 174. 2. Jayaprakasha G K, Negi P S, Jena B S and Rao L (2007) J Food Composition and Analysis 20: 330 – 338. 3. Kulicis T, Radonic A and Katalinic V (2004) Food Chem 85: 633 – 640. 4. Marinova E M and Yanishlieva V N (1996) Food Chem 58: 245 – 248. S. Marongiu B et al. (2007) J Agric Food Chem24: 10022 – 10027.

**PE5**

**Effect of Salt Stress on Growth and Essential oil of Matricaria chamomilla L.**

Dadkhah A

 Ferdowsi University of Mashhad, College of Agriculture, Shirvan, Khorasan Shomali, Iran

A pot experiment based on complete block design was carried out to investigate the effect of salinity on growth traits and essential oil content of chamomile (*Matricaria chamomilla* L.). Four levels of salinity, including control (0 mM), 50, 150 and 250 mM NaCl and CaCl2 in 5:1 molar ratio were used. Result indicated that increased salinity caused reduction in plant height, number of branches per plant, number of flowers per plant. Increased salinity also significantly decreased plant dry weight, flower dry weight and essential oil content. The highest values of growth traits such as number of flower per plant, flower dry weight and essential oil content were observed under control condition (non-salinity stress). The effect of salinity on flower dry weight is greater than other traits. Flower dry weight of plants at low (50mM) level of salinity was decreased 12.2% compared to control (non-stressed plant) while essential oil content increased 18.2% at the same salinity level. At the highest level of salt stress (250mM) flower dry weight and essential oil content was decreased by 79.8 and 45.5% compared to non-stressed plants, respectively. Number of flower per plant was decreased by 16.1 and 69.2% at lowest (50 mM) and highest (250 mM) salinity concentration respectively. Salinity affects flowering time of plants. Flowering time of non-stressed plants started 50 days after plant transplanting while flowering time of plants treated by 250 mM salinity started 64 days after seedlings transplanting to pots. Acknowledgement: I would like to express my appreciation for research deputy of the Ferdowsi University of Mashhad for financial support. The author is grateful to Mr Hamid Eskandari BSc student of medicinal plant production for his excellent assistance.

**PE6**

**Effect of Salinity on Germination and Seedling Growth of Four Medicinal Plants**

Dadkhah A

 Ferdowsi University of Mashhad, College of Agriculture, Shirvan, Khorasan Shomali, Iran

This experiment was conducted in germinator in order to study the effects of water potential on seed germination, rate of germination and seedlings growth of four medicinal plants (*Coriandrum sativum* L., *Portulaca oleracea* L., *Matricaria multiflora* and *Coriandrum sativum* L.). The experiment was carried out based on completely randomized design with three replicates. Results showed that the effect of water potential on germination percentage, rate of germination, root and shoot length were significant. With decreasing water potential, germination percentage and rate of germination declined but the response of plant were differ. Germination of *Portulaca oleracea* was not affected by decreasing water potential whereas others significantly decreased. The effect of salt composition was significant on rate and percentage germination. The percentage of germination at lower water potential (-0.37 MPa) which made by NaCl + CaCl2 significantly was higher than the same water potential made by only NaCl and CaCl2. Although, percentage and rate germination of *Portulaca oleracea* was not affected by different water potential, seedling growth of *Portulaca oleracea* significantly decreased. Acknowledgement: I would like to express my appreciation for research deputy of the Ferdowsi University of Mashhad for financial support.

**PE7**

**Effects of Different Level of Nitrogen and Phosphorous Fertilizers on Yield Quantity and Quality of Matricaria recutita**

Dadkhah A, Amini Dahaghi M, Rasam G

 Ferdowsi University of Mashhad, Mashhad, Iran

In order to investigate the effects of different levels of nitrogen and phosphorous fertilizers on qualitative yield and some quality components of German chamomile (*Matricaria recutita* L.), a factorial experiment was based on a randomized complete block design with four replications. The experiment was carried out in the Medicinal Plant Research Farm of Shirvan College of Agriculture in 2007. Nitrogen had three levels (0, 100, 200 kg/ha as source of urea) and phosphorous also had three levels including 0, 100 and 200 kg/ha. The experiment was carried out in a randomized complete block design with four replications. Results showed that the effects of water potential, type of salt on germination percentage, rate of germination, root and shoot length were significant. With decreasing water potential, germination percentage and rate of germination declined but the response of plant were differ. Germination of *Portulaca oleracea* was not affected by decreasing water potential whereas others significantly decreased. The effect of salt composition was significant on rate and percentage germination. The percentage of germination at lower water potential (-0.37 MPa) which made by NaCl + CaCl2 significantly was higher than the same water potential made by only NaCl and CaCl2. Although, percentage and rate germination of *Portulaca oleracea* was not affected by different water potential, seedling growth of *Portulaca oleracea* significantly decreased. Acknowledgement: I would like to express my appreciation for research deputy of the Ferdowsi University of Mashhad for financial support.
of nitrogen and phosphorous fertilizers to the soil imposed a significant effects (p < 0.01) on plant height, number of branches per plant, number of flower per plant and flower yield. The main effects of nitrogen fertili-
zer appeared in improvement of vegetative growth of plants. Plants treated with 200 kg nitrogen per hectare and 60 kg phosphorous per hect-
tare significantly produced the highest flower yield per square meter. Fertilizer did not affect the percentage of chamazulene. However, plant treated with 200 kg nitrogen and 60 kg phosphorous produced highest essential oil and chamazulene per square meter due to higher flower yield.

Acknowledgement: I would like to express my appreciation for research deputy of the Ferdowsi University of Mashhad for financial sup-
port.


Enantiomeric composition of α-pinene in essential oils of leaves and unripe cones of
Juniperus communis L.

Loziene K, Labokas J
Institute of Botany of Nature Research Centre, aliu˛j u˛E zˇeru˛
LT-08406 Vilnius, Lithuania

The leaves and unripe cones of common juniper (Juniperus communis L.) were sampled from 11 habitats across Lithuania. The essential oils of leaves and cones from 110 samples (+trees) each were isolated by hy-
drodistillation in a European Pharmacopoeia apparatus during two hours. The enantiomers of α-pinene were separated by the chiral-phase capillary GC and identified by matching their retention times to the optically pure analytical standards. The average content, the min-max values and the variation coefficient of (1R)+(α)-α-pinene were 74 ± 1%,
7 – 92% and 16.4% in leaves and 69 ± 2%, 0 – 96% and 24.9% in unripe cones, respectively; the average content, the min-max values and the variation coefficient of (1S)-(α)-α-pinene were 26 ± 1%, 8 – 93% and
45.8% in leaves and 31 ± 2%, 4 – 100% and 55.7% in unripe cones, respec-
tively. It was established that the most of samples of leaves and unripe cones of the studied J. communis individuals were rich in (1R)+(α)-
α-pinene, while (1S)-(α)-α-pinene dominated in 2.7% samples of leaves and
10.8% those of unripe cones only. However, the (1S)-(α)-α-pinene absolutely predominated in leaves of two J. communis individuals. The absolute predomination of the (1R)+(α)-α-pinene was not detected neither in leaves nor in unripe cones. Acknowledgement: This research was funded by a grant (No. MIIP-56/2010) from the Research Council of Lithuania.

Antifungal activity and chemical composition
of Mentha cervina L. essential oils.

Gomes A¹, Delgado P², Timóteo T², Rodilla J³, Silva L³
¹Department of Química, Universidade da Beira Interior,
Rua Marquês d’Avila e Bolama, 6200-Covilhã, Portugal;
²Escola Superior Agrária, Quinta da Sra. de Mércules 6001 –
099 Castelo Branco, Portugal; ³Departamento de Química,
Universidade de Evora, Rua Romão Ribeiro, 6001-901,
Portugal; ⁴Unidade de Materiais Têxteis e Papeleiros,
Universidade da Beira Interior, Rua Marquês d’Avila e
Bolama, 6200-Covilhã, Portugal

Mentha cervina L. is a very aromatic plant with a characteristic flavour,
which can be found on some regions from central eastern and south-
ern Portugal. In the present paper, we analysed the chemical composition of essential oils from fresh and dried leaves of M. cervina and its antifungal activity against strains of Aspergillus niger, Penicillium sp. and Fusarium oxysporum isolated from soils. M. cervina was collected in Almaceda – Vila Velha de Ródão, Central Eastern of Portugal during the flowering period. Yellowish essential oils were obtained in a yield 1.1% and 1.8%
(±/v/v) to fresh and dry plant, respectively. Major component of the oils was identified as pulegone (78.0 and 80.4%). Both essential oils of M. cervina at the doses of 10 μL, inhibited totally the growth of the tested fungi. Doses of 5 μL of each essential oil also showed activity against the fungi strains used in this work, in particular against Penicillium sp. Tak-
ing into account the high level of pulegone observed in both essential
oils and the antimicrobial activity of this compound reported by Duru [1], these results suggest that this compound could be the main responsible component for the antifungal activity observed. References:
43 – 48.

The effect of drying temperature, storage and distillation times on the essential oil content and composition of anis hyssop (Agastache foeniculum). The treatments were two levels of temperature (ambient temperature and 40°C), two levels of material storage time (immediately after drying and distillation) and two levels of distillation time (2 and 4 h). The treatments were arranged in factorial design in base of Complete Randomized De-
sign (CRD) with three replications. The findings show that essential oil content was declined with increasing temperature degree and storage time. Distillation time did not have a significant effect on the composition of the essential oil extracted from anis hyssop.

The volatiles obtained by hydrodistillation and methanol extraction of the aerial parts of Ferulago carth ochorus Boiss. et Hausskn. and Levisticum officinale Koch., two Umbelliferae species of Iran, were analyzed by GC and GC/MS. The oil and the extract obtained by hydrodistillation and extraction of F. carth ochorus were characterized a high amount of mono-
terpene hydrocarbons (93.8% and70%, respectively). The main components of the oil and extract were (Z)-α-pinene (13.1% and 6.0%), α-Phellandrene (12.7% and 8.3%) and β-
-Phellandrene (10.9% and 8.8%), respectively. The water distilled oil and methanol extract of the air-dried Levisticum officinale, were also both rich in monoterpene (42.8% and 52.9%, respectively). In the oil, α-terpi-
line acetate (40.5%) and β-Phellandrene (16.7%) were the main constitu-
ents, whereas in the extract, β-Phellandrene (23.0%), naphthalene
(20.6%) and γ-terpinene (12.1%) were the major components. Refer-
Oil Res 15: 143 – 144. 3- Sedaghat S, Khorrossai M, Masoudi S, Larjani K and

Major volatile compounds of 50 Thymus taxa
naturally grown in Antalya region of Turkey.

Karaç K¹, Elmasulu S², Kürkçüoğlu M³, Ince AG¹, Chun A¹,
Onus A¹, Bayer KHC²,² Turgut K¹
¹Akdeniz University, Faculty of Agriculture, 07059 Antalya, Turkey; ²Ankara University, Faculty of Pharmacy, 26470 Esiktepe, Turkey; ³King Saud University, College of
Science, Botany and Microbiology Department 11451
Riyadh, Saudi Arabia

A large number of medicinal and aromatic plant species naturally grown in the Mediterranean Basin of Turkey contain secondary metabolites
that are used in the food, pharmaceutical, cosmetic, and pesticide in-
dustries [1 – 5]. This study used 50 taxa consisting of 9 species or sub-
species of the genus Thymus grown wild in Antalya area in the Mediter-
nanean part of Turkey to determine their volatile compounds. The major constituents of the volatile constituents noted in six taxa of Thymus
longicaulis C.Presl subsp. chaubardii (Boiss. & Heldr. ex Rchb.f.) Jalas var. chaubardii were borneol, nerol, geraniol, thymol, γ-terpinene, 1,8-
cineole and linalool. The main components of these taxa of T. silypeus Boiss. subsp. silypeus var. rosulans (Borbas) Jalas

References:
Oil Res 15: 143 – 144. 3- Sedaghat S, Khorrossai M, Masoudi S, Larjani K and
wereα-pinene,1,8-cineole,β-caryophyllene,α-terpineneand interme-
diol. Threetaxa ofT. sylvestrex Boiss. subspxyloides in the Mediterranean basin of Turkey contain and produce essential oil [1]. Fresh aerial parts of koalz are used as a flavoring agent for local dairy products. Ourprevious studies showed that this plant is a rich source of furanocoumarins like xanthotoxolin and Angelisins (2). In this study the essential oil of the leaves, roots and fruits of the plant obtained by hydrodistillation and analyzed by GC and GC/ MS. Average yieldsof essential oil were 1.5%, 2.5%, 0.3%, for leaves, roots and fruits respectively. The most abundant components of the leaves were trans-sabinene (22.5%), α-phellandrene (19.0%), linalool (7.3%) and cis-cimine (6.8%). The root essential oil was characterized by high amounts of α-phellandrene (20%), α-pinene and α-terpinolene (12%). The main components of the fruits were α-phellandrene (17.1%), γ-terpinene (16.9%), limonene (13.2%) and α-terpinolene (11.2%). References: 1) Niguel S (2007) Flora of Iran. Research Institute of For-

are 63 taxa of eight Origanum species grown in the Mediterranean region of Antalya, Turkey were DNA typed using microsatellite markers, and oil compositions of these taxa were determined using methods described in [2,3,4,5]. All the 8 Origanum species were separated from one another according to classical taxonomic groups using DNA markers. Individuals of two O. vulgare L. subsp. hirtum (Link) latsw., two O. ma-

jorana L., two O. sylvestre P.H.Davis and two O. saccharum P.H.Davis taxa could not be differentiated in the DNA typing studies. There were high levels of similarities between a dendrogram obtained from DNA markers and oil composition types among the taxa studied. O. bilgeri P.H.Davis consisted of two chemotypes (carvacrol and α-thujene) and they were clearly separated by DNA analyses. O. husnak-

baseri H.Duman, Aytat et A.Duran was also separated from other species and it was the only species containing trans-sabinene hydrate. Taxa collected from Elmali location of O. onites L. were linalool types and they were distinctly separated from other individuals within the species. O. majorana consisted of two chemotypes (carvacrol and linalool). In conclusion, present study indicated that chemotypes could be identified using DNA markers. Thus, DNA markers developed in this study could be used in the identification of species in herbal mixtures, selecting the individual plant for desired oil compositions and the most importantly these DNA markers are valuable in Origanum improvement programs. Acknowledgement: This work was supported by the Scientific and Technological Research Council and The Scientific Research Projects Coordination Unit of Akdeniz University. References: 1) Ince AG, Karaca M (2006) Sci Food Agric 89: 168 – 176, 2) Karaca M et al. (2005) Anal Biochem 343: 353 – 355, 3) Karaca M et al. (2008) Sci Food Agric 88: 2508 – 2516, 4) Ince AG et al. (2010) Biochem Genet 48: 83 – 95, 5) Cos-
Ferula gummosa Boiss. from Umbelliferae family (Bardide in Persian and Galbanum in English) is one of the most important medicinal plants of Iran mountains area which has industrial applications, too. The northern steeps with 2000 – 4000 altitude and soils of well drained and deep, rich Iran mountains area which has industrial applications, too. The northern steeps with 2000 – 4000 altitude and soils of well drained and deep, rich

**PE16**

Essential oil variability and trichomes morphology from *Lavandula pedunculata* (Mill.) Cav. grown at *Mata Experimental do Escaroupim* (Portugal)


The Experimental Forest of Escaroupim [*Mata Experimental do Escarou-pim* (MEE), Salvaterra de Magos, Portugal], is a protected forest area with over 175 years and under the Total Forestry Regime since 1901. *Lavandu-la pedunculata* Cav. is an aromatic herb in the Iberian Peninsula [1], and frequent in the understory of the MEE Pinus, Quercus, Ulmus and Eucalyptus spp. forests. In the present work, the essential oils and trichomes morphology from flowering aerial parts of two populations of *L. pedunculata* collected in two years were evaluated. The essential oils were isolated by hydrodistillation, and residue obtained by GC and GC-MS [2]. The indumentum of *L. pedunculata* field grown plants was studied by LM and SEM, according to [3]. *L. pedunculata* essential oils were obtained in an average yield of 2% (v/w); Thirty six components were identified, representing 97 – 99% of the total essential oils, which were dominated by fenchone (62 – 70%) and 1,8-cineole (6 – 28%). cis-Verbenol (traces-5%), camphor (1 – 5%) and limonene (traces-4%) were also relatively abundant. Previous studies also showed essential oils fenchone-, 1,8-cineole- and camphor-rich [2,4,5]. *L. pedunculata* showed a morphologically complex indumentum of i) non-glandular uni- and bi-cellular unbranched trichomes and multi-cellular branched trichomes of the stellate type; ii) peltate and capitate trichomes, the last with three different morphological types; iii) multi-cellular branched stellate type with only glandular arms and iv) multi-cellular branched stellate type with both glandular and non-glandular arms. These results are in agreement with a previous study on the indumentum of *L. pedunculata* and in vitro-grown plants [5]. Acknowledgement: Telémuno Nunes, Paula Pires, A. Sofia Borges, Prof. Ana Monteiro. References: 1. Morales R (2010) Flora Iberica, Lavandula. Vol. XII. Real Jardim Botânico. CSIC. Madrid 2. Matos F et al. (2009) J Essent Oil Res 21: 327 – 336. 3. Antunes T, Sevi-nate-Pinto I (1991) Flora 85: 65 – 70. 4. Zussarte M et al. (2009) Chem Biodivers 6: 1283 – 1299. 5. Zussarte, M et al (2010) Ind Crops Prod 32: 580 – 587.

**PE17**

Comparative assessment on efficiency and compounds of *Ferula gummosa* Boiss. essential oils at two different harvesting areas of Alborz mountains in Iran

Gavrilov A, Hossetini Gezir A, Pananich AR, Shakare R

1International Desert Research Center, University of Tehran, Tehran, Iran; 2Behbahan Higher Education Complex, Traditional Pharmacy, Faculty of Traditional Medicine, Tehran University of Medical Sciences and Medicinal Plant Research Center, Tehran 1417614441, Iran; 3Research Institute of Forests & Rangelands, Tehran, Iran

*Ferula gummosa* Boiss. from Umbelliferae family (Bardide in Persian and Galbanum in English) is one of the most important medicinal plants of Iran mountains area which has industrial applications, too. The northern steeps with 2000 – 4000 altitude and soils of well drained and deep, rich of humus and different quantity of lime are the best growth region for Alborz, as ecological investigation have been shown. Galbanum exudes sap-like exudate which contains oils, 60 – 75% resins and 5 – 30% carbohydrates, roughly1. It must be noticed that no alkaldoids and phenol compounds have been found; except some trace of saponin and tannin. Non-carbohydrate portion of Galbanum can be extracted with ethanol. Incineration is used to determine the quality of exudates, results in ash content that must not exceed 10%. In this study, the percentage and essential oil components of Galbanum were investigated. The oleo-gum-resin was submitted to steam distillation. The essential oil was subjected to column chromatography. Gas chromatography was done and careful analysis by GC and GC-MS. Essential oils were isolated by hydrodistillation, and residue obtained by GC and GC-MS. The oils yield for leaves and flowers of S. virgata were 21.1%, germacrene-D (13.2%), bicyclogermacrene (7.0%), α-caryophyllene (8.2%). The Major components of flowers oil were β-caryophyllene (24.6%), 1,8-cineole (23.3%), β-thujone (20.7%) and α-thujone (17.1%) as major components in this plant. The essential oil, having a very strong odor and acid taste, is described as neurotoxic due to the high thujone content [3]. The composition of the volatile oil in *Artemisia spicigera* varies widely according to geographical location, climate, day length, soil type and cultivar [1]. References: 1. Aleskerova AN et al. (1986) Khim Prir Soedin 1:116 – 117. 2. Bremer K and Humphries C J (1993) Bull Br Mus (Nat Hist) Bot 23: 71 – 171. 3. Miller Y et al. (1981) Clinical Toxicology 18: 25 – 32. 4. Naseri HR et al. (2009) Cytologia 74(1): 56 – 64.

**PE18**

Essential oil composition of *Artemisia spicigera* C. Koch

Naseri H1, Azarnivand H, Jalili A, Sefidan P2

1Department of Coexisting with Desert, International Desert Research Center, University of Tehran, Tehran, Iran; 2Research Institute of Forests & Rangelands, Tehran, Iran

Many species of Salvia are aromatic and rich in essential oils and are called Marjoram goli in Persia (1). Some species are used in cosmetics, perfume, pharmacy and aromatherapy and as species (2). Salvia virgata Jacq. and *S. verticillata* L. (Lamiaceae) were collected from Chalus (Gach-sar), in Mazandaran Province, in north of Iran. Hydrodistilled essential oils from the leaves and flowers of these plants were analyzed by GC and GC-MS. The oils yield for leaves and flowers of S. virgata were 0.15% and 0.19% (v/w), 19 and 30 compounds of the essential oils were identified respectively. The main constituents of the leaves oil were phytol (29.1%), β-caryophyllene (19.2%), caryophyllene oxide (17.0%) and hexadecane acid (8.2%). The Major components of flowers oil were β-caryophyllene (21.1%), germacrene-D (12.3%), bicyclogermacrene (10.3%), α-caryophyllene (6.7%) and β-pinene (6.7%). The oils yield for leaves and flowers of *S. verticillata* were 0.1% and 0.12% (v/w); which 54 and 36 compounds were identified respectively. β- Caryophyllene (13.6%), β-phellandrene (12.9%), germacrene-D (11.5%), β-pinene (7.5%) and α-humulene (5.6%),

**Chemical Composition and Antioxidant Activity of Salvia virgata Jacq. and S. verticillata L. Volatile Oils from Iran**

Sarbanon S1, Moammi F1, Kamalinejad M2, Yassa N1

1Department of Pharmacognosy, Faculty of Pharmacy, Tehran University of Medical Sciences and Medical Plant Research Center, Tehran 1417614441, Iran; 2Department of Traditional Pharmacy, Faculty of Traditional Medicine, Tehran University of Medical Sciences and Medical Plant Research Center, Tehran 1417614441, Iran; 3Department of Pharmacognosy, Faculty of Pharmacy, Shahid Beheshti University of Medical Sciences, Tehran, Iran

Salvia virgata Jacq. and *S. verticillata* L. (Lamiaceae) were collected from Chalus (Gach-sar), in Mazandaran Province, in north of Iran. Hydrodistilled essential oils from the leaves and flowers of these plants were analyzed by GC and GC-MS. The oils yield for leaves and flowers of *S. virgata* were 0.15% and 0.19% (v/w), 19 and 30 compounds of the essential oils were identified respectively. The main constituents of the leaves oil were phytol (29.1%), β-caryophyllene (19.2%), caryophyllene oxide (17.0%) and hexadecane acid (8.2%). The major components of flowers oil were β-caryophyllene (21.1%), germacrene-D (12.3%), bicyclogermacrene (10.3%), α-caryophyllene (6.7%) and β-pinene (6.7%). The oils yield for leaves and flowers of *S. verticillata* were 0.1% and 0.12% (v/w); which 54 and 36 compounds were identified respectively. β-Caryophyllene (13.6%), β-phellandrene (12.9%), germacrene-D (11.5%), β-pinene (7.5%) and α-humulene (5.6%)}

Planta Med 2011; 77: 1229–1472 Georg Thieme Verlag KG Stuttgart · New York · ISSN 0032-0943
were dominant substances of leaves oil. Spathulene (23.6%), β-caryophyllene (17.2%), caryophyllene oxide (16.4%) and sabine (8.4%) were the main compounds of flowers oil. Antioxidant capacities of the oils were measured using DPPH (2, 2-diphenyl-1-picrylhydrazyl). Results showed that the antioxidant properties of 50 μl of S. virgata flowers oil was equal with 200 μg/ml of BHA (P < 0.05), but flowers oil of S. verticillata and leaves of S. virgata and S. verticillata were not as potent as BHA (P > 0.03). References: 1. Mozaffarian V (1996) A Dictionary of Iranian Plant Names. Farhang Moaser Publisher, Tehran. p. 207. 2. Ozcan M et al. (2003) Flav Frag J 18: 325 – 327.

Lemon thyme (Thymus x citriodorus (Pers.); Schreb. (synonym T. fragrans citrius, T. serpyllum citriodorus) is widely grown as ornamental plants in Turkey and has no natural distribution. It's a hybrid of Thymus vulgaris L. and Thymus pulegioides L. Compared with other Thyme species, Thymus citriodorus has a herbaceous oblique structure and rhizome growth. By means of this growth pattern, the species was firstly cultivated in a field experiment and its yield and quality parameters were recorded in semiarid conditions. Field trials were conducted in the Central Research Institute for Field Crops, Ankara, at complete randomized block design with four replications during the years of 2009 and 2010. Total yield of green herb, drug herb and drug leaf were 3769 – 4707 kg/da, 1193 – 1475 kg/da and 742.3 – 844.2 kg/da, respectively. The species has been widely accepted as traditional medicine among people. We report on the essential oil composition of Thymus kotschyanus cultivated in Hamadan. Aerial parts of dry plant were steam distilled for three hours using a Clevenger-type system. Essential oils were dried with anhydrous sodium sulfate and kept in amber vials at 4°C until chromatographic analysis. The essential oil was isolated in yield of 0.6% (w/w) the yellowish oil.


**Phytochemical analysis of essential oil from Rosmarinus officinalis L. of Iran**

Rosemary (Rosmarinus officinalis L.) belongs to the family Lamiaceae (Labiateae) and has been a very important medicinal and aromatic plant since earliest times. It is a small evergreen which grows in most Mediterranean countries, southern Europe and in the littoral region through Minor Asia areas wildly. The main producers are Italy, Dalmatia, Spain, Greece, Turkey, Egypt, France, Portugal and North Africa. Rosemary is a food flavoring and medicinal herb for its powerful antibacterial, antifungal properties, and as a chemo preventive agent. Owing to its antioxidant properties of leaves, R. officinalis has been widely accepted as one of the spices with the highest antioxidant activity. Rosemary essential oil is also used as an antibacterial, antifungal and anticoncancer agent [1, 2, 3]. The aerial parts of R. officinalis grown at Hamadan in the west of Iran were hydrodistilled for 3 hours, using a Clevenger-type apparatus to yield 2.08% (w/w) of pale yellow oil. The essential oil was dried over anhydrous sodium sulphate and stored in a sealed vial at +4°C until analysis. The oil was analyzed by GC and GC-MS. The constituents of the essential oil were identified by comparison of their mass spectra and retention indices (RI) with those given in the literature and authentic samples [4]. Twenty-nine compounds were characterized in the essential oil of Rosmarinus officinalis, representing 98.5% of the oil, of which α-pinene (22.4%), 1,8-cineole (10.8%), verbeneine (10.2%), camphor (9.9), camphone (8.4%) were found to be the major components.

**Analysis of the essential oil of Thymus kotschyanus from Iran**

The genus Thymus (Labiateae) consists of about 215 species of herbaceous perennials and subshrubs. This genus is represented in Iranian flora by 14 species, four of which are endemic. Studies indicating Thymus species have strong antibacterial, antifungal, antiviral, antiparasitic, spasmolytic and antioxidant activities [1, 2, 3] Among them, Thymus vulgaris L. and Thymus kotschyanus Boiss. et Hohen known as two members of thyme species is used as traditional medicine among people. We report on the essential oil composition of Thymus kotschyanus cultivated in Hamadan. Aerial part of dry plant were steam distilled for three hours using a Clevenger-type system. Essential oils were dried with anhydrous sodium sulfate and kept in amber vials at 4°C until chromatographic analysis. The essential oil was isolated in yield of 0.6% (w/w) the yellowish oil.


**Determination of the chemical main composition and anti bacterial effectiveness of the volatile oils in two in Syrian wide spread plants Microcera rubestris L., Mentha viridis L. (Lamiaceae)**

Microcera rubestris L., Mentha viridis L. (Lamiaceae), two widespread plants in Syria are commonly used in herbal medicine. The aim of this research was to determine the chemical composition and antibacterial activities of the volatile oils in dried leaves of these plants. Using water distillation method, the volatile oils were obtained and their main chemical composition was determined using GC/MS. To determine the effectiveness of the volatile oils against bacteria, disk diffusion methods, MIC and MBC, were used [2]. The results showed that the yield of volatile oil was 1.2% in Microcera rubestris (main components β-caryophyllyene 49% and piperitone oxide 29%), the best antibacterial effect was against Staphylococcus aureus and 1.5% in Mentha viridis (main components carvone 53% and limonene 21%, the best antibacterial effect against Micrococcus luteus).
The effects of different drying methods (natural method, oven and microwave) on drying time, essential oil content and composition of Savory (Satureja hortensis L.), this experiment was carried out during 2008. The experimental design was completely randomized block design with three replications and treatments were: two temperatures: 50 and 70°C, six microwave powers: 100, 180, 300, 450, 600 and 900 w and drying in shaded and sunny area. The drying process was continued until the mass of the sample reduced to a moisture content of about 0.10 on a dry basis or 10% on a wet basis. The results indicated that different treatments of drying had a significant effect on drying time and essential oil content. Minimum and maximum drying times (4.5 minutes and 96 hours respectively) obtained at 900 w microwave power and drying in shaded area. The maximum essential oil content (3%) obtained at drying by 70°C and drying in shaded area and minimum content (0.9%) obtained at drying in sunny area. 100 and 300 w microwave powers had average content of essential oil (2/3%). Maximum carvacrol content (63.9%) obtained at 300 w microwave drying. Maximum γ-terpinene content (28.2%) obtained at drying by 70°C that it had little difference with 50°C, 100 and 300 w. According to the results, because of reduction of drying time and suitable essential oil content and composition in drying by low microwave powers, these methods counseled. References: 1. Szumny A et al. (2009) Food Engin 97(2): 253 – 260. 2. Soysal Y (2004) Food Engin 89(2): 167 – 173.

Chemical composition variation in essential oils of Calamintha hispidula (Boissier and Reuter) Mazrae, endemic in North-eastern Algeria; Sehdi M1, Lahouel M2

Laboratory Phytochemistry and Pharmacology, Faculty of Sciences, University of Jijel, Ouled Aissa BP 98 Jijel 18000 Algeria; 2Laboratory Toxicology Department of Molecular and Cellular Biology, Faculty of Sciences, University of Jijel, Ouled Aissa BP 98 Jijel 18000 Algeria

The essential oils from three samples at the full blossom stage of wild Calamintha hispidula Boiss. and Reut. M. native of the mountain of Tex- anna, (Algeria), (one sample taken at the altitude of 625 m south facing and the other two at 526 m and 620 m north facing), have been ex-tracted by hydrodistillation and analysed by GC-MS. The oil yileds were 1.06%, 0.59% and 1.49%, respectively. The main essential oil constituents were isomenthone (68.7%, 68.2% and 57.9%), pulegone (18.1%, 15.1% and 22.2%), and piperitone oxide (13.6%, 6.6% and 22.2%), respectively. The variation in the yields of essential oils was considerable between the two altitudes 526 m, 620 m north facing. Isomenthone was found to be the major constituent in all cases. Acknowledgement: The authors are grateful to Mr Gerhard de Bèlair Lecturer at the University of Anaba for species identification and writing assistance, and Mr Desdous Abderrachid for the GC/MS analysis.

Investigation on Development of Zein Antimicrobial Edible Film and Essential oil of Zataria multiflora Boiss. on Camphor, Salvia enteralidis, Listeria monocytogenes, Escherichia coli and Staphylococcus aureus Ghasemi S1, Khosravi Darani K2, Haji Seyed Javadi N1, Moradi M3, Romiehie A4, Esmaeili S5

1Department of Food Technology Research, National Nutrition & Food Technology Research Institute, Shahed Beheshti Medical University. P.O.Box: 19395 – 4741 Tehran, Iran; 2Department of Food Hygiene and Quality Control, Faculty of Veterinary Medicine, Uromia University, P.O. Box: 1177, Uromia, Iran; 3Iran Polymer and Petrochemical Institute, Pajouhesh Blvd, km-15, Tehran-Karaj Freeway, Tehran, Iran; 4Traditional Medicine & Materia Medica Research Center, Shahid Beheshti University of Medical Sciences P.O.Box 14155 – 6354, Tehran, Iran

Active packaging is a type of packaging that can control or react to things arranged inside (2). An antimicrobial Active Packaging is made by incorporating antimicrobial agents in food packages. Zataria multiflora Boiss. is a plant that belongs to the Lamiaceae family and grows...
only in Iran, Pakistan and Afghanistan (1). The antimicrobial activities of the plant are also well established against a wide variety of bacteria (3). In this study zein is based on the films that contain essential oil of Zataria multiflora (0, 1%, 2%, 3% and 4% w/v) and glycerol (30% v/v).

The objective of this study was to determine the effectiveness of the edible film in inhibiting Salmonella enteritidis. The main constituents of the essential oils were determined by the DPPH test. The essential oil of T. minuta exhibited the highest antioxidant in vitro (EC_{50}= 0.8 mg/ml), compared to T. elliptica (EC_{50}= 3.4 mg/ml) and T. filifolia (EC_{50}= 20.2 mg/ml). Mosquito larvicidal tests were runned against third instar larvae of Aedes aegypti, considered an important vector of dengue and yellow fever. Essential oil from T. filifolia showed the strongest larvicidal activity (LC_{50}= 47.7 ppm), compared to T. minuta (LC_{50}= 52.3 ppm), and T. elliptica (LC_{50}= 111.0 ppm). The essential oil of Tagetes filiformia might be considered as a natural alternative to chemical larvicides for the control of Ae. aegypti.


**PE29**

Effect of different solvents for seed oil extraction of Melia azedarach L. (Meliaceae) on insecticidal activity of this plant extract

Zamani Dehqahobi K1, Ahmadi K2, Salari E1, Najmzadeh H3
1Department of Plant Protection, Faculty of Agriculture, Shahid Bahonar University of Kerman, Kerman, Iran; 2Researcher of Young Researchers Society, Shahid Bahonar University of Kerman, Kerman, Iran; 3Department of Plant Protection, Faculty of Agriculture, Shahid Bahonar University of Kerman, Kerman, Iran

Developing of new pesticides from botanical pesticides has been attempted in the past [1]. Botanical pesticides are safe to use in different propose and IPM program [2]. Melia azedarach L. (Meliaceae) is characterized by containing allelochemicals compounds, with a high level of bioactivity against insect. In this study M. azedarach seeds were collected from Kerman, Iran. The seeds were milled and powdered mechanically using commercial electrical stainless steel blender to extract their oil. The experiments were directed to determine the effect of different solvents on insecticidal activity of seed oil of M. azedarach on 3–4-day-old individuals of Aphis fabae Scopoli. The oil was extracted with different solvents. The solvents were included acetic acid, aceton and n-Hexane. All experiments were conducted by topical test bioassay in laboratory, at 25 ± 1°C temperature, relative humidity of 60 ± 10% and 16 hours of artificial light at an intensity of about 4000 lux. Water and DMSO (Dimethyl sulfoxide) were used as control treatments. The results indicated that the concentration 80 μl/ml, the mortality of A. fabae treated with n-Hexanec seed extract of M. azedarach after 24 hours, was more than 72%, while it was 40.75% and 60% in acetic acid seed extract of M. azedarach and acetic acid seed extract of M. azedarach respectively. The mortality of A. fabae treated with n-Hexanec seed extract of M. azedarach was significantly higher than acetic acid seed extract of M. azedarach. It could be concluded that some plant extracts may be applicable as a safe insecticide to aphid’s control. References: 1. Isman M (2005) Biopesticides of Plant Origin. Lavosier Tech & Doc. Paris. 2. Raja N et al. (2001) Stored Prod Res 37: 127–132.

**PE30**

Chemical composition, Antioxidant and Mosquito larvicalic activities of essential oils from Tagetes filifolia, Tagetes minuta and Tagetes elliptica from Peru

Ruíz C1, Cachay M2, Domínguez M2, Velásquez C3, Espinoza G1, Ventosilla P3, Rojas B1
1Unidad de Investigación en Productos Naturales, Laboratorios de Investigación y Desarrollo, Universidad Peruana Cayetano Heredia, Lima, Perú; 2Instituto de Medicina Tropical Alexander von Humboldt, Universidad Peruana Cayetano Heredia, Lima, Perú

The chemical compositions of the essential oils from the aerial parts of Tagetes filifolia Lag. ("Anís serrano"), Tagetes elliptica Sm. ("Chinchito") and Tagetes minuta L. ("Huacayata") from Peru were determined by gas chromatography-mass spectrometry. The main constituents of the essential oils were: trans-anethole (88.2%) and methyl chavicol (10.9%) for T. filifolia; trans-ocimene (51.7%), cis-tagetone (17.7%) and cis-ocimene (7.7%) for T. minuta; and 6,7-epoxy myrcene (31.9%), dihydrotagetone (22.0%), trans-ocimene (12.0%) and cis-tagetone (8.0%) for T. elliptica. Antioxidant activities of the essential oils were determined by the DPPH test. The essential oil of T. minuta exhibited the highest antioxidant in vitro (EC_{50}= 0.8 mg/ml), compared to T. elliptica (EC_{50}= 3.4 mg/ml) and T. filifolia (EC_{50}= 20.2 mg/ml). Mosquito larvicidal tests were runned against third instar larvae of Aedes aegypti, considered an important vector of dengue and yellow fever. Essential oil from T. filifolia showed the strongest larvicidal activity (LC_{50}= 47.7 ppm), compared to T. minuta (LC_{50}= 52.3 ppm), and T. elliptica (LC_{50}= 111.0 ppm). The essential oil of Tagetes filiformia might be considered as a natural alternative to chemical larvicides for the control of Ae. aegypti.

**PE31**

Chemical composition of the oil of Cicutic virosa L. from Kazakhstan

Ishmuratova S1, Ozek T2, Ozek G3, Baser KH2,3
1Zhezkazghan Botanical Garden, Zhezkazghan, Karagandinskaya Oblast, 100600, Kazakhstan; 2Department of Pharmacognosy, Faculty of Pharmacy, Anadolu University, 26470 Eskisehir, Turkey; 3Department of Botany and Microbiology, College of Science, King Saud University, 11451, Riyadh, Saudi Arabia

The present work is a part of our ongoing research into the volatiles of Apiaceae species. A well-known toxic plant, water hemlock, Cicutic virosa L. is widely distributed in the temperate regions. Herbal parts of C. virosa were collected in Karkaraly Mountain (Kazakhstan) during blossoming in 2009. The essential oil (yield 0.1%) was obtained by hydrodistillation using a Clevenger type apparatus. Chemical composition of the oil was analyzed by GC-FID and GC/MS methods. The oil was found to be rich in sesquiterpenic compounds with α-farnesene (22.7%), α-humulene (5.4%), humulene epoxide II (5.9%), caryophyllene oxide (3.4%), germacrene D (3.2%) and (ZE)-α-farnesene (3.6%) as major constituents. Among the monoterpeneres myrcene (7.8%) was detected in high percentage. Fatty acids were represented mostly by hexadecanoic acid (8.4%). To the best of our knowledge, the essential oil of C. virosa from Kazakhstan was not investigated previously.

**PE32**

Sulphur containing volatiles of Barbarea vulgaris W.T. Aiton from Kazakhstan

Rakhmadieva SB1, Ozek G2, Marenich M3, Baser KH2,3
1Department of Chemistry, L.N. Gumilyov Eurasian National University, 010008 Astana, Kazakhstan; 2Department of Pharmacognosy, Faculty of Pharmacy, Anadolu University, 26470 Eskisehir, Turkey; 3Department of Botany and Microbiology, College of Science, King Saud University, 11451, Riyadh, Saudi Arabia

Aim of the present study was the investigation of the chemical composition of Barbarea vulgaris W.T.Aiton volatiles. The plant material was collected in Akmolinskaya and Karagandinskaya provinces of Kazakhstan in 2009. Different plant parts (flower, leaf and herb) were subjected to microdistillation to obtain the volatiles. The volatiles were then analyzed by GC-FID and GC/MS methods. The most of samples were rich in sulfur containing compounds. Methyl (methylthio) methyl disulfide (14.5%), dimethyl trisulfide (11.2%), dimethyl sulfide (3.4%) were detected in the herb of B. vulgaris from Akmolinskaya province. Chemical composition of the flower and leaf volatiles was found to show some differences. Isopropyl isothiocyanate (36.7%) was the main constituent in flower, while phytol (25.7%), hexadecanoic acid (9.3%), hexahydrofarne nesyl acetone (7.4%), dodecanic acid (5.5%) and isopropyl isothiocya nate (5.3%) were detected in the leaf. It should be noted that another sample of B. vulgaris from Akmolinskaya province was found to be very different in volatile composition. Namely, bornenol (20.3%), camphene (13.5%), bornyl acetate (8.0%) and germacrene D (5.4%) were the major constituents. In the flower and leaf of B. vulgaris from Karagandinskaya province, methallyl cyanide (50.5% and 12.9%), 3-butenyl isothiocyanate (15.6% and 43.8%) and isoeugenol cyanide (4.2% and 0.9%) were the major
volatile compounds. To the best of our knowledge, the volatiles of B. vulgaris from Kazakhstan were not investigated previously.

**PE33**

Essential oil composition of different populations of *Thymus caryophyllus* from South and South-East in Serbia: Hyssopus officinalis L., Satureja kitabeili L. and Nepeta nuda L.

**PE34**

Antioxidant activity of the essential oils of five species of the family Lamiaceae: *Stiosievic D*, *Stojicevic S*, *Karabegovic F*, *Velickov D*, *Djordjevic S*, *Lazic M*.

**PE35**

Essential oil composition of *Ducrosia assadii* aerial parts and fruits from Iran: *Yasoo N*, *Alahi P*, *Mozaffarian V*.

**Whitening effect and antioxidant activity of essential oils from *Cryptomeria japonica* Kim S, Lee S, Gwak K, Lee F, Choi F.

**References:**

1. Anon. (2004) PDR for Herbal Medicines, 3rd Ed., Thomson PDR at University, Pharmaceutical Science Branch, Tehran, Iran; 2. Department of Agriculture, Medicinal Plants and Drugs Research Institute, Shahid Beheshti University, Tehran, Iran; 3. Department of Research and Development (R&D), Khorraman Pharmaceutical Co., Khorramabad Industrial City, 68135–579, Khorramabad, Iran. Essential oils of the aerial dried aerial parts of seven populations of *Thymus caryophyllus* collected from different localities of Iran were used as standard. Essen-

idant assays, scavenging of 2,2-diphenyl-1-picrylhydrazyl (DPPH) [2] were used. The essential oils were isolated in an airtight container, protected from light, until the analysis. Two antioxidant assays, scavenging of 2,2-diphenyl-1-picrylhydrazyl (DPPH) [2] and FRAP (Ferric Reducing Antioxidant Power) assay [3] were used to evaluate vitamin C antioxidant activities, were used as standard. Essential oil of *Thymus serpyllum* L. exhibited the highest antioxidant activity (EC$_{50}$ = 0.69 ± 0.14 µl/ml), while the lowest activity was determined for *Hyssopus officinalis* L. oil (EC$_{50}$ = 47.50 ± 11.62 µl/ml). Compared with vitamin C (EC$_{50}$ = 0.04 ± 0.05 µg/ml), all essential oils were of lower DPPH antioxidant activity. In the FRAP assay, the reducing power decreased in the following order: *Thymus serpyllum* L> *L-ascorbic acid>* *Origanum vul-

gare* L>Satureja kitabeili L> *Nepeta nuda* L> *Hyssopus officinalis* L. Our results confirm that the traditional use of medicinal and aromatic plants in mitigating oxidative stress is an initiator of many diseases. Acknowl-

**Essential oil composition of *Ducrosia assadii* aerial parts and fruits from Iran:**

1Department of Pharmacognosy, Faculty of Pharmacy, Tehran University of Medical Sciences and Medicinal Plant Research Center, Tehran 1417644111, Iran; 2Islamic Azad University, Pharmaceutical Science Branch, Tehran, Iran; 3Research Institute of Forests and Rangelands, Ministry of Jahed-e-Agriculture, Tehran, Iran.

Thymus species are well known as medicinal plants because of their biological and pharmacological properties. *Thymus caryophyllus* is an endemic species of Iran. Essential oils of the air-dried aerial parts of Thymus populations from different localities of Iran were isolated by hydrodistillation with a yield of 0.41 – 2.9% (w/w). The essential oils were analyzed by combination of GC and GC-MS techniques. Oxygenated monoterpenes were the main group of constituents in all samples, carvacrol (24.9 – 97.6%), Thymol (25.6 – 36.9%), p-cymene (2.9 – 16.1%), and c-terpine (1.3 – 8.1%), represented as the major compounds.

Species in the family Lamiaceae are praised medicinal and aromatic plants. They are used against various inflammations, stomach problems, as expectorant, as well as spices [1]. The aim of the present study was to evaluate the antioxidant activity of essential oils from wild growing herbs in South and South-East in Serbia: *Hyssopus officinalis* L., *Origanum vulgare* L., *Satureja kitabeili* Wierz. ap. Heuff., *Nepeta nuda* L., and *Thymus serpyllum* L. The essential oils were isolated by hydrodistillation in a Clevenger-type apparatus. The resulting essential oil was dried over anhydrous sodium sulfate, filtered and stored at +4°C in a well-filled, airtight container, protected from light, until the analysis. Two antioxidant assays, scavenging of 2,2-diphenyl-1-picrylhydrazyl (DPPH) [2] and FRAP (Ferric Reducing Antioxidant Power) assay [3] were used to evaluate vitamin C antioxidant activities, were used as standard. Essential oil from *Thymus serpyllum* L. exhibited the highest antioxidant activity (EC$_{50}$ = 0.69 ± 0.14 µl/ml), while the lowest activity was determined for *Hyssopus officinalis* L. oil (EC$_{50}$ = 47.50 ± 11.62 µl/ml). Compared with vitamin C (EC$_{50}$ = 0.04 ± 0.05 µg/ml), all essential oils were of lower DPPH antioxidant activity. In the FRAP assay, the reducing power decreased in the following order: *Thymus serpyllum* L> *L-ascorbic acid>* *Origanum vul-

gare* L>Satureja kitabeili L> *Nepeta nuda* L> *Hyssopus officinalis* L. Our results confirm that the traditional use of medicinal and aromatic plants in mitigating oxidative stress is an initiator of many diseases. Acknowl-

**Whitening effect and antioxidant activity of essential oils from *Cryptomeria japonica***


**References:**


The aim of this study was to investigate whitening effect and anti-oxi-
dation effect by determining the tyrosinase inhibition activity, DPPH radical scavenging activity and superoxide dismutase (SOD) like activity of essential oil from *Cryptomeria japonica* D.Dom. Essential oil of *C. japonica* was extracted by steam distillation of Clevenger-type. Essential oil of *C. japonica* was divided into crude oil and 6 fractions by column chromatography and thin layer chromatography. Crude oil and 6 fractions of *C. japonica* essential oil inhibited the oxidation of L-tyrosine and L-dihydroxyphenylalanine (L-DOPA) by mushroom tyrosinase. In tyrosi-

nase inhibitory activity of essential oils of *C. japonica*, the activity was effective at the fraction A (L-tyrosine: 86.76%, L-DOPA: 88.45%) and B (L-tyrosine: 87.53%, L-DOPA: 85.03%). In examination of anti-oxidation activity, the *C. japonica* essential oils were determined using the free radical and stable reductant, 1,1-diphenyl-2-picrylhydrazyl (DPPH) and pyrogallol. Fraction B (44.11%), C (86.91%) and D (44.40%) were highly effective of DPPH radical scavenging, and in examination of SOD like activity, fraction B (96.19%) was appeared as extremely high effec-
tive. Fraction B of *C. japonica* essential oil, effective fraction of whitening activity and antioxidant activity, was mainly consisted of bornyl acet-
ate and neuzukol, which are terpenoids having hydroxyl group. These compounds were inhibition of acting on tyrosinase in melanin biosyn-
thesis and block up DPPH radical scavenging and anti-oxidation by sup-
Salvia is the largest genus of the family Lamiaceae with ca. 900 species distributed around the world. Its centre of origin is considered to be south west and central Asia (1). Salvia is represented in Turkey by 97 species including 4 subspecies and 8 varieties. The rate of endemism in Turkey is 52.5% with 51 species (2). Salvia fruticosa Mill. has a wide distribution in Turkey and its dried leaves are sold in local markets for consumption as herbal tea and dried leaves are exported especially to European Countries. We have distilled essential oils from samples collected from 20 localities by water distillation and analyzed them by GC and GC/MS techniques. Oil yields in the samples varied between 2.0% to 3.0% and the main components were characterized as 1,8-cineole (20.7% to 46.9%), b-caryophyllene (6.0% to 16.9%) b-pinene (5.3% to 11.3%), and camphor (2.8% to 17.5%). References: 1. Hedge I C (1992) A Global Survey of the Biogeography of the Lamiaceae. In: Flora of Turkey and the East Aegean Islands, vol. 5. Edit., P.H. Davis, Edinburgh University Press, Edinburgh, UK. 2. G/Cöner A, et al. (2000) Flora of Turkey and the East Aegean Islands, vol. 5. Edit., P.H. Davis, Edinburgh University Press, Edinburgh, UK. 3. Aslan, S., et al. (2001) Flora of Turkey and the East Aegean Islands, vol. 5. Edit., P.H. Davis, Edinburgh University Press, Edinburgh, UK. 4. Turkoglu I et al. (2003) Bot J Linn Soc, 143:207 – 212

Vitex agnus-castus L. (Lamiaceae) is widely distributed in Turkey, mainly in coastal areas of the West and the South West. The fruits are used in folk medicine to treat illnesses and the essential oil obtained from the fruits is known as a substitute for kekik oil in Turkey (1). Essential oil compositions of the fruits of Vitex agnus-castus collected from 5 different regions, Balikesir (Aynoluk) 1, Mugla (Bodrum) 2, Antalya (Manavgat) 3, Edirne (Enez) 4 and Zonguldak (Devrek) 5 were analyzed by GC and GC-MS to document the variability in their composition. The results indicate chemovariability in oils of the fruits sourced from different sites. There are also marked differences in contents of the major constituents. Sample 2 and 3 contains 1,8-cineole [17% (1), 13.2% (2)], sabine [12.8% (2), 12.1% (3)], b-caryophyllene [12.7% (2), 11.4% (3)] and bicyclogerma-cene [11.0% (2), 12.1% (3)] as major constituents respectively. Sabine [15.4%), 1,8-cineole (17.3%) and bicyclergemacene (12.1%) are the major constituents together with (Z)-b-farnesene (13.5%) in the sample 1. The highest content of bicyclogerma-cene (22.1%) is shown in sample 4. Sample 5 is the only one which contains α-pinene (10%) as the main constituent. References: Reference: 1) Baytop T (1999) Türkiye‘de Bitkiler ile tedavi (Therapy with plants in Turkey), 2nd ed. Nobel Tip Kitabevleri Ltd, Istanbul


Phoenix dactylifera L. essential oil: Chemical composition, antimicrobial and insecticidal activities

Demirci B1, Alqausoumi S2, Al Rehaily AJ2, Al Yahya MA2, Yunufoglu HS1, Tabanca N3, Wedge DE3, Demirci F4, Becnel JJ5, Bernier UF5, Basar KHC3,4
1Department of Pharmacognosy, College of Pharmacy, Al Kharj University, Al Kharj, Saudi Arabia; 2Department of Pharmacognosy, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia; 3Department of Pharmacognosy, College of Pharmacy, Al Kharj University, Al Kharj, Saudi Arabia; 4USDA-ARS, Natural Products Utilization Research Unit (NPRU), Thad Cochran National Center for Natural Products Research, University of Mississippi, University, MS 38677, USA; 5USDA-ARS-Center for Medical, Agricultural, and Veterinary Entomology, Gainesville, FL, 32608 USA; 6King Saud University, College of Science, Botany and Microbiology Department, 11451- Riyadh, Saudi Arabia

Date palm, Phoenix dactylifera L. (Arecaceae), is very common in the Arabian Peninsula. The essential oil of P. dactylifera from the spathe was obtained by hydrodistillation. The oil was subsequently analysed by GC and GC-MS, simultaneously. Overall, 16 components were characterized representing 99% of the oil. 3,4-Dimethoxytoluene (73.6%) and 2,4-dimethoxytoluene (5.5%) were found as the main components. First the antimicrobial activity of the essential oil was determined using the broth microdilution method against various human pathogens, where a low inhibitory range was observed (MIC 1000 µg/ml). The oil was evaluated further for antifungal activity against the strawberry anthracnose-causing fungal plant pathogens Colletotrichum acutatum, C. fragariae and C. gloeosporioides using the direct overlay bioautography assay. As a result the essential oil showed no antifungal activity at 80 and 160 µg/spot concentrations compared to standard antifungal agents. In addition, the oil was subsequently investigated for its insecticidal properties against Aedes aegypti. The oil showed repellent activity against the yellow fever mosquito A. aegypti using the “cloth patch assay” with 0.051 mg/cm², however, the oil exhibited weak activity against the mosquito’s first instar larvae in a high throughput bioassay and adult topical assay. As a conclusion, the Phoenix essential oil and other fractions may have potential against parasites for further evaluation.
Rosa damascena Mill. is one of the most important Rosa species, for its wide application in perfumery and cosmetics, and as a valuable natural drug possessing diverse effects (1). Therapeutic activities of R. damascena oil and water have been reported both in traditional Iranian medicine and modern pharmacological studies (2). The essential oil is secreted by epidermal cells in petals of Rosa species, but no histological data is available on the mode of secretion in this genus. The present research has focused on the secretory structure in R. damascena petals in relation to flower development. Flowers were collected at four successive ontogenetic stages from Shiraz, south western Iran. Petals were fixed with glutaraldehyde and osmium tetroxide, dehydrated in acetone and embedded in resin. Semithin sections were stained with toluidine blue. The two petal surfaces were distinguishable from the stage with the emerging intensely colored petals. They differed by the shape and content of the epidermal cells. The upper epidermal cells revealed a very dense cytoplasm, and numerous small vacuoles in half open flowers. Their polyphenolic content diminished during flower development. At full bloom, the vacuolar polyphenolic compounds had totally disappeared and only a few cells had a dense cytoplasm. Structural features of the epidermal cells suggest the petals of the half open flowers to be in the most active secretory phase. Further analytical studies will correlate these histological data with the essential oil secretion process during R. damascena flower development. References: 1. Libster (2002) Delmar’s Integrative Herb Guide for Nurses. Delmar’s Thamson Learning, Albany. 2. Rakshashdeh et al. (2007) Iranian Journal of Pharmaceutical Research 6: 193 – 197.

Mastic oil is the essential oil of mastic (masticia), the resin of Pistacia lentiscus L. var. chia Duham (Anacardiaceae), uniquely produced in the Greek island of Chios. It is a valuable product with a small annual yield (~300 kg), as well as mastic itself. Its characteristic odor and its estab-

Ajowan (Trachyspermum ammi Sprague) fruits (Apiaceae) accumulate essential oil in compartments referred to as canals (vittae). Tammi is one of the most important herbs used in Ayurveda (1). Iranian traditional herbal medicine recommends the use of Ajowan fruits also as digestive stimulant. A high proportion of thymol has been selectively demonstrated by Magnetic Resonance Imaging in the essential oil of mature Ajowan fruits (2). It has also been reported that different harvest stages affect the number of oil components in Ajowan fruits (3). The present research has focused on the relationship between the secretion process and reproductive development in Tammi. Flowers and fruits were collected from wild growing plants at 6 ontogenic stages. Samples were fixed with glutaraldehyde and osmium tetroxide, dehydrated in acetone and embedded in resin. Semithin sections were stained with toluidine blue. Six canals were distinguishable in the ovary wall of the partly open flowers. A single layer of orderly arranged cells represented the schizogenous cavity. The cytoplasm of these cells increased in density, and reached the highest level in flowers at full bloom. The immature fruit exhibited the features of the most active secretory phase, i.e., higher amount of the secreted material lining the cavity and maximum width of the canal. The cells underwent post-secretory phase throughout the fruit maturation. They were compressed, with a degenerative protoplast in dry mature pericarp. Results of the present histological study confirm previous analytical data (3) and recommend the use of immature stage for the best efficiency of Ajowan. References: 1. Pathak et al. (2010) Journal of pharmacy Research 3(4): 895 – 899. 2. Gersbach & Reddy (2002) Annals of Botany 90: 253 – 257. 3. Saharkhiz et al. (2005) Journal of Essential Oil Bearing Plants 3:300 – 303.

Structural and developmental studies on secretory epidermis in Rosa damascena Mill. petals
Rajaei H, Mousavi F
Biology Dept, College of Sciences, Shiraz University, 71454, Shiraz, Iran

Quality profile of Chios mastic (mastitia) essential oil
Paraschos S1, Magiatis P1, Skaltsounis A1, Smyrnioudis P2
Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Athens, Athens, Greece; 2Chios Mastiha Growers Association, Kardamada, Chios, Greece

Structural changes in secretory canals associated with fruit development in Ajowan (Trachyspermum ammi Sprague)
Jafari S, Rajaei H
Biology Department, College of Sciences, Shiraz University, 71454, Shiraz, Iran.

Trichomes in Echium amoenum Fisch & Mey petals: A micromorphological survey
Movaghary S, Rajaei H
Biology Department, College of Sciences, Shiraz University, 71454, Iran

Echium amoenum Fisch & Mey (Boraginaceae) grows widely in the northern highlands of Iran. Dried petals of E. amoenum flowers have long been used for their anxiolytic, sedative, anti-inflammatory and analgesic effects in Iranian folk medicine (1). Phytochemical studies revealed a variety of substances of which rosmarinic acid and flavonoids showed antioxidant activity in humans (2). Inhibition of humoral antibody synthesis has also been reported (3). All the published studies on E. amoenum have concentrated on the therapeutic uses and/or the phytochemical analyses. No botanical data is available and the secretory structure has not been reported so far. This study was carried out to provide elements on the morphology and localization of the secretory structure, with regard to floral development of E. amoenum. Petals were collected from Ghazvin, at four developmental stages. Petals were double fixed in glutaraldehyde and osmium tetroxide, dehydrated in acetone, air dried in hexamethyldisilazan, coated with gold and viewed under the scanning electron microscope. The youngest floral buds were densely covered with protective non glandular trichomes of different length. The same trichomes covered the outer epidermis of the young petals emerging from the sepals. Short stalked capitate trichomes, with one globular secretory head were observed between the protective hairs. During development, the number of non glandular trichomes decreased, but capitate trichomes increased in number, reaching their maximum in 3.5 cm long petals at full bloom. Further histochemical studies will elucidate the variable nature of the secreted material, as well as the phases of the secretion process. References: 1. Zargari A (1996) Medicinal Plants, vol. 5. Tehran University Publications. 2. Ranjbar A et al. (2006) eCAM 3(4): 460 – 473. 3. Amirghofran Z et al. (2000) Iranian Journal of Medical Sciences 2(3 & 4): 119 – 124.

Abstracts | 59th International Congress of the GA | 4th–9th September 2011, Antalya, Turkey

Planta Med 2011; 77: 1229 – 1472 Georg Thieme Verlag KG Stuttgart · New York · ISSN 0032-0943
Murraya paniculata (Linn.) Jack, syn. M. exotica Linn, known as orange jessamine, belongs to the family Rutaceae and is commonly grown in gardens is commonly grown in gardens for its glossy green foliage and large clusters of fragrant flowers (1). This plant has been used in ethnomedicine. Infusion of the leaves and flowers of M. exotica is tonic and stomachic. It is said to be aromatic, refrigerant, digestive, and beneficial in rheumatic fever, coughs, giddiness, hysteria, thirst, and burning of the skin (2,3). The essential oil was obtained by hydrodistillation, were analyzed by gas chromatography-mass spectrometry (GC-MS). The antioxidant activity was evaluated using several in vitro studies. The results showed that the essential oils tested differed in their chemical compositions although there is coincidence in the most abundant constituents.

The analysis of Murraya paniculata volatile oil showed the presence of eighteen compounds identified, accounting for 95.1% of the total amount. The major component of both oils was found the Caryophyllene (30%), with the other components in lesser amounts. The antioxidant activity has shown good activity for the inhibition of primary and secondary oxidation products in crude Curcubita oil added at the concentration of 0.02% which were evaluated using peroxide, thiobarbituric acid, p-anisidine values. Moreover, it was further supported by complementary antioxidant assay in linoleic acid system, comparable with synthetic and natural origin antioxidants (butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), carvacrol and timol). References: 1. Roig JT (1974) Plantas medicinales, aromáticas o venenosas de Cuba. Ed. Científico-Técnica. La Habana. 2. Jiangsu New Medical College (1977) Dictionary of Chinese Herbal Medicine; Shanghai Science & Technology Press: Shanghai, China. 3. Pery LM (1980).Medicinal Plants of East and Southeast Asia: Attributed Properties and Uses; MIT Press: Cambridge.

Rosmarinus officinalis L. (Labiatae) is an evergreen shrub native in Mediterranean region which has been used as condiments mainly with meat dishes. It has been cultivated in many herbal gardens also in Korea and used for herbal remedies and etc. To develop the new natural antibiotics from the plant resources against antibiotic-resistant Staphylococcus aureus, the antibacterial activities of the essential oil compounds compared to current antimicrobial drugs. As the results of the experiments, the Rosmarinus oil showed significant inhibiting activities against most of the tested strains with minimum inhibiting concentrations (MICs) between 0.5 – 16.0 mg/ml. Potential synergism between 0.26 and 1.00. In conclusion, the combination of essential oil or 1,8-cineole with antibiotics could be used to reduce the effectiveness of the oil compounds compared to current antibiotic drugs. As the results of the experiments, the Rosmarinus oil showed significant inhibiting activities against most of the tested strains with minimum inhibiting concentrations (MICs) between 0.5 – 16.0 mg/ml. Potential synergism was identified when antibiotics were combined with the oil. The activity of erythromycin, norfloxacin, or oxacillin, against antibiotic-susceptible and also against -resistant strains of S. aureus was enhanced significantly by combination with Rosmarinus oil and its main component, 1,8-cineole with fractional inhibition concentration indices (FICs) between 0.26 and 1.00. In conclusion, the combination of R. officinalis essential oil or 1,8-cineole with antibiotics could be used to reduce the effective dose of antibiotic and to modulate the resistance of S. aureus strains. Acknowledgement: This work was supported by National Research Foundation of Korea Grant funded by the Korean Government (2010 – 07 – 1-M020 – 0006). References: 1) Shin S (2005) Kor J Pharmaco-cog 36: 252 – 256 2) Shin S (2010) Yaktakh Hoeji 54: 122 – 125

Brazil presents the highest floristic genetic diversity of the world. Among the native plants of Brazil is Ocimum sellos Benth., an herbaceous annual plant of Lamiaceae family. This medicinal specie has been used as anti-diarrheic, antispasmodic and anti-inflammatory and these properties have been observed in pre-clinical tests. Leaves of Ocimum sellos were collected in a private property in Pato do Alferes district (Rio de Janeiro State) in 11/2010, and dried at room temperature (25 °C + 2) at shade conditions. Essential oil was obtained by hydrodistillation (Cle-verger-type apparatus) for 4h and analyzed by GC-MS (Shimadzu, QP 5050-D8-5 capillary column – 30 m x 0.25 mm x 0.25 µm). Carrier gas was Helium (1.7mL/min); split ratio: 1:30. Temperature program: 50 °C, rising to 180 °C at 5 °C/min, 180 °C, rising to 260 °C at 10 °C/min. Injector temperature: 240 °C and detector temperature: 230 °C. Identifications of chemical compounds were made by matching their mass spectra and Kovat’s indices (IK) values with known compounds reported in the literature. In the essential oil were found 16 chemical compounds. The major compound characterized was methyl-chavicol (35.3%), followed by trans-caryophyllene (1.8%), germacrene-D (2.9%), bicyclogermacrene (3.3%), germacrene B (0.5%) and spathulenol (0.6%). Anethole was not observed in this essential oil. This results showed that this queptom type is similar to the one observed by Martins [1], founded in Víçosa- Minas Gerais State (Brazil) References: [1] Martini ER (1998) in Ming et al. Plantas Medicinais, Aromáticas e condimentares: avanços na pesquisa agronômica. UNESP. Botucatu. p. 97 – 126.
Chemical composition and antibacterial activity of the essential oil of *Achillea filipendula* (Asteraceae)  
KiyanoSpor V, Fekhari A, Ashghari B, Yousefzadi M
Department of Phytochemistry, Medicinal Plants and Drugs Research Institute, Shahid Beheshti University, G. C. Evin, Tehran, Iran;  
Department of Technology Incubator Center, Qom University of Medical Sciences, Qom, Iran;  
Department of Chemistry, Faculty of Sciences, Shahid Beheshti University, G. C. Evin, Tehran, Iran;  
Department of Biology, Faculty of Science, Tarbiat Modaress University, Tehran, Iran.

There are 19 *Achillea* species available in the Iranian flora and 7 of them are endemic. Various part of different species of the genus *Achillea* are widely used in folk medicine due to numerous pharmacological properties, such as antimicrobial, antiinflammatory, antiallergic and antioxidant activities. The antimicrobial activities of the essential oils and various extracts from several *Achillea* species have been reported before. As far as our literature survey confirms, the antimicrobial activities of leaves and flowers essential oil of *Achillea filipendula* Lam. (Asteraceae) have not been studied before. Although there was a report concerning the chemical analysis of flowers essential oil, the chemical composition of leaves oil has not been investigated previously. The chemical composition and antibacterial activity of the essential oils of the leaves and flowers of *Achillea filipendula* were investigated. Essential oils were isolated by hydrodistillation and analyzed by GC and GC-MS. Overall, 95.3% and 92% of the constituents were characterized for the leaf and flower oils. The main components of the leaves and flowers were: 1,8-cineole (17.2 – 19.0%) and chrysanthanol acetate (18.5 – 19.3%), respectively. The antibacterial activity of the essential oils against seven gram positive and gram negative bacteria were investigated and high antibacterial activity was observed. References: 1. Simon JE, Chadwick AF and Craker LE (1984) Herbs: An Indexed Bibliography 1971 – 1980. Elsevier, Amsterdam. 2. Konemann (1999) Botanica. Gordon Cheers Publication, Hong Kong. p.1020. 3. Esmaeili A et al. (2006) Flavour Fragr J 21: 253 – 256. 4. Mishurova SS, Abbasov RM, Malinovskaya, TA and Mamedalieva L. has recorded at the treatment of 173.2 kg ha -1 compared with control treatment (0.13% &0.015 g plant-1). 28 constituents were identified in the EO. The main constituents (a-cadinol, D-and y-cadinene) increased with K level increased. The highest amount of main constituents is-cadinol (33.11%), D-cadinene (18.41%), and y-cadinene (9.99%) produced from the 346.4 kg ha -1 treatment compared with other and control treatments. Alcohols are the major constituents of the heavy oxygenated compounds (HOC) of Calendula EO. a-Cadinol represents the highest concentration among the alcohols. This indicates that Calendula EO grown under K belongs to the a-cadinol chemotype.

**Antimicrobial Activities in Cultivated *Origanum vulgare* subsp. hirtum Populations of Different Origin**  
SancaktarSoglu S, Abaci O, Tınmaz AB, Usta AH
Ilgaz University, Agriculture Faculty Field Crops Department,76000, Iğdır, Turkey;  
Ege University, Science Faculty, Microbiology Department;  
Atatürk Horticultural Central Research Institute

*Origanum* L. genus belongs to Lamiaceae (Labiatae) family. It has antimicrobial activities on high rates. Especially *Origanum vulgare* L. has very efficient antimicrobial activities because of the high essential oil with its main components; thymol and carvacrol. *Origanum vulgare* L. subsp. *hirtum* (Link) Lettswaart, main components of the essential oil are carvacrol, thymol, y-terpinene and p-cymene. This study was conducted in order to determine antimicrobial activities in cultivated *Origanum vulgare* subsp. *hirtum* populations of different origin, and to correlate harvest times with antimicrobial activity. Species of bacteria tested were *Staphylococcus aureus* ATCC 6538, *Staphylococcus epidermidis* ATCC 12228, *Streptococcus faecalis* ATCC 29212, *Bacillus cereus* ATCC 10231, *C. parapsilosis* ATCC 29212, *C. albicans* ATCC 10231, *C. tropicalis* RRR 665, *K. krausei* ATCC 6258, *C. parapsilosis* ATCC 2219, *C. dubliniensis* CD 36, *Aspergillus fumigatus* NRRL 2999, *CLSI (The Clinical Laboratory Standards Institute)* broth microdilution method was used for the determination of MIC (Minimum inhibition concentration). MIC was determined according to the CLSI M27-A2 for Candida species, CLSI M38-A microdilution for *Aspergillus fumigatus* and CLSI M2-A7 microdilution for bacteria.

**Effect of potassium nutrition on essential oil of *Calendula officinalis* L. flowers**  
Ahmed KA  
Department of Cultivation and Production of Medicinal and Aromatic Plants, National Research Centre, Dokki, Giza, Egypt.

Effects of potassium (K) by rates of 0.00 (control), 115.5, 173.2, 231.00, 288.7 and 346.4 kg ha -1 on essential oil (EO) extracted from marigold (*Calendula officinalis* L.) flower heads were investigated by GC and GC/MS. The highest accumulation of EO (0.29% &0.095 g plant-1) was recorded at the treatment of 173.2 kg ha -1 compared with control treatment (0.13% &0.015 g plant-1). 28 constituents were identified in the EO. The main constituents (a-cadinol, D-and y-cadinene) increased with K level increased. The highest amount of main constituents is-cadinol (33.11%), D-cadinene (18.41%), and y-cadinene (9.99%) produced from the 346.4 kg ha -1 treatment compared with other and control treatments. Alcohols are the major constituents of the heavy oxygenated compounds (HOC) of Calendula EO. a-Cadinol represents the highest concentration among the alcohols. This indicates that Calendula EO grown under K belongs to the a-cadinol chemotype.

**Composition of Artemisia abrotanum and *A. pontica* Essential Oils and Their Repellent Activity against *Aedes aegypti***  

1National Center for Natural Products Research, The University of Mississippi, University, MS 38677 USA;  
2Department of Pharmacology, Faculty of Pharmacy, Anadolu University, 26470 Eskişehir, Turkey;  
3Coastal Research and Extension Center, Mississippi State University, South Mississippi Branch Experiment Station,  
4USDA-ARS Center for Medical, Agricultural, and Veterinary Entomology, Gainesville, FL 32608 USA;  
5USDA-ARS Natural Products Utilization Research Unit, University of Mississippi, University, MS 38677 USA;  
6National Center for Natural Products Research, The University of Mississippi, University, MS 38677 USA;  
7Department of Pharmacognosy, School of Pharmacy, The University of Mississippi, University, MS 38677 USA;  
8Department of Pharmacognosy, College of Pharmacy, King Saud University, 11451 Riyadh, Saudi Arabia;  
9Department of Entomology, College of Science, King Saud University, 11451 Riyadh, Saudi Arabia.

Mosquito-borne diseases such as malaria, encephalitis, and Yellow, Dengue, and Rift Valley fevers are diseases that result in significant morbidity and mortality in humans and livestock globally. Currently, the development of natural product-based insecticides and repellents are under exploration to increase and improve our ability to protect humans from mosquito bites, and ultimately to reduce the incidence of mosquito-borne illnesses [1]. We have undertaken a collaborative research project to discover new natural compounds for personal protection and control of mosquitoes. *Artemisia abrotanum* L. leaves have reportedly been used as a moth and insect repellent. Therefore, we evaluated *Artemisia abrotanum* and *A. pontica* L. essential oils for mosquito repellent activity against *Aedes aegypti*. *Artemisia oils obtained by hydrodistillation of aerial parts were analyzed by gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS). The main *Artemisia* oil constituents were as follows: A. abrotanum: 32.6% 1,8-cineole, 13.5% borneol, 10.2% presilphiperfolan-9-ol and 8.0% p-cymene; A. pontica: 35.6% artemisia ketone, 30.1% y-thujone, 22.3% 1,8-cineole and 3.7% y-thujone. *Artemisia abrotanum* oil showed repellent activity down to a minimum effective dosage of 0.219 mg/cm 2 (€ 0.143) using cloth patch assay. Whereas *A. abrotanum* oil exhibited no repellent activity at the highest concentration tested, 0.375 mg/cm 2. Our research into exploring the repellency of specific compounds in the *A. abrotanum* oil will continue and be expanded to include other mosquito vectors and pesticide resistant mosquito strains. Acknowledgement: This study was supported by a grant from the Deployed War-Fighter Protection (DWFP) Research Program and the U.S. Department of Defense through the Armed Forces Pest Management Board (AFPMB), and by a grant from the Mississippi Agricultural and Forestry Experiment Station. References: 1. Hoel D et al. (2010) Wingbeats 21(1); 19 - 34.
Fresh flowers of Rosa damascena Miller var. trigintipetala Dieck cultivated in Tait, Saudi Arabia are the source of Tait Rose Oil. The oils were sourced from two dealers in Riyadh, Saudi Arabia in 2011. They were analysed by GC and GC/MS. Both oils gave a similar profile with quantitative differences. The main components characterized were citronellol (23 – 28%), geraniol (14 – 20%), nonadecane (11 – 16%), nerol (6 – 11%), linalool (8%) and heneicosane (7%), resp.

Application of vibrational spectroscopy in the quality assessment of Buchu oil obtained from two commercially important Agathosma species (Rutaceae)

Agathosma species (Rutaceae) are medicinal shrubs used traditionally to treat renal and chest ailments. In addition, Buchu oil (from two South African species; Agathosma betulina (P.J. Bergius) Pillans and Agathosma crenulata (L.) Pillans) is an important ingredient in flavour and fragrance formulations. The use of vibrational spectroscopy as possible alternatives to conventional chromatographic techniques for the rapid and inexpensive assessment of Buchu oil was investigated. Samples of A. betulina (55) and A. crenulata (16) were collected from natural populations and cultivation sites in South Africa. The essential oil was scanned on NIR, MIR and Raman spectrometers. The spectral data was processed using orthogonal partial least squares discriminate analysis (OPLS-DA). Using GC-MS data, calibration models were developed for the MIR, NIR and Raman spectral data to predict the major compounds in Buchu oil. The results showed that OPLS-DA technique is a useful tool in the differentiation of Agathosma species using a non-targeted approach. Identification of wave regions that contain peaks separating the two species was possible. The PLS calibration model developed using MIR data was the best with R² = 0.96; R²Y = 0.88 and Q²Ycum = 0.85 for the quantification of six oil constituents. The model showed high predictive power (R² = 0.85). These results illustrate the potential of MIR spectroscopy as an expensive and auxiliary alternative to predict the major compounds in commercially important Buchu oil. Acknowledgement: The financial support of National Research Foundation (SA), Tshwane University of Technology and Jagiellonian University is gratefully acknowledged. S. Chicken Nationals (Cape Town) are thanked for logistic arrangement to source plant material. References: 1. Van Wyk B-E, Wink M (2004) Medicinal and Aromatic Plants of the World. Briza publications, Pretoria. 2. Simpson D (1998) Scott med j 43:189 – 191 3. Turpie JK, Heydenrych BJ, Lambeth SJ (2003) Biol Cons 112: 233 – 251.

Investigation on the improvement of essential oil distillation efficiency by changing the osmotic potential of distillation system

Shahriar S, Azizi M
Department of Horticulture, College of Agriculture, Ferdowsi University of Mashhad, Mashhad, Iran

Just as different environmental factors influence the amount and quality of essential oil, changes in osmotic potential of the extraction system can also be effective on the amount and quality of essential oils extracted. The purpose of this experience was possible increasing the efficiency of essential oil extraction by changing the osmotic potential of extraction system using NaCl. The research was conducted on the four important medicinal plants including, Ammi, Peppermint, Thyme and Eucalyptus. The research was set as completely randomized block design with three replications. The results showed that the osmotic potential of the extraction system affect oil extraction efficiency significantly. The highest essential oils (in all samples) obtained using NaCl salt solution at 3.5 w/v that adjusts the extraction temperature of the system equal to 98.5 °C. The essential oil content of Eucalyptus, Thyme, Ammi and Peppermint were 2.717, 3.233, 3.25 and 2.167 v/v respectively. According to the results of this study osmotic potential of the extraction system affect extraction efficiency of essential oils. Keywords: extraction efficiency,
A. excelsa. Use of either species as traditional medications via extracts that are obtained by boiling, either as a tea or soup, is common in rural communities in KwaZulu-Natal. Medicinal plants have not only therapeutic properties but are also used in traditional phytotherapy, particularly for the prevention and treatment of respiratory diseases.

Evaluating the use of wild medicinal plants in the traditional therapy of respiratory diseases in high mountain region of W. Balkan Peninsula.

The use of wild medicinal plants in the traditional therapy of respiratory diseases in high mountain region of W. Balkan Peninsula.

PF1

Phytochemical properties of Aspilia africana leaf

Aspilia africana (Pers.) C.D.Adams is used in herbal medicine for the perceived presence of some bioactive components in the leaves. Scientific reports suggest that different crude extracts of the plant contain specific bioactive constituents that could have varied effects on its biological activities. This study evaluated the phytochemical activities in the meal; aqueous, chloroform and ethanolic extracts of A. africana leaves. The anti-microbial activity of the four samples of A. africana were tested on nine micro-organisms of six bacteria and three fungal strains using the agar well diffusion technique. Results of the phytochemical screening and subsequent quantification revealed the presence of high amount of some bioactive compounds; saponins, tannins, alkaloids, flavonoids, terpenoids and phenols, but the absence of steroids (leaf meal and all extracts), phlobatannin (chloroform and ethanolic extracts) and cardiac glycoside (ethanolic extract) in the A. africana leaf products. Though the chloroform leaf extract had higher concentrations (P < 0.05) of these phytochemicals, significant (P < 0.05) improvements were observed in the chemical composition of the aqueous and ethanolic extracts. The anti-microbial activities observed indicated that biological activities were dependent on the types of extractants and the concentrations of principles present such as alkaloids and tannins. These results are in agreement with other studies [1].

PF2

A comparative analysis of two medicinal plants used to treat common skin conditions in South Africa

Naidoo KK, Coopoosamy RM
Mangosuthu University of Technology, Dept. of Nature Conservation, Umlazi, Durban, South Africa

Infectious dermatological diseases are a common occurrence in southern Africa. Plants showing dermatological properties are highly sought after due to their ability to stop bleeding, speed up wound healing and to soothe skin exposed to burns. An attempt was made to validate the use of Haworthia limifolia Marloth and Aloe excelsa A.Berger against microbial properties from extracts of leaves against five gram positive, four gram negative bacteria and six species of fungi. All gram positive bacteria were inhibited by both the ethyl acetate and acetone extracts for leaves of H. limifolia. However, only one gram negative bacteria was inhibited by the same extracts. Ethyl acetate extract of A. excelsa was only effective against three gram positive bacteria whilst acetone extract was effective against all bacteria except for Shigella sonnei and Enterobacter aerogenes. Both ethanol and aqueous extracts of H. limifolia and A. excelsa showed antifungal activity. H. limifolia extracts showed greater antibacterial activity than A. excelsa whilst A. excelsa showed greater antifungal activity than H. limifolia. Use of either species as traditional medicine will therefore depend on the type of infection or condition presented by the patient.

PF3

An ethnobotanical survey of medicinal plants used by traditional healers in Durban, South Africa

Coopoosamy MR, Naidoo KK
Mangosuthu University of Technology, Durban, South Africa

Medicinal plants have been extensively used for the treatment of infectious diseases by majority of the world’s population. Many of the rural communities in KwaZulu-Natal have no access to western medical practitioners and rely on traditional medicines for their cures. It has been noted that approximately 20% of the plants found in the world have some pharmacological properties. An ethnobotanical survey of medicinal plants used for various treatments including stomach ailments, skin diseases, blood purifiers, rashes, burns and other infections used in KwaZulu-Natal, South Africa was conducted through the use of structured questionnaires. Respondents included traditional healers, herbalists and herb sellers. The information collected revealed that 25 plant species belonging to various families are currently being exploited for their curing properties. The most frequently used parts are the leaves followed by root, rhizome or bulb. Stems, flowers and fruits are seldom used. The survey has indicated that Traditional healers administer their medications via extracts that are obtained by boiling, either as a tea or concoction.
Bioactivity Guided Evaluation of Antinociceptive and Anti-inflammatory Properties of Cnestis furrugineus Vahl ex DC (Connaraceae) N. Shukla 1, S. Eid 1, M. Ouchfoun 1, A. Brault 3, D. Vallerand 4
1Division of Pharmacology, Central Drug Research Institute Lucknow Uttarpradesh, India; 2Division of Medicinal Plant Process Chemistry, Central Drug Research Institute Lucknow Uttarpradesh, India; 3Department of Pharmacology, College of Medicine, University of Lagos, Lagos, Nigeria

Cnestis furrugineus Vahl ex DC (Connaraceae) (CF) is a shrub widely used in traditional African Medicine (TAM) for the treatment of various painful and inflammatory conditions. This study sought to isolate, identify and investigate the anti-inflammatory and antinociceptive activity of the active constituents of CF through bioassay guided fractionation. The crude methanolic root extract of CF was sequentially fractionated into four sub extracts (chloroform, ethylacetate, n-butanol and the remaining aqueous fraction). The aqueous fraction having shown significant inhibition of inflammation and pain was subjected to fractionation through successive column chromatography on silica gel 60 – 120 mesh, eluted with a gradient of CHCl3 – MeOH. Fifty five factions were collected; fractions with similar TLC profile were grouped into seven major fractions (1 – 7). Fraction 4 being most active in bioassay was rechromatographed to yield CF-2 and CF-5. The effect on inflammatory mediators was studied in rat astrocotyelma cells (C6), nitric oxide release in culture supernatant, ROS in cells and TNF-α in cell lysate were estimated. The methanolic extract, aqueous and n-butanol fraction showed significant (p < 0.05, p < 0.01, p < 0.001) inhibition of acute inflammation, peripherally and centrally mediated forms of pain in the bioassay procedures. The aqueous/n-butanol fraction afforded two active major compounds, identified as amentoflavone (CF-2) and an amino acid compound (CF-5) following various spectroscopic experiments. CF-2 and CF-5 significantly attenuated nociception, inflammation, nitrite acid compound (CF-5) following various spectroscopic experiments. CF-2 and CF-5 significantly attenuated nociception, inflammation, nitrite acid compound (CF-5) following various spectroscopic experiments.

Blighia unijugata Baker (Sapindaceae) is a small to medium-sized tree up to 30 m tall widespread in tropical Africa. The leaves are eaten as vegetable and various part of the tree are considered to have sedative and analgesic properties and are used in traditional medicine for the treatment of rheumatism, kidney pain and muscular stiffness. The macerated twigs, leaves, flowers and fruit as a fish poison by the coastal people in Nigeria and there is a high correlation between plants employed as fish poison or soap substances and molluscicidal activity. Powdered pericarp was macerated with 50% ethanol, filtered and the filtrate concentrated to dryness under vacuum to yield 10.30 g of the dried extract and out of this 9.80 g was dissolved in water and partitioned between ethyl acetate, butanol and water to give 2.77 g of ethyl acetate, 2.81 g of Butanol and 3.35 of water fractions respectively Snails for the experiment were collected from streams that have not been subjected to either synthetic or poison or soap substances and molluscicidal activity. They were allowed to acclimatize in the laboratory and were fed on lettuce for 7 days before any treatments were given. Efforts are being made to study the molluscicidal activity of Blighia unijugata Baker.

**PF10**

Molluscicidal Potential of the Fruit Pericarp of *Blighia unijugata* Baker Against Biomphalaria pfeifferi

Agboola OA1, Ajeyi CO2, Adesegan SA3, Adesanya SA3

1Niger Delta University, Wilberforce Island, Bayelsa State, Nigeria; 2University of Lagos, Lagos, Nigeria; 3Obafemi Awolowo University, Ile-Ife, Nigeria

**PF11**

Antibacterial and anticancer activity of kaurenoic acid from root bark extract of *Annona senegalensis*

Okoye TC1, Akah PA1, Omeje EO2, Okoli CO1, Nworo SC1

1Department of Pharmacology and Toxicology, Faculty of Pharmaceutical Sciences, University of Nigeria, Nsukka, Nigeria; 2Department of Pharmaceutical and Medicinal Chemistry, Faculty of Pharmaceutical Sciences, University of Nigeria, Nsukka, Nigeria

The antibacterial [1] and anticancer [2] activities of extract of *Annona senegalensis* Pers. (*Annonaceae*) have been reported. Bioactive-guided fractionation of the methanol-methylene chloride root extract of *A. senegalensis* afforded a potent antibacterial ethyl acetate fraction (EF) which on further fractionation, gave two active sub-fractions F1 and F2. F1 yielded a lipophilic liquid component while F2 on purification, precipitated a white crystalline compound, ASI, that was characterized by proton NMR and X-ray crystallography as kaur-16-en-19-oic acid. F1 was precipitated a white crystalline compound, ASI, that was characterized by proton NMR and X-ray crystallography as kaur-16-en-19-oic acid. 1-Naphthalenemethanol, 6,6-dimethyl-bicyclo[3.3.1]hept-2-ene-2-ethanol, 3,3-dimethyl-2-[3-methylbuta-1,3-dienyl] cyclohexan-1-methanol and 3-hydroxyandrostan-17-carboxylic acid. Agar well diffusion method, using a 0.5 McFarland standard, was employed to obtain the MIC’s for F1 and ASI. The MIC’s against clinical isolates of *B. subtilis* subsp. *subtilis* has been reported.

**PF12**

Microscopic and histochemical characterization of leaves of the medicinal plant *Ocimum obovatum*

Naidoo Y, Kasim N, Nicholas A

School of Biological and Conservation Sciences, University of KwaZulu-Natal, PBag X5401, Durban, 4000, South Africa

Ocimum obovatum E. Mey. subsp. obovatum var. obovatum has been valued for its hair restorative properties for decades on the African continent. The member of the Lamiaceae is also traditionally prescribed as a remedy for infantile abdominal cramps and a hot water extract of the leaves is used to treat epigastric conditions in children. Commonly known as ‘cat’s whiskers’, the aromatic plant can be seen growing along the KwaZulu-Natal coastline and the Western Cape of southern Africa. *Ocimum obovatum* is also common in Zimbabwe and Swaziland as well as northern and west Africa [1]. The medicinal properties of the plant are attributed to the essential oils supposedly produced and secreted by appendages on the foliar surfaces referred to as trichomes [2]. Traditional light and electron microscopy studies revealed the presence of two types of glandular trichomes and one type of non-glandular trichome across all stages of leaf development. The glandular trichomes were classified as large, four-celled peltate trichomes and smaller capitate trichomes. The latter were further classified into two subtypes: Type I capitate trichomes with a single basal cell and two head cells and Type II capitate trichomes with a single basal and stalk cell and an ovoid head cell. Histochemical and phytochemical studies showed that essential oils of a terpenoid nature were present in the head cells of glandular trichomes. Flavonoids, triterpenoids, tannins, saponins, fixed oils and fats, phenolics and cardiac glucosides were also detected in a crude ethanolic extract of the leaves using phytochemical test methods. Acknowledgement: The National Research Foundation (South Africa) is gratefully acknowledged for the funding of this research. References: 1. Hutchings et al. (1996) Zulu Medicinal Plants: An Inventory. University of Natal Press, Pietermaritzburg. 2. Werker et al. (1993) Annals of Botany 71: 43.

**PF13**

Effect of drying methods on the antioxidant activity of *Anacardium occidentale* L. (Cashew)

Jaiswal VS1, Taithe PA1, Gabhe SY1, Vaidya AB2

1Department of Pharmaceutical Chemistry, C.U. Shah College of Pharmacy, S.N.D.T Women’s University, Mumbai-400 049, India; 2C.I.M.R. Centre of Reverse Pharmacology in Traditional Medicines, Kasturba Health Society, Vile Parle (w), Mumbai-400 056, India.

Studies on the drying characteristics of Cashew leaves are scarce in the literature; particularly the traditional sun drying properties as well as oven drying properties of plants are not adequately investigated. The aim of the work was to determine the sun, oven, shade dried and fresh leaves drying characteristics of Cashew and to compare the effect of the same on the antioxidant property of the extracts. The extracts of leaves exposed to various drying conditions were prepared using various solvents. The phytochemical test were carried out for various compounds prepared in order to ascertain the presence/absence of various phytoconstituents. The effect of drying conditions on the antioxidant activity of the extracts, the extracts were evaluated for their antioxidant effect by DPPH. Assay, Greiss assay and determination of total phenol content.
The Phytochemical investigations of the extracts revealed the presence of tannins and phenolics, saponins, flavonoids, alkaloids, steroids and sugars. From the results obtained, it was observed that shade dried leaves contained the maximum content of polyphenols. And the order of content to polyphenol was found to be Sun Dried leaves > Fresh Leaves > Oven Dried leaves > Shade dried leaves. Acknowledgement: The authors thank ICMR, New Delhi, India for funding the research project.


Antihyperglycaemic and antihyperlipidaemic effects of *Raphia hookeri* root extract on alloxan induced diabetic rats

Muboka GO, Olgonboni SO, Bango AE

1Department of Anatomy, Lagos State University College of Medicine, Ikeja, Lagos, Nigeria. 2Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Lagos, Ido- Araba, Lagos, Nigeria. 3Department of Anatomy, Faculty of Basic Medical Sciences, Obafemi Awolowo University, Remo Campus, Ikenne, Nigeria

The antihyperglycaemic and antihyperlipidaemic effects of *Raphia hookeri* G.Mann & H.Wendl. (RH) root extract used for diabetic treatment were evaluated against alloxan diabetic rats. Adult rats weighing 150 ± 10 g were fasted for 18 hrs and induced with diabetes for three days using alloxan monohydrate (150 mg/kg body weight). Animals with blood glucose level >= 250 mg/dl were used. Diabetic rats were divided into four groups and treated as follows: groups 1 – 3 received graded doses of RH (50, 100 and 200 mg/kg) by gavage; group 4- glibenclamide (10 mg/kg); groups 5 and 6 as normal and diabetic controls. Each comprised of 5 rats. Blood was collected at days, 0, 3, 5, 7, 9, 11, 13 and 15 and analyzed for glucose by oxidase method; Lipid profile, by modified enzymatic procedure; Insulin assay, using Diagnostic Automation Kit 2 and glycated haemoglobin by standard protocol 3. Results showed that the extract exhibited significant (P < 0.05) dose dependent decrease in glycaemia from day 3 to 15 with highest dose exerting 87% decrease at day 15. RH dose at 500 mg/kg on normal rat caused hypoglycaemia after 4hrs with maximum decrease (54.7%) observed after 6 hrs. Total cholesterol, triglycerides and low density lipoprotein cholesterol levels decreased with dose while high density lipoprotein cholesterol showed dose increase. RH stimulated insulin secretion and equally exerted significant increase in glycated haemoglobin (HbA1c). Photomicrograph of RH treated showed significant beta cell survivors. RH exhibited antihyperglycaemic and antihyperlipidaemic effect on lipid homeostasis. Acknowledgement: Prince Musibau Sikiru a herbalist at Sagamu, Ogun State, Nigeria, for assisting with the plant material.

Ethnobotanical survey and antifungal activity of plants identified for the management of opportunistic fungal infections in HIV/AIDS patients in the Amathole District of the Eastern Cape Province, South Africa

Otang WM, Grierson DS, Ndip RN

1Department of Botany, School of Biological and Environmental Sciences, Faculty of Science and Agriculture, University of Fort Hare, Private Bag X1314, Alice 5700, South Africa. 2Department of Biochemistry and Microbiology, School of Biological and Environmental Sciences, Faculty of Science and Agriculture, University of Fort Hare, Private Bag X1314, Alice 5700, South Africa

In a study to document plants used to treat opportunistic fungal infections (OFIs) seen in HIV/AIDS patients in the Eastern Cape, South Africa, ethnobotanical information was obtained through questionnaires and conversations with 22 traditional healers and 101 HIV/AIDS patients. Thirty two plant species, belonging to 26 families, were identified as being used for this purpose. For two frequently used plants, Arctitis arctoides and *Acanthopanax senticosus*, validation by recording antifungal effects and minimum inhibitory concentrations (MICs) of their hexane, acetone and water extracts against 10 opportunistic fungi, using agar well-diffusion and broth micro-dilution methods. Among the 6 plant extracts, all the hexane and acetone extracts were active against at least one of the fungi with zones of inhibition varying from 8 to 23 mm (control: 14 – 27 mm). For both plants the lowest MICs were obtained with the hexane extracts (*A. arctoides*: 0.005 mg/ml against *Trichophyton mucosum* and *G. bicolor*: 0.04 mg/ml against *A. fumigatus*). The inhibitory activity of the active extracts, based on the mean inhibition diameters, was in the order: *A. arctoides* (hexane) > *A. arctoides* (acetone) > *G. bicolor* (hexane) > *G. bicolor* (acetone). The most susceptible fungi were *Candida glabrata*, *C. krusei* and *Microsporum canis*, while *C. albicans* and *M. gypseum* were not susceptible to any of the extracts even at 5 mg/ml which was the highest concentration used. This study not only documents thirty two plants used, but validates the use of two of these plants in traditional medicine for the management of OFIs in HIV/AIDS patients. Acknowledgement: The Govan Mbeki Research and Development Centre of the University of Fort Hare is acknowledged for financial support for the research and conference attendance.
showed that all doses of leaf extract could recover the damaged islet cells in diabetic rats in a dose dependent manner. The pancreatic islets of diabetic rats receiving the extract were larger and the cells within the islets were rounder and less congestive when compared to diabetic control rats. Conclusion, the results of this study suggested that Morus alba leaf extract could reduce blood glucose levels and improve the histological features of pancreatic islets in diabetic rats. Acknowledgement: This research was financially supported by Faculty of Medicine, Thammasat University Research Fund.

Hepatoprotective effect of the ethanolic extract of Anethum graveolens L. on paracetamol-induced hepatic damage in rats

Ahmat N 2, Suganuma T1, Kitahara K2, Fuji M2
1Department of Pesticide Chemistry, Faculty of Agriculture (Elshatby) Alexandria University, Alexandria 21545, Egypt; 2Department of Biochemistry and Applied Biosciences, Faculty of Agriculture, Kagoshima University, 1-21-24 Korimoto, Kagoshima 890-0065, Japan

The activity of Raphia hookeri G.Mann & H.Wendl. (RH) seed extract was investigated on exogenous induced prostatic enlargement. Adult male rats weighing 200 ± 10g/kg were induced with BPH by exogenous administration of testosterone and estradiol in staggered doses (three times a week) for three weeks (1). The induced animals were divided into five groups. Groups 1 and 2 received the extract at 50 and 100 mg/kg body weight by gavages for forty five days; group 3- fenasteride (0.1 mg/kg); group 4- was left untreated for forty five days; group 5- (negative control) was sacrificed immediately after induction. Group 6 received the extract (100 mg/kg) and the steroid hormones simultaneously whereas group -7 was normal control. Prostate specific antigen (PSA) and testosterone levels were determined from blood serum. The oxidative activity, Catalase (CAT), Superoxide dismutase (SOD), Lipid peroxidation and glutathione (GSH) were assayed as described by Rukumani et al[2]. The result showed significant decrease (P < 0.05) in PSA level in RH treated compared to the negative control. There was also decrease in testosterone level in RH treated. The levels of CAT and SOD (Table 1) in RH treated were comparable to normal. However, GSH showed comparably higher level to normal while the extract peroxidative activities decrease slight. Prostatic tissue morphology of the extract treated (Fig.1) showed extensive shrinkage while hypertrophy of prostate gland occurred in the untreated (Fig. 2). RH effectively reduced enlarged prostate mass, lowered PSA and testosterone levels and also exhibited anti-oxidative activity. Acknowledgement: Prince Musibau Si-kiri, herbalist, Ogun State, Nigeria assisted with the plant material. References: (1) Bernoulli J (2008) An experimental model of prostatic inflammation for drug discovery. Medica – Odontologica. (2) Rukumani et al. (2004) J Pharm Sci 72: 274 – 283.
Abstracts | 59th International Congress of the GA | 4th–9th September 2011, Antalya, Turkey

Pounds from fraction D will be identified and their antiinflammatory properties will be investigated.

**Table 1:** Antinociceptive activity for petroleum ether fraction of Mitrigia calabura

<table>
<thead>
<tr>
<th>Fraction (mg/kg)</th>
<th>First phase Percentage</th>
<th>Second phase Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>A (300)</td>
<td>79.667 ± 3.585</td>
<td>2.6</td>
</tr>
<tr>
<td>B (300)</td>
<td>74.833 ± 1.922</td>
<td>8.6</td>
</tr>
<tr>
<td>C (300)</td>
<td>57.167 ± 2.056</td>
<td>30.1</td>
</tr>
<tr>
<td>D (300)</td>
<td>27.667 ± 2.418</td>
<td>66.2</td>
</tr>
<tr>
<td>E (300)</td>
<td>42.167 ± 3.381</td>
<td>48.8</td>
</tr>
<tr>
<td>F (300)</td>
<td>81.82 ± 3.26</td>
<td>0.01</td>
</tr>
<tr>
<td>G (300)</td>
<td>74.333 ± 1.429</td>
<td>9.2</td>
</tr>
</tbody>
</table>


This study aims to investigate the protective effect of the Polygonum odoratum L. on acetaminophen-induced liver injury in rats. Soparn P, Nantasenawasuk S, Ittarwas R, Thappia A. Kasisingon J

Department of Preclinical Science, Faculty of Medicine, Thammasat University, Rangsit Campus, Khlong Luang, Pathum Thani, 12110 Thailand

This study investigates the protective effect of the Polygonum odoratum L. on acetaminophen-induced liver injury in rats. 30 male Sprague Dawley rats were divided into 5 groups. Group I, II and III were given 5\% Tween 80 whereas group IV and V were given 500 and 1000 mg/kg body weight/day of the plant extract, respectively for 7 days. On day 8th, animal in group II-V were received acetaminophen 3 g/kg body weight; group II also received N-Acetyl Cystein (NAC) 400 mg/kg body weight at 2 hours after acetaminophen administrated. All animal were sacrificed on day 9th and blood samples were collected for the determination of Aspartate transaminase (AST), Alanine transaminase (ALT), Malondialdehyde (MDA) and nitrite formation. AST, ALT, MDA and nitrite levels were significantly higher in rats treated with acetaminophen alone compared with normal control (p < 0.05). Pre-treatment of the animal with the extract and administration of NAC significantly reduced oxidative stress and liver injury induced by acetaminophen as shown by reduction of MDA, nitrite levels, AST and ALT levels compared to the rats treated with acetaminophen alone (p < 0.05). In conclusion, the result of this study demonstrated that pre-treatment with the ethanolic extract of Polygonum odoratum can ameliorate oxidative stress and liver injury induced by acetaminophen. Acknowledgements: This research was financially supported by Faculty of Medicine, Thammasat University Research Fund.

An ethno-phyto study was conducted as a part of the local knowledge study which was carried out in 2010. The target participants were the Livestock owners in the arid Jordanian Badia region. The objective of the study is to document the traditional knowledge in using wild plants in treating health problems in order to conserve this valuable knowledge from loss; to identify the key plant species used and to calculate the Informant Consensus Factor (ICF) for the health disorder categories. The data was collected from interviewing 70 participants, 21% of them were women, the women interviews were very important as they are consid- ered experts in the field of the local medicinal plants. The participants were interviewed face to face and few focus groups were conducted. A questionnaire that helps in the data gathering was prepared, video recording was taken to show the procedures that the local communities were using in their process. A total of 48 plant species are used by local Bedouins for medicinal purposes, the majority of these are native to the study area, e.g. Artemisia judaica L., Cirsium coloxycninum (L.) Schrad., Dicroas anethifolia Boiss., Ecballium elaterium (L.) A.Rich., Paronychia spp and Rheum palustrenum Feinbr. The study showed that the plant species with the highest use value is Artemisia herba-alba. Moreover, the highest value of Informant Consensus Factor (ICF) was scored for Jaun- dice disease (0.87) followed by Gastrointestinal disorders (0.86) and dental disorders (0.81). This may indicate the high incidental occur- rences of these diseases and the lack of dental care services in the rural areas.

Current status, roles and challenges of traditional medical practitioners involved in management of diabetes mellitus in Nigeria

Jegede IA

National Institute for Pharmaceutical Research and Development, Abuja, Nigeria.

Patients suffering from diabetes mellitus in Nigeria have resulted to consulting Traditional Medical Practitioners (TMPs) to manage their health conditions. There are no available data on the role and status of traditional medicine practice in the management of the disease in the country. A study was initiated in 2009, to document this in six geogra- phical zones of the country. Data was collected through oral interviews in the local languages of over 90 practitioners and responses document- ed in a specially designed questionnaire along with prior informed consent form and entered into a database. This paper intends to high- light the results obtained which include collection of over 80 recipes in various forms, (mostly of plant based), preliminary screening which yielded 4 most active recipes along with pharmacognostic standards, adequate referral system of the practise, low percentage of women involved in the practice, inadequate educational background of practi- tioners, good understanding of disease diagnosis, inadequate record keeping and improved shelf life of products. Challenges include need for training on standardization methods of thier products and practise, establishment of botanical gardens due to derestoration, establishment of clinics and more opportunities for product regisstration. These results are required to aid the promotion, standardization and integration of the practise into National Health Care System. Acknowledgement: The authors wish to acknowledge the STEPB Project of the World Bank for award of Research Grant for this study as part of a larger study.
properties using in vitro and in vivo models. The effect of 1,1-diphenyl-2-picrylhydrazyl (DPPH) antiradical activity on ethanol, petroleum ether, ethylacetate, butanol, and water fractions of *C. abdum* was determined. The ethylacetate fraction was purified in column chromatography which led to the isolation and characterization of a myricetin rhamnoside [2]. The structure was elucidated by NMR and mass spectrometric techniques. Furthermore, ethanol extract was administered to five groups of eight rats per group. The positive control animals were administered with vehicle on the first four days, and with the vehicle and CCl4 on the fifth, sixth and seventh day [3]. The animals in the treatment category were respectively administered (by gastric intubation) with 500, 1000 and 1500 mg/kg bw of extract & distilled water for the first four days, and with distilled water, extract and CCl4 on the last three days. Animals were anaesthetized and blood samples were collected for some antioxidant assays. Petroleum ether fraction showed the least antiradical activity (4057.5 ± 809.6 g/kg) while ethyl ether fraction exhibited the highest activity (414.4 ± 92.0 g/kg). Myricetin rhamnoside also exhibited an excellent radical scavenging activity (314.1 ± 60.2). *C. abdum* fraction exhibited significant (p < 0.05) differences on the activity of malondialdehyde, catalase, and reduced glutathione. The plant therefore possesses antimicrobial and antioxidant activities and could be employed as natural antioxidant boosters.

**PF27**

Possible mediators underlying Linalool effect on HepG-2 but not primary hepatocytes: Comparative study

Usta J1, Shatha S2, Rach A1, Yolla B1, Omar R2, Sawson K2, Karam E1
1Department of Biochemistry and Molecular Genetics, Faculty of Medicine, American University of Beirut, Beirut, Lebanon; 2Department of Biology, Faculty of Arts and Sciences, American University of Beirut, Beirut, Lebanon

Linalool is the major component of coriander-sativum seeds. We have recently reported [1] a 100% decrease in the viability of HepG2 treated with 2μM linalool. No effect was observed with other cell lines. Linalool resulted in a decrease in the ATP and GSH levels; increase in ROS; and inhibition of ETC-complexes I and II. ROS are known to affect level of UCP-2 and ANT. Recent report showed Leukemias cells treated with linalool induced apoptosis mediated by P-53 up-regulation [2]. We investi-gate the effects of linalool on 10ry-hepatocytes, variation in ALT and AST expression in HepG2 and 10ry-hepatocytes. Viability of 10ry-hepatocytes (3), treated with varying concentration of linalool was determined using MTT assay. Expression of P53, ANT, & UC2 in 10ry-hepatocytes was compared to those of HepG2 cells, using western blotting and was expressed relative to GAPDH. We report that: a) 10ry-hepatocytes were not sensitive to linalool treatment; Comparing 10ry-hepatocytes to HepG2 cells, a 50 fold of linalool concentration was needed to demonstrate a 100% b) Increase in P53 expression was obtained in Hep-2 cells whereas P53 was not detected in 10ry-hepatocytes; c) De-regulation of the expression of ANT & UC2 in 10ry-hepatocytes. Linalool effect is specific to HepG2 cells but had no significant effect on 10ry-hepatocytes. There is a role of P53, and the mitochondrial proteins ANT and UC2 in rendering HepG2 cells more sensitive. Bio-transformation into toxic metabolites of linalool by HepG2 cells, but not 10ry-hepatocytes, may not be disregarded. Acknowledgement: Medical Practice Plan and University research Board at the American University of Beirut References: 1. Usta J et al. (2009) Chem- Biol Interact 180: 39 – 46 2. Gu Y et al. (2010) Toxicology 268:19 – 24 3. Schaffner I et al. (2005) Assay Drug Dev Technol 3(1):27 – 38

**PF28**

The aqueous root extract of *Aristolochia ringens* (Vahl.) prevents chemically induced inflammation

Aigbe FR, Adeyemi OO
Department of Pharmacology, Faculty of Basic Medical Sciences, College of Medicine, University of Lagos, P.M.B. 12003, Idiaraba, Surulere, Lagos, Nigeria.

*Aristolochia ringens* Vahl Aristolochiaceae belongs to a family with many medicinal uses, but also reported toxic (1). Based on its use in traditional medicine, the antiinflammatory activity of the aqueous root extract of *Aristolochia ringens* (AR; [10 – 100 mg/kg p.o.]) was evaluated using the carrageenaan and egg albumin induced rat paw oedema (2), formaldehyde induced arthritis inflammation (3) and xylene induced mouse ear oedema methods (2). AR (10 – 50 mg/kg) produced a dose-dependent decrease in rat paw oedema in the carrageenaan and egg albumin induced models at all doses and concentrations studied. The maximum inhibition effects of AR (50 mg/kg), 57.1% and 65.6% in both experiments were comparable to the 57.9% and 63.9% of standard drugs, indomethacin and diclofenac (10 mg/kg p.o.) respectively. AR (10 – 50 mg/kg) also dose dependently inhibited the arthritis paw oedema induced by formalde-hyde over the 10 day period of study. The percentage inhibition by AR (50 mg/kg) was greater than the 40.8% inhibition by diclofenac (10 mg/kg i.p.). AR (10 – 50 mg/kg) also produced a significant (p < 0.05) dose dependent inhibition of mouse ear oedema, with a peak effect at 50 mg/kg of 84.78%, which was greater than the 65.21% inhibition by dexamethasone (1 mg/kg). No mortality was observed in 24 hours, with AR (up to 10 g/kg p.o.), but an LD50 of 453 mg/kg was obtained with the intraperitoneal route of administration in mice. Results suggest that the aqueous root extract of *Aristolochia ringens* possesses antiinflammatory activity, inhibiting chemically induced inflammation. Acknowledge-ment: Department of Pharmacology, Faculty of Basic Medical Sciences, College of Medicine, University of Lagos, Nigeria. Chijioke M.C. References: 1. Pacheco AG et al. (2009) Molecules 14: 1245 – 1262. 2. Adeyemi OO et al. (2002) Fitteraprac 73: 375 – 380. 3. Hosseinzadeh & Younesi (2001) BMC Pharmacol 2: 7 – 14.

**PF29**

Medicinal plants and their traditional uses in Kabylia (Algeria): an ethnobotanical survey

Meddour R, Meddour OS, Derrida A
Department of Agronomic Sciences, Faculty of Biological Sciences and Agronomical Sciences, University of Moulaoud Mammeri, Tizi Ouzzo, Algeria

This study aims to assess ethnobotanical knowledge in Kabylia, focusing on the use of traditional medicinal plants, at eight rural municipalities in the department of Tizi Ouzou. This region has remained relatively isolated and agro-industrial development is not led to a significant decline in traditional practices, including the use of plants in traditional medicine. Ethnobotanical information was gathered using a questionnaire among herbalists, traditional healers and local populations in the study area. Overall, 98 vascular plants were identified and recorded, a large majority of them live in a wild habitats (forests and wetlands, espe-cially), with the exception of 6 crops. They belong to 48 families, the most represented are the Lamiaceae (13 species) and Asteraceae (12 species). The many diseases listed in the survey are grouped into 10 major disease groups. The most pathologies treated are those of the digestive system (40 plants), skin diseases (29), circulatory system (24) and respiratory system (21). In contrast, the visual system, too precious, is treated with a single plant (*Oxism basilicum* L.). The toxicity of some herbs used over the 10 day period of study. The many medicinal plants are often multipurpose plants (food, flavor, feed, veterinary, crafts, etc.). Moreover, 31 of these wild plants yet still have an interest in food for rural populations. Finally, a large majority of medicinal plants used in Kabylia are also known for their therapeutic properties in the Mediterranean basin. For example, 73.5% of the plants of this study are cited by the project Rubia. References: Gonzalez-Tejero MR et al. (2008) J Ethnopharmacol 116: 341 – 357.

**PF30**

Evaluation of anti-fertility of *Lawsonia inermis* L. (Lythraceae) roots found in Kaduna State, Nigeria

Agamu A, Samagoro C, Nuhu H
Department of Pharmacognosy and Drug Development, Ahmadu Bello University, Zaria, Nigeria

Traditional contraceptive practices and use of medicinal plants is a common occurrence in Kaduna State, Nigeria [1]. The use of *Lawsonia inermis* L. (Lythraceae) roots was evaluated for antifertility activity. Extraction of the plant root and phytochemical studies of the extract were carried out according to methods described Evans [2]. Apparently healthy female and male Wistar rats were used. Pre-implantation and mating ratio using methods of Ambali et al. [3] were carried out. Determination of implantation sites was by method of Cavieres et al. [4], determination of Corpora Lutea was the method described by Arman-da et al. [5] and the effect of extract on weight of the rats were also determined. It was observed that the extract effect on contracility of isolated rat uterus was less than oxtocin. There were loss of implantation sites and decrease in body weight. The number of implantation sites showed dose-response relationship significantly (P<0.05) among
the dose of extract and to standard drug (ethylin estradiol). There was also significant (p=0.05) difference observed in the number of corpora lutea in all experimental and control groups. Similarly, there was significant (p=0.05) difference observed in all the experimental and control groups on percentage pre-implantation loss. Since extracts gave positive tests for steroids, and sex hormones being steroidal compounds, the plants’ sterols (phytosterols) may be suspected to be responsible for the anti-fertility effects of the extract. The finding may explain the traditional use of the plant as an anti-fertility agent. Acknowledgements: The authors appreciate the support of Dr. S. F. Ambali of the Department of Veterinary Physiology and Pharmacology, Ahmadu Bello University, Zaria, Nigeria. References: 1. Samagoro C (2010) MSc thesis, A.B.U., Zaria, Nigeria 2. Evans C (2002) Trease and Evans Pharmacognosy. Saunders Elsevier Ltd. London. 3. Ambali S F et al. (2010) Agric Biol J N Amer 1(2): 152 – 155 4. Cavender F M et al. (2002) Environ Health Perspect 110(11): 1081 – 1085 S. Armanda-Dias L et al. (2001) Braz J Med Biol Resour 34(9): 1209 – 1215

**PF31**

**Assessment of wound healing, anti-inflammatory and antioxidant activities of Helichrysum graveolens (Bieb.) Sweet**

**Sunwar F, Kupeli Akkol E, Sarker SD, Rees H, Baykal T, Yevlaia E**

1Department of Pharmacognosy, Faculty of Pharmacy, Gazi University, Etiler 06330, Ankara, Turkey; 2Department of Pharmacy, School of Applied Sciences, University of Wolverhampton, MM Building, Molineux Street, Wolverhampton WV1 1SR, UK; 3Department of Pathology, Faculty of Veterinary Medicine, Afyon Kocatepe University, 03030, Afyonkarahisar, Turkey; 4Faculty of Pharmacy, Yeditepe University, Atasehir 34755, Istanbul, Turkey

Helichrysum graveolens (Bieb.) Sweet is used for the treatment of jaundice, as a wound-healing and diuretic agent in Turkish folk medicine. In order to prove the claimed utilization of the plant, effects of the extracts and the fractions were investigated by using the in vivo linear incision and circular excision wound models. Antioxidant and anti-inflammatory activities, which are correlated to wound healing activity, were also evaluated by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging assay and the acetic acid-induced increased vascular permeability model, respectively. The methanolic extract, which demonstrated potent antioxidant and anti-inflammatory activities, which are correlated to wound healing activity, were also evaluated to study the potential use of the extract for further investigation of new antibacterials in the fight against resistant pathogens. Acknowledgement: Robert Leo Skov, SSI, the National Reference Center in Denmark, for strains and helpful advice. Arjie Onder and Betil Asar for technical support and Sara Nleam for valuable knowledge during plant collection.

**PF32**

**Chilean medicinal plants traditionally used for wound healing therapy studied for activity against resistant Staphylococcus aureus strains**

**Holler JG 1, Slotved HC 2, Gfflzman A 1, Mølgaard P 1, Tor Udom S 1, Hiriote W 1, Pinmai K 1, Sireratatwong S 1**

1Department of Pharmacognosy, Faculty of Pharmacy, Gazi University, Etiler 06330, Ankara, Turkey; 2Department of Medicinal Chemistry, University of Copenhagen, Copenhagen, Denmark; 3Department of Microbiological Surveillance and Research, Statens Serum Institut, Copenhagen, Denmark

Plants traditionally used for wound healing therapy by the Huilliche people in Chile were investigated for their activity against a selection of Staphylococcus aureus strains. S. aureus is a frequently encountered pathogen in skin infections and ethnomedicinal knowledge on treatment of infected wounds may prove valuable in the search for anti-staphylococcal compounds. 30 plant samples of 24 species were collected in the Valdivian rainforest west of Osorno in Chile. Material was extracted with three different organic solvents and antibacterial activity against susceptible and resistant S. aureus was evaluated. An agar-overlay diffusion assay and a MIC-determination were utilized for comparative purposes. Total phenolics and tannins were determined and antibacterial contribution of the tannins evaluated. Extracts of 19 species were active against susceptible S. aureus at 100 μg extract. At the same concentration 16 species showed activity against resistant S. aureus. Extracts without tannins rendered only six samples active. The MIC-determination showed antibacterial activity of 20 extracts on all eight strains, and the highest effect was 64 μg/ml. Species Aristotelia chilensis (Mol) Stuntz, Baccharis magellanica (Lam.) Pers., Baccharis sphaerocephala Hook et Arn., Berberis buxifolia Lam. and Crinodendron hookeriunum Gay being among the most active. Activity against multidrug resistant Vanthia strain was remarkable with 36 active extracts. The results support Huilliche traditional knowledge, and the hypothesis that their wound healing plants are potential sources of anti-staphylococcal agents. These results will form the basis for a selection of plant species for further investigation of new antibacterials in the fight against resistant pathogens. Acknowledgement: Robert Leo Skov, SSI, the National Reference Center in Denmark, for strains and helpful advice. Arjie Onder and Betil Asar for technical support and Sara Nleam for valuable knowledge during plant collection.

**PF33**

**In vitro antiplasmodial activities and cytotoxicity of water extracts of Piper rostratum Roxb., Sida rhombifolia Linn. and Tiliacora triandra (Colebr.) Diels**

**Tor Udum S1, Hirote W1, Pinmai K1, Sireratatwong S2**

1Division of Microbiology and Immunology, Department of Preclinical Sciences, Faculty of Medicine, Thammasat University, Thailand; 2Division of Pharmacology, Department of Preclinical Sciences, Faculty of Medicine, Thammasat University, Thailand

This study aims to evaluate the in vitro antiplasmodial activity and cytotoxicity of Piper rostratum Roxb., Sida rhombifolia Linn. and Tiliacora triandra (Colebr.) Diels. Piper, herbs traditionally used to treat malaria in Thailand. The water extracts of these Thai medicinal plants were tested for their antimalarial activity by assessing their ability to inhibit the uptake of [3H]hypoxanthine into the multidrug-resistant strain Plasmodium falciparum K1. The antiplasmodial activity was expressed by the concentration that inhibited 50% of parasite growth (IC50). Cytotoxicity of the extracts was determined on Vero cells, and the Cytotoxicity Index (CI = IC50 on Vero cells/IC 50 on Plasmodium falciparum) was calculated to evaluate the safety of tested extracts. Tiliacora triandra (Colebr.) Diels. was the only one of three plants that showed the in vitro antimalarial activity (IC50 = 43.43 ± 0.90 μg/ml) with good Cytotoxicity Index (CI = 5.92) whereas Piper rostratum Roxb. and Sida rhombifolia Linn. did not show this activity. Further study is needed to evaluate an in vivo antimalarial activity of Tiliacora triandra (Colebr.) Diels. extract. Acknowledgement: This study was supported by The Annual Government Statement of Expenditure for Thammasat University.

**PF34**

**Total phenolics content of the ethyl acetate extract of Salvia tomentosa**

**Onay M1, Coruh N2, Celep P3, Dogan M4**

1Middle East Technical University, Department of Biochemistry, Ankara, 06800, Turkey; 2Middle East Technical University, Department of Chemistry, Ankara, 06800, Turkey; 3Middle East Technical University, Department of Biology, Ankara, 06800, Turkey

Salvia generally including flavonoids is one of the largest genera in Lamiaceae family and this genus has 900 species. It is composed of 88 species in Turkey and mostly benefited in the treatment of skin infections, colds, stomach ache, headache and tuberculosis. In the present study, aerial part of Salvia tomentosa Mill. was used and extraction procedure was applied to its ethyl acetate extract. Yield was obtained as percentage (%). In addition, it was investigated for its total phenol content (TPC) and performed by Singleton and Rossi method with a few modifications. Gallic acid was used as standard and results were expressed as micrograms of total phenolics including of extract as gallic acid equivalents (GAE). In conclusion, its TPC was found as 103.75 ± 4.32 GAE/mg of extracts. Each datum was calculated as a average of duplicate measurements acquired from at least three separate experiment sets. According to this result, when its total phenol content was compared with other Salvia species, S. tomentosa showed higher TPC than that of other Salvia species. References: 1. Kelen M, Tepe B (2008) Biosourse Technology 99: 4096 – 4104. 2. Kivrak I, Dur ME, Öztürk M, Mercan N, Harmandar M, Topçu G (2009) Food Chemistry116: 470 – 479. 3. Singleton V L, & Rossi J A (1965) American Journal of Enology Viticulture 16:144 – 158.
1Department of Biology, Faculty of Science, Chulalongkorn University, 254 Phayathai Road, Pathumwan, Bangkok 10330, Thailand; 2Department of Biotechnology, Faculty of Science, Chulalongkorn University, 254 Phayathai Road, Pathumwan, Bangkok 10330, Thailand; 3Department of Chemistry, Faculty of Science, Chulalongkorn University, 254 Phayathai Road, Pathumwan, Bangkok 10330, Thailand; 4Laboratory of Biochemistry, Chulabhorn Research Institute, Vibhavadee-Rangsit Highway, Laksi, Bangkok 10210, Thailand

Proteomics are commonly used in direct protein study of tissues, cells and living organisms for functional component analysis. This technique is widely applied in biological science because it provides more information on living systems than the genomics approach. Proteomics are applied by researchers for medical proteomics, pharmaceutical proteomics and plant proteomics. The principles of proteomics comprise of 4 main steps: protein separation, protein identification, protein quantitative and protein analysis. Application of this technique in soybean (Glycine max) has established reference map in nodule cytosol in which 69 glycolysis enzymes has been found [1]. In soybean leaf, a total of 71 unique proteins are identified [2]. High levels of flavonoids in soybean leaf are confirmed to be sensitive to UV-B at the proteomics level [3]. Since very few proteomics study is established in herbal plants, we thus will focus on the proteomics study of *Pueraria mirifica* Airy Shaw & Suvatav, or A. lindleyi [4], an indigenous Thai medicinal plant is traditionally consumed for the treatment of menopausal symptoms belonging to legume family the same as soy bean. The plant phytoestrogens and their estrogenic activity have long been investigated. This will enable an analysis into the key proteins related with metabolite production in the Thai herbal plant tissues. Acknowledgement: Thailand Research Fund DBGS180025, Department of Biology, Department of Chemistry, Department of Biotechnology, Laboratory of Biochemistry, Chulabhorn Research Institute References: 1. Oehrle NW, Sarma AD, Waters JK,Emerich DW (2008) Phytochemistry 69: 2426-2438. 2. Xu C, Garrett WM, Caperna TJ, Natarajan S,JK, Emerich DW (2008) Phytochemistry 69: 2431-2440. 3. Xu C, Sullivan J, Garrett WM, Caperna TJ, Natarajan S (2008) Phytochemistry 69: 38-48.

**PF36**

**Phytoconstituent of Petroleum Ether Extract of Atriplex lindleyi Moq. aerial Part and Its Hepato-Renal protection**

Matloob AA*, El Souda SY**, Hamed MA**

1Pharmacognosy Dept., National Research Center, Cairo, 12622, Egypt; 2Chemistry of Natural compounds Dept, National Research Center, Cairo, 12622, Egypt; 3Therapeutic Chemistry Dept, National Research Center, 12622, Cairo, Egypt

The work aimed the detailed description of the lipid profile and hepato-renal protective effect of *Atriplex lindleyi* Moq. aerial part against bromobenzene (BB) intoxication in rats. Column chromatography of petroleum ether (60 – 80) extract and GC/MS analysis of the unsaponifiable matter and fatty acid methyl esters were qualitatively and quantitatively investigated. Oxygenated and non-oxygenated hydrocarbons, alkaloidal, phenolic, steroidal and triterpenoidal compounds were identified in the petroleum ether extract. GC/MS analysis of fatty acid methyl ester led to identification of 20 compounds. In vivo examination of the petroleum ether extract against bromobenzene (BB) intoxication using hepatocan as a reference drug was included for the five groups of albino rats were selected in this study. Group1: normal control group, group 2: i.p injected with BB (460 mg/kg b.wt) two times/week for three weeks, group 3: received oral doses of plant extract (150 mg/kg b.wt) at the same time and duration of BB injection. Group 4: served as group 3 and treated with hepatocan(medical Union Pharmaceutics company, Egypt) (100 mg/kg b.wt.) as a reference drug. Group 5: received plant extract only. The Drastic changes observed after BB intoxication in liver function enzymes (AST, ALT and ALP), hepatic cell organelles marker enzymes (SD, LDH, G-6-Pase, AP and 5’ -nucleotidase), kidney disorder parameters (creatinine and urea) and certain antioxidants; glutathione, lipid peroxide and superoxide dismutase. Treatment with petroleum ether extract improved all biochemical parameters under investigation as well as the histopathological chromatogram of liver and kidney. The petroleum ether of *A. lindleyi* contains bioactive compounds exhibiting hepatoprotective effect. References: 1. El-Sharky AS, Newary AA, Kamel MA, Eweda SM (2009) Food Chem Toxicol 47(7): 1584 – 90. 2. Shaker E, Mahmoud H, Mnaa S (2010) Food Chem Toxicol 48(3): 803 – 6. 3. Said O, Fulder S, Khalil K, Azaizeh H, Kaisis E, Saad B (2008) Evid Based Complement Alternat Med 5(4): 421 – 428.

**PF38**

**Diospyros lotus L. fruit extract protects G6PD-deficient erythrocytes from hemolytic injury in vitro and in vivo: prevention of favism disorder**

Habibi E*, Azadbakht M*, Hosseinimehr S†, Shokrzadeh M*, Ahmatt A†

1Department of Pharmacognosy, Faculty of Pharmacy, Tehran University of Medical sciences, Tehran, Iran; 2Department of Medical Chemistry, Mazandaran University of Medical Sciences, Sari, Iran; 3Department of Medicinal Chemistry, Mazandaran University of Medical Sciences, Sari, Iran; 4Student Research Committee, Faculty of Pharmacy, Traditional and Complementary Medicine Research Center, Mazandaran University of Medical Sciences, Sari, Iran

Favism is a life-threatening hemolytic crisis that can result from the ingestion of fava beans by susceptible individuals who have G6PD enzyme deficiency. The aim of this study was to evaluate the protective effect of Diospyros lotus L. fruit extract against the hemolytic damage induced by fava beans extract in both G6PD enzyme-deficient human and rat erythrocyte in vitro and in vivo models. In vitro model, venous blood samples were obtained from five subjects with known G6PD deficiency, which was also confirmed with standard techniques. Erythro-
cyte hemolysis was induced by fava beans extract in the presence and absence of Diospyros lotus fruits extract. The hemoglobin release in the supernatant, as well as the value of the hematocrit was determined by recording optical density at 540 nm in a spectrophotometer and microhematocrit, respectively. In vivo model, G6PD enzyme deficiency was induced in rats by intraperitoneal injection of DHEA (Dehydroepiandrosterone) (100 mg/kg), a specific G6PD enzyme inhibitor, for 35 consecutive days (2). Then the animals were pre-treated with different doses of Diospyros lotus (500, 750, 1000, and 1500 mg/kg) by oral administration for seven consecutive days after induction of G6PD deficiency. Rats were administered orally on the seventh day with Vicia faba beans extract (40 mg/kg b.w.), the blood was removed for evaluation of its value of erythrocyte hematocrit and hemoglobin after one hour. Our results have shown that Diospyros lotus fruits extract with an antioxidant activity has protective effect against hemolytic damage induced by fava beans extract in both G6PD-deficient human and rat erythrocytes. 

Acknowledgement: The in vitro modeling of this investigation was the subject of Pharm. D thesis of Emran Habibis as a student of the Mazandaran University of Medical Sciences. This research was supported by a grant from the Research Council of Mazandaran University of Medical Sciences. References: 1. Neto EC et al. (1993) Hum Genet 91: 293 – 294. 2. McIntosh MK (1993) Nutrition 123: 216 – 224.

PF39

Ethnopharmacological evaluation of male contraceptive efficacy of Dendrophthoe falcula in albino rats

Kachhawa JB1, Gupta RS2, Sharma KK1

1Molecular Developmental Biology Laboratory, Department of Zoology, Maharshi Dayanand University, Ajmer- 305002 (Rajasthan) India; 2Centre for Advanced Studies, Department of Zoology, University of Rajasthan, Jaipur- 302004 (Rajasthan) India

Search for male-antifertility agent in plants remains a potential area of investigations. Though, antispermatogenic activity has been reported in some plants, only few are reported. Therefore, present study was undertaken to evaluate the contraceptive-efficacy of Dendrophthoe falcula (L.f.) Ettingsh. in male albino rats as reported in folk remedies. Shade-dried stems of D. falcula were extracted in methanol and then fractionated with different solvents. A part of isolated-fractions were also processed for various phytochemical-techniques to identify active constituents. Findings of pre-
Schistosomiasis, a parasitic disease caused by trematode flatworms of the genus Schistosoma, represents a growing concern in the Sub-Saharan Africa, where up to 80% of the population is infected. Mirazid®, a commercial drug obtained by combination of two solvent extracts of Myrrh, the oleo-gum-resin from the stem of Commiphora molmol, is marketed in Egypt since 2001 as an alternative treatment for schistosomiasis [1]. However, recent independent studies question its efficacy. All experiments conducted with Myrrh so far are either in vivo tests or clinical trials, but no in vitro data is available. In order to shed light into controversy around Myrrh, two commercial Myrrh samples (from S. Africa and M. East) were extracted and/or combined to extract components when contaminated with MeOH (A+B) b) hydrodistillation to yield volatile oil c) for DPPH photometric method, BHT was used as positive standard. We investigated the four herbal extracts by HPLC-MS for the presence of polyphenolic compounds, we also assessed the antioxidant activity by the DPPH photometric method, BHT was used as positive standard. The current study explains that Myrrh has antischistosomal potential, but the origin of this study was to evaluate their potential to stimulate the wound healing process. 

PF42 In vitro effect of Myrrh extracts on the viability of Schistosoma mansoni larvae
Karamustafa SD1, Mansour N2, Demirici B3, Anki A4, Bayer MTC2, Bickle Q2, Tasdemir D1
1Centre for Pharmacognosy & Phytotherapy, The School of Pharmacy, University of London, London WC1N 1AX, UK.
2Department of Infectious & Tropical Diseases, London School of Hygiene & Tropical Medicine, London WC1E 7HT, UK.
3Department of Pharmacognosy, Faculty of Pharmacy, Anadolu University, 26470 Eskisehir, Turkey.
4CAMAG Laboratory, Sonnenmattstrasse 11, 4132 Muttenz, Switzerland.

Evaluation of four traditional Romanian medicinal plants as wound healing agents
Alexandra V1, Necula R2, Ghița G2, Gaspar A3, Toma A4, Tuna R5, Calle IE
1National Institute of Research and Development for Biological Sciences, Department of Cellular and Molecular Biology, Bucharest, Romania;
2Faculty of Pharmacy, Gr. T. Popa University of Medicine and Pharmacy, Iassy, Romania;
3Faculty of Biology, AL I. Cuza University, Iassy, Romania;
4NIURDS/”Neurála” Biological Research Centre, Piatra Neamț, Romania;
5University of Piatra Neamț, Piatra Neamț, Romania.

PF43

PF44

Study of apoptosis induction effects of traditional remedies and quality control strategies
Ho Huyhn T1, Nguyen T2, Nguyen Thai H1, Nguyen T2, Nguyen T1, Tran N1
1Department of Genetics, University of Science, 227 Nguyen Van Cu St., Dist. 3, Ho Chi Minh City, 70000, Vietnam;
2Department of Genetics, University of Education, 280 An Duong Vuong St., Ho Chi Minh City, 70000, Vietnam

Traditional medicine is an important part of health care system in Vietnam [1]. Nevertheless, lack of scientific and therapeutic evidences as well as quality control system limit its development. Many anticancer producers used in cancer therapy act by induce apoptosis in cancer cells [2]. In addition to chromotographic analysis with standard compounds, biological response fingerprinting are suggested for quality control of traditional formulations [3]. We investigated five traditional remedies reported in traditional pharmacopoeia as having anticancer effects. We determined apoptosis inducing capacity and cell cycle arrest of these remedies and their components on HeLa cells by DNA fragmentation assay, fluorescence microscopy, caspase activity assay and flow cytometry-based method. We showed that the modified remedy “Hoang Lien Giao Doang Thang” (HLGD) caused cell death by inducing apoptosis, independently of caspase-3 activation. Three components, Coptis sinensis Franch, Scutellaria baicalensis George and Phellodendron amurense Rupr have higher cytotoxicity than the whole remedy on HeLa cells. Microarray data analysis performed on HeLa cells treated with HLGD for 24 and 36 hours showed differential, increased or decreased, expression of 408 genes. Some overexpressed genes – DDIT3, TRIB3, FAM129A, STC2, GDF15, SERPIN2E2 were reported as involved in ER-stress. Expression level of these genes was confirmed by real-time RT-PCR. Real-time RT-PCR amplification of these genes were furthered to set up biological fingerprints. Bicalin and berberin were used as chemical fingerprints through chromatographic analysis. These fingerprints could be considered for quality control purposes of the remedy. Acknowledgement: These work was supported by grants from the Department of Science and Technology – Ho Chi Minh City. We are grateful to Prof. Sangho Lee and the Microarray Platform from Sungkyunkwan University Singapore for microarray experiments: 1.WHO (2002), WHO traditional medicine strategy 2002 – 2005. 2. Fulda S (2010) Planta Med 76(11): 1075 – 9 3. Chavan P, Joshi K, & Patwardhan B (2006) eCAM: 1 – 11

Evaluation of the effects of Parinari curtulifolia seed and Anchoheista vogelii root extracts individually and in combination on postprandial and alloxan-induced diabetes in animals
Ogbonnia SO1, Mhoka GO2, Anyika EN3, Lediju OK1, Ota DA1
1University of Lagos, Lagos, Nigeria; 2Lagos State University, Lagos, Nigeria

Parinari curtulifolia Planck. ex Benth. seed and Anchoheista vogelii Planck.roots extracts mixture (1:1) have been used locally for the treatment of diabetes. The postprandial effects were evaluated on albinino rats (20) randomly distributed into four groups. Each received orally 500 mg/kg of the extract mixture, P. curtulifolia and A. vogelii respectively and the control 0.5 ml (2% w/v) acacia solution. Blood glucose levels were monitored at 30, 60, and 120 min. intervals as described by Ogbonnia et al (1). Twenty five diabetic albino rats with plasma glucose>= 200 mg/dl were randomly divided equally into five groups and treated orally for 30 days as follows: Groups I, II and III received orally 500 mg/kg body weight of the mixture of P. curtulifolia and A.vogelii respectively while group IV received glibenclamide 600 μg/kg weight (2), while V diabetic control received 0.5 ml acacia solution. Results showed a significant reduction (p<0.05) in postprandial plasma sugar level after 30min in all treatments. Also significant reductions (p<0.05) in the plasma glucose, LDL-cholesterol, AST and ALT levels, and increase in HDL-cholesterol were observed in the treated diabetic groups. The pancreas tissue of diabetic animals treated with the extract mixture showed marked necrotic changes while pancreatic tissue of diabetic untreated animals showed more severe necrosis of beta cells which formed mass of amorphous osinophilia. The glibenclamide treated animals showed...
In vitro screening of selected medicinal plants against Schistosoma mansoni larvae
Karamustafa SD1, Mansour N2, Bickle Q3, Tasdemir D3
1Centre for Pharmacology & Phytotherapy, The School of Pharmacy, University of London, 29-30 Brunswick Square, London WC1N 1AX, UK; 2Department of Infectious & Tropical Diseases, London School of Hygiene & Tropical Medicine, Keppel Street, London WC1E 7HT, UK

Schistosomiasis, caused by various members of the trematode flatworms (Schistosoma species) is the second most important parasitic disease next to malaria. Resistance and low susceptibility towards praziquantel, the only available schistosomicidal drug, urge the search for new drugs. This study was aimed at assessing the in vitro schistosomicidal effects of several medicinal plants traditionally used for the treatment of schistosomiasis or other helminths [1]. The crude MeOH extracts of selected plants Artemisia absinthium L. and A. abrotanum L. (aerial parts) Phytoallacea dodecandra L. (roots), Curcuma longa L. (roots), Zingiber officinalis (roots), Panax ginseng L. (roots), fruits and leaf extract of the fruits) and Citrus reticulata Blanco (peels) were tested against juvenile worms (schistosomulae of S. mansoni) by using the standard visual larval assay. All crude extracts, except P. granatum and C. reticulata exhibited significant antischistosomal effect. The highest activity was displayed by C. longa (IC50 = 4.06 μg/mL), followed by A. abrotanum and Z. officinalis extracts with IC50 values of 11.1 μg/mL and 11.73 μg/mL, respectively. In the next step, the crude MeOH extracts were subjected to a liquid-liquid partitioning scheme between aqueous solvents (n-hexane, dichloromethane, ethylacetate, and 70% ethanol) and the prominent compounds in ethyl acetate extract of M. edulis were identified as indole alkaloids. The lipohilic subextracts retained the initial schistosomicidal activity at 100 μg/mL, and the subextracts were retested in the same assay. Generally, the lipohilic subextracts retained the initial schistosomicidal potential, whereas the aqueous MeOH subextracts were mostly inactive at 100 μg/mL concentration. The current study highlights the potential of plants against Schistosoma infections and confirms their use in traditional medicine. It also warrants phytochemical studies on the active plants to identify their active principles. References: 1. Sanaa AA (2011) Res Med Plant Sci 5:1–20.

Changing of some elements during phonological stages in Nitraria Schoberti L.
Shakeri R1, Naseri HR2, Pourrezaee J3, Yousefi Khanghah S3, Shakeri R1, Mehrabian Z4
1Department of Environmental Science, Natural Resources Faculty, Behbahan High Education Complex, Behbahan, Iran; 2Department of Coexisting with Desert, International Desert Research Center, University of Tehran, Tehran, Iran; 3Department of Rangeland Science, Natural Resources Faculty, Behbahan High Education Complex, Behbahan, Iran; 4Department of Forestry, Natural Resources Faculty, University of Tehran, Tehran, Iran

Nitraria Schoberti L. from Nitrariaceae as pharmaceutical, industrial and fodder plants can tolerate harsh environmental conditions especially sandy and saline soil. This plant is natural sources of some important chemical components like schobarine nitrate, dihydrodinitrate, nitramine, nitroxine and etc. More than 25 Mha of salt affected land exists in Iran and this plant can offer an economic and practical alternative towards achieving saline land and water resources in order to produce natural chemical components for pharmaceutical industries. Changing in some elements (Nitrogen, Phosphor, Sodium, Potassium, and Calcium) during flowering and forming seed stages (2009) were investigated. Samples of the aerial part of Nitraria Schoberti collected in the Kashan region were dried, sized and analyzed in Laboratory. The results showed that levels index values including P, K and Na were significantly differed among phenological stages but Na did not show any difference between two stages. Decreasing in P, K and N showed chemical component of this plant could change by sequence of phenological stages and finally these changes affect on quality of fodder and medicinal properties.

PF48
Phytochemical characterization of antymycobacterial crude extracts from medicinal plants traditionally used in Mozambique
Luo X1, Pires D2, Almeida IA3, Godinho B3, Mulhovo S4, Duarte A1, Anes F3, Ferreira M1
1Institute of Medicines and Pharmaceutical Sciences (IMEDUI), Faculdade de Farmacia, Universidade de Lisboa, Av. das Forças Armadas, 1600 – 083, Lisboa, Portugal; 2Centre of Patogenezie Molecular, Unidade das Retroviruses e Infecções Associadas e Instituto de Medicina Faculdade, Faculdade de Farmacia, Universidade de Lisboa, Av. das Forças Armadas, 1600 – 083, Lisboa, Portugal; 3Department of Microbiology, Faculty of Medicine, University of Zaragoza, C/Domingo Miral s/n, 50009 Zaragoza, Spain, and CIBER Enfermedades Respiratorias (CIBERES), Spain; 4Department of Ciências Agropecuárias, Escola Superior Técnica, Universidade Pedagógica, Campus de Lhanguene, Av. de Moçambique, 2140261 Maputo, Mozambique

A number of medicinal plants have long been used by traditional healers to treat tuberculosis and related diseases in Mozambique [1,2]. The present study was aimed to evaluate selected medicinal plants for their in vitro antymycobacterial activity, and reveal the main classes of compounds which might account for the observed activity. Four organic solvents (n-hexane, dichloromethane, ethylacetate, and 70% ethanol) were used for the sequential extraction. Decoction of each plant material was prepared according to traditional use. Different species of mycobacteria, namely, M. smegmatis, M. smegmatis, M. avium, and M. tuberculosis were employed to screen extracts by broth microdilution method. The cytotoxicity against human macrophages from the monocyte THP-1 cells was also evaluated. Overall, n-hexane extracts of Maerua edulis Gilg & Gilg-Ben, and Securidaca longepedunculata Fresen, ethyl acetate extract of Tabernaemontana elegans Stapf and dichloromethane extract of Zanthoxylum capense (Thunb., Hary, were found to possess considerable activity against M. bovis BCG and M. tuberculosis H37Ra with MIC = 15.6 – 62.5 μg/mL Tabernaemontana elegans ethyl acetate extract displayed strong activity against M. tuberculosis H37Rv (MIC 15.6 μg/mL) as well as potent cytotoxic effect on the THP-1 cells (IC50 < 4 μg/mL). Based on 1 H NMR spectroscopic analysis, major compounds in both Maerua edulis and Securidaca longepedunculata n-hexane extracts were linear chain unsaturated fatty acids. Zanthoxylum capense dichloromethane extract contained more complex constituents (mostly phenolic compounds), and the prominent compounds in ethyl acetate extract of Tabernaemontana elegans were identified as indole alkaloids. Keywords: Antymycobacterial activity, medicinal plants, Mozambique, tuberculosis


PF49
Genome wide expression analysis of the effect of wwcw, a traditional Korean herbal formula, on rat intracerebral hemorrhage
Cho S1, Kim H2, Lim C3, Lim S4
1Division of Pharmacology, School of Korean Medicine, Pusan National University, Gyeongnam, Republic of Korea; 2Department of Medicine, Graduate School, Dongguk University, Gyeonggi-do, Republic of Korea; 3Department of Nursing, School of Public Health, Far East University, Chungbuk, Republic of Korea

Woo-whang-chong-shim-won (WWCW) is a traditional Korean herbal formula which is commonly used for treating patients with hypertension, arteriosclerosis, coma and stroke in China and Korea. WWCW is composed of various kinds of chemical components, it would be difficult to isolate major components having pharmaceutical effect. Therefore, high throughput screening systems such as microarray analysis is essential procedure to elucidate the molecular effects of herbal extract on animal disease model. In this experiment, we measured the effect of WWCW on ICH in rat using microarray technology. ICH was induced by injection of total RNA was isolated. Hierarchical clustering was implemented using CLUSTER and TREEVIEW program, and for Ontology analysis, GOSTAT program was applied in which p-value was calculated by Chi square or Fisher’s exact test based on the total array element. WWCW-treatment restored the gene expression...
altered by ICH-induction in brain to the levels of 76.0% and 70.1% for up- 
and down-regulated gene, respectively. Co-regulated genes by ICH mod- 
el of rat could be used as molecular targets for therapeutic effect of drug 
Int Med 30(3): 594 – 60. 3. Andrew IS et al. (1979) Progress in Cardio-

**PF50**

Isolovanoid biosynthesis in *Pueraria mirifica* leaves

Jungsukcharoen J1, Cherdshewasart W2, Sanguanich P3

1 Department of Biotechnology, Faculty of Science, Chulalongkorn University, 254 Phayathai Road, Pathumwan, Bangkok 10330, Thailand; 2 Department of Biology, Faculty of Science, Chulalongkorn University, 254 Phayathai Road, Pathumwan, Bangkok 10330, Thailand; 3 Department of Chemistry, Faculty of Science, Chulalongkorn University, 254 Phayathai Road, Pathumwan, Bangkok 10330, Thailand.

*Pueraria mirifica* Airy Shaw & Suvatav. is a Thai indigenous herb with long-term consumption among Thai menopausal women for menopausal treatment. Researches in the tubers of this plant are mostly focused on their estrogenic potency and application to human health. The tubers are sources of active ingredients including the potent estrogenic miroes-
trol and deoxymiroestrol, and also isolovanoids, however, the plant tubers showed limited growth rate. Isolovanoids are the abundance sec-
dary metabolites in *P. mirifica* [1], [2] which play important roles in 
estrogenic effects [3] in animal assays, especially daidzein and genistein are potent anti-cancer, including breast cancer [4]. This group of chemi-
cals is also needed for dietary supplement and cosmetic products. The 
plants produce a lot of leaves during their growth and development. In 
this study, the leaves were collected for 12 consecutive months and 
tubers were collected for every 4 months. The leaves were dried and 
extracted for isolovanoids in the absence of chlorophyll for HPLC ana-
lysis together with the dried tubers. The analysis revealed that plant 
leaves contain significant amount of isolovanoids. Thus it would initiate 
only on isolovanoid extraction industry but also commercialized products derived from these chemicals. In addition, proteomics 
approach is introduced in our study which resulted in finding some 
interesting appearance proteins in the plant tubers. Acknowledgement: 

**PF51**

Larvicidal and antimarial activity of some Zulu medicinal plants

Opoku AR1, Nethengwe ME2, Dhludla P3, Madida K1

1 Department of Biochemistry and Microbiology, University of Zululand, P/B X 1001, KwaDlangezwa, 3886, South Africa; 2 Division of pharmacology, University of Cape Town, Private Bag X 3, Rondebosch, 7701, South Africa; 3 Department of Biochemistry, University of KwaZulu-Natal, Durban 4000, South Africa.

*Gardenia thunbergia* T.A Sprague, *Siphonochilus aethiopicus* (Schweif.) B.L. Burt, *Schottia bruchypetala* Sonon, *Acros calamus* L., *Withania somnifera* (L.) Dunal in DC., *Elaeodendron transvalens* (Butt. Davy) R.H. Archer, *Hypoxis hemerocallidea* Fisch., C.A. Mey. & Ave-Lal., *Vernonia adonisoides* Sch. Bip. Ex Walp.and *Acanthusperus austral* (Loefl.) Kunzente are medicinal plants commonly used by traditional healers in South Africa to treat malaria. Aqueous, dichloromethane and methanol extracts of these plants were screened for larvicidal, antioxidant, in vivo antipryretic, and in vitro antiplasmodial activities. The plant extracts either killed or reduced spontaneous movement in *Culex quinquesfascatus* larvae after 24 hours following treatment. Methanol extracts exhibited antioxidant (DPPH, ABTS scavenging, Fe2+ chelating) activity, albeit to varying degree of efficiency. The dichloromethane and methanol extracts significantly (p<0.05) reduced pyrexia with activity increasing in a concentration dependent manner. The antimalarial activity against chloroquine sens-
itive strain of *Plasmodium falciparum* (D10) showed that the methanol 
extracts of *G. thunbergia*, *V. adonisoides* and the dichloromethane extracts

of *E. transvalens*, *A. austral* and *W. somnifera* were active (IC50 of 1.04 –
5.07 μg/ml). The results suggest that these plants contained constituents that could be developed as potent antimalarial drugs (mosquito larvi-
cide, anti-fever and anti-plasmodial). Possibly, the compounds target metabolic pathways common to the *G. quinquesfascatus* larvae as *P. falciparum*. Acknowledgement: University of Zululand Research Com-
mittee Medical Research Council, South Africa.

**PF52**

Ethnobotanical studies in Astor valley, Nanga Parbat, Pakistan

Jeabem A, Begum F

Environmental Sciences Department, Fatima Jinnah Women University, Rawalpindi, Pakistan

Astor valley adjoin the eastern part of Nanga Parbat (Pakistan) consists of more than 100 villages. Local communities are dependent on surrounding plant resources for fruits, vegetables, fodder, shelter and healthcare. Data was collected through questionnaire and interview with 30 respondents from three villages, Kehamadas, Gorikot and Harcho. In total 33 plant species were recorded along with their local names, part used, purpose and diseases treated. The most commonly used medicinal plants are *Mentha longifolia* Hudis. (Philiklees), *Thymus linears* Benth. (Tumuros), *Saussurea petiolata* Komarow ex Lipsch. (Mumiran), *Berberis lyceum* Ishki (Ishkieen), *Cichorium intybus* L. (Chitay iskaniji), *Swertia petiolaria* Royle (Mumiran), *Viola pilosa* Blume (Lilium), *Ferula narthech* Boiss. (Sup), *Bergenia ciliata* Stearn (Sanzspur). The medicinal plants are used for stomach problem, cough, asthma, acidity, dysentery, pneumonia, cold, fever, blanking, itching, pain, dental diseases, swelling, anti-allergic, burning, skin problems, typhoid etc. The area depleted, due to over-exploitation of herbs and trees should be pro-
tected completely and attempt should be made to cultivate these plants at different localities and elevations. There is need to assess and identify the factors affecting biodiversity and indigenous knowledge system for mountain natural resource utilization and conservation. Acknowledge-
ment: We are thankful to the people who participated in the study. Re-

**PF53**

The determination of total phenolics and flavonoid contents, and antioxidant activity of some sage populations of *Salvia fruticosa* Mill., *Salvia pomifera* Mill. and *Salvia tomentosa* Mill. in the Marmara region of Turkey

Ergönül SS1, Karık U1, Baser KH2,3

1 Atatürk Bahçe Kultürleri Merkez Arastirma Enstitüsü, Yalova, Turkey; 2Anadolu Üniversitesi, Eczacilik Fakultesi, Farmakognosi Ana Bilm Dalı Eksesiner, Turkey; 3Botany and Microbiology Dept. College of Science – King Saud University P.O. Box 2455 – Riyadh 11451- Saudi Arabia

Methanolic extracts of 40 different population of three species of Salvia (Salvia fruticosa Mill. 20 samples, Salvia pomifera Mill. 5 samples and *Salvia tomentosa* Mill. 15 samples) were analyzed for their antioxidant properties. Samples were collected from different natural ecological areas in Marmara Region in Turkey. The antioxidant capacity (TAC) was investigated with the 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method and expressed as trolox equivalents (TE). The amount of total phenolics was determined by using Folin-Ciocalteu method and Flavonoid contents in the extracts were determined by a colorimetric method. The TAC values of the spices ranged from 288.57 to 3608.32 μmol (TE)/100 g dw. The total phenolic and flavonoid content ranged from 488.07 to 5277.97 mg of gallic acid equivalents (GAE)/100 g DW and 664.03 to 4046.77 mg of catechin equivalents (CE)/100 g DW, respectively.
The antioxidant activity and properties of 12 wild mushrooms (Lactarius piperatus (L.) Pers., Tricholoma caliginatum (Viv.) Ricken, Amanita caesar- ea (Scop.) Pers., Lactarius delicious (L.) Gray, Lactarius salmonicolor R. Heim & Leclair, Cantharellus cibarius Fr., Hydnum repandum L., Picoa lefebvrei (Pat.) Maire, Ramaria aurea (Schaeff.) Quel., Lactarius semisan-guifluus R. Heim & Leclair, Craterellus cornucopioides (L.) Pers., Laccaria laccata (Scop.) Fr.) collected from Turkey were evaluated. Their metha-nolic extracts were used to determine antioxidant capacity (TAC), total phenolics and flavonoids. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activities were measured to evaluate antioxidant capacity of the extracts and expressed as trolox equivalents (TE). The amount of total phenolics was determined by using Folin-Ciocalteu method and Flavonoid contents in the extracts were determined by a colorimetric method. Wild mushrooms were found to be high in antioxidant phyto-chemicals, such as phenolics (575.10 – 2156.40 mg GAE/100 g DW), fla-vonoids (103.01 – 346.53 mg CE/100 g DW). The TAC values of the spices ranged from 525.32 to 1603.95 (µmol TE)/100 g DW, and the antioxidant activity was found to vary in the order: Hydnum repandum L > Ramaria aurea (Schaeff.) Quel. > Lactarius salmonicolor R. Heim & Leclair > Crater- ellus cornucopioides (L.) Pers. > Lactarius delicious (L.) Gray > Lactarius piperatus (L.) Pers. > Picoa lefebvrei (Pat.) Maire > Tricholoma caliginatum (Viv.) Ricken > Amanita caesarea (Scop.) Pers. > Cantharellus cibarius Fr. > Laccaria laccata (Scop.) Fr. > Lactarius semisanguifluus R. Heim & Leclair.

Identification of proteins in preparations of Candida species used in homeopathic medicinal products

Preparations with Candida albicans and C. parapsilosis have been safely used as active substances in isopathic remedies for over 30 years. Isopathy is a special kind of homeopathy [1]. Water-soluble fractions after cell mill treatment and different purification steps are obtained from both yeast and named e volume cellulae (lyophil., steril.) [2]. D 3 to D 5 potencies of these starting materials are used both yeast and named e volumine cellulae (lyophil., steril.) [2]. D 3 to D 5 potencies of these starting materials are used and Microbiology Dept. College of Science – King Saud University P.O. BOX 2455 – Riyadh 11451- Saudi Arabia.

In vitro activity and chemical characterization of an apolar fraction of Morus alba leaf hot water extract

White mulberry (Morus alba L.) leaf is a well known traditional medicine of type II diabetes, a progressive disease with a broad spectrum of complications which has increasing incidence worldwide. Most typically it is taken as tea, often in combination with other phytotherapeutics. Many constituents were found to contribute to the anti-diabetic activity of mulberry leaf, including inosinsugars, flavonoids and related com-pounds, glycoproteins and ecldy steroids. Moreover, the role of pheny-lpropanes and megestagmine glycosides was also hypothesized by our group [1,2]. Here we report the investigation of a fraction of mulberry leaf hot water extract obtained by solvent-solvent partition between water and dichloromethane. A significant increase in the 24 h glucose consumption of fully differentiated adipocytes was found when treated with 50µg/mL of fraction with 0.32µM insulin or rather 200–350µg/mL without it, as compared to the corresponding controls. In the latter case, the activity was similar to that of 50µg/mL rosiglitazone that was used as positive control. Chemical composition of the pleasant odour, only fraction was investigated by using HPLC-DAD, GC-MS, GC-FID and LC-MS/MS. The main constituent (GC-FID: 56.2%) is suggested to be a chain-saturated cinnamaldehyde derivative, and benzyl alcohol, ethyl benzoate, t-cinnamic acid, p-hydroxyacetophenone, t-coniferyl alcohol and sinapyl alcohol were also identified as minor constituents. Attempting to perform in vitro activity guided isolation, further fractionation was done by using rotational planar chromatography. All fractions ob-tained were found inactive, which may suggest synergy between certain constituents. In vivo investigation of antidiabetic activity is currently in process. Acknowledgement: This project was supported by the Hungarian Na-tional Research Fund (OTKA; PD 75383), the New Hungary Develop-ment Plan (TÁMOP-4.2.2.-08/1-2006-0001 and TÁMOP-4.2.1.-09/1-2011-KONV-2010-0005) and by the grant from the National Science Council of Taiwan (NSC 9823140803701MY3). References: 1. Hunyadi A et al. (2007) Planta Med 73: 941. 2. Hunyadi A et al. (2008) Planta Med 74: 1117.
Phytochemical Study from Sonchus arvensis L. Leaves for Standardizing Traditional Medicine Extract

Sonchus arvensis L. leaves are empirically used as a traditional medicine for asthma, cough, anti-inflammation and diuretic [1,2]. To ensure quality through identification and standardization of its extract, fingerprint/phycotechnical study is needed. In this research, the phytochemical study was carried out by TLC (Thin Layer Chromatography) scanner and HPLC (High Performance Liquid Chromatography). From the results, n-hexane extract showed a better separation with toluen: ethyl acetate (93:7 v/v) and had specific retention factor 0.80; 1.30; 2.18 (254 nm) and 0.88; 1.29; 2.14 (366 nm). Chloroform extract showed specific retention factor 1.13; 1.30; 2.14 (254 nm) and 0.88; 1.29; 2.14 (366 nm). Otherwise, clear separation of ethyl acetate extract was shown in chloroform: toluen: ethanol (4:4:1 v/v/v) with specific retention factor 0.98; 1.44; 1.9; 2.34; 2.49 (254 nm) and 0.98; 1.94; 2.34; 2.50 (366 nm). From HPLC chromatogram at 254 nm, using acetoniitrile-phosphoric acid mixture showed specific retention time at 2.46; 4.09; 4.83; 7.69; 10.02; 11.06; 11.73 minute for hexane extract and 3.66; 5.84; 6.98 minute for ethyl acetate extract. In conclusion, the specific retention time from both extracts can be used as fingerprint for standardization of traditional medicine extract of Sonchus arvensis leaves. References: 1. Foster S. & Duke J A (1995) A Field Guide to Medicinal Plants. Eastern and Central N. America. Houghton Mifflin Co., ISBN 0395467225. 2. Xu et al. (2008) Food Chemistry 111: 92 – 97.

PF58

Effects of Angelicae Gigantis Radix (AGR) on Polycystic Ovary Induced by Estradiol Valerate in rats

Kim H, Choi E, Chung H, Jeung Y, Shin D, Cho S
School of Korean Medicine, Pusan National University, Pusan, Korea

Angelicae Gigantis Radix (AGR) is the most frequently used medicinal plants for patients with gynecological problems, especially pregnancy (1,2). This study was designed to investigate the effects of (AGR) on Polycystic Ovary (PCO) induced by Estradiol valerate (EV) in female rats. We investigated the effects of AGR on Changes in body weights and food and water uptake for 5 weeks. In addition, we examined the effects on ovary weights. Finally, we also observed histopathological changes in PCO rats. In our results, AGR administration group reduced ovary/body weight ratio to normal levels, which were lowered by induction of PCO (3). In histopathological observation, formation of cysts was suppressed in AGR group compared with non-treated PCO group. In conclusion, these results suggest that AGR can be used for patients with PCO to prevent formation of cysts. Further study and recommendation of AGR are necessary.

PF60

Evaluation of antimicrobial effects of three traditional medicinal plants from Iran

Yassa N1, Tofighi Z2, Molazem M1, Aliaalamghany F2, Shahverdi A3, Samadi N1
1Department of Pharmacoogy and Medicinal Plant Research Center, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran 14174 – 14411, Iran.; 2Department of traditional Pharmacy, Faculty of traditional medicine, Tehran University of Medical Sciences, Tehran, Iran.; 3Department of Pharmaceutical Biotechnology and Medical Nanotechnology, Research Center, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran.; 4Department of Drug and Food Control, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran.

There is growing interest in use of plants as natural antimicrobial agents because they do not induce antibiotic resistance which is usually happened with the synthetic antibiotics. Therefore, there is a need to develop alternative antimicrobial drugs for the treatment of infection diseases from various sources such as medicinal plants (1). The antimicrobial effects of different fractions of seed extract of Securigera sicuridea L., fresh petal of Rosa damascena Mill. and aerial parts of Tripleurospermum disciforme Sch.Bip. extract were examined against four gram positive and four gram negative bacteria and 2 fungi, which were obtained...
from Department of Drug and Food Control, Faculty of Pharmacy, Tehran University of Medical Sciences. The seed of Securigera securidaca, petals of Rosa damascena and top flowered of Tripleurospermum disciforme were collected in September, May and July 2009 around the Fars, Gilan and Tehran Provinces of Iran, respectively. The antibacterial and antifungal activity were studied by cup plate diffusion method as described by Warnock DW (2) and Soybean Casein Digest Agar and Sabouroud Dextrose Agar were used as medium for the growth of bacterial and fungal strains, respectively. The petroleum ether and chloroform fractions of S. securidaca showed antibacterial activities against Staphylococcus aureus and Pseudomonas aeruginosa, while methanol fraction had no antibacterial effects. R. damascena extract had antibacterial activities against Bacillus cereus, Staphylococcus aureus, Staphylococcus epidermidis and Pseudomonas aeruginosa. T. disciforme extract demonstrated antibacterial effects against Staphylococcus aureus and Staphylococcus epidermidis. All the fractions of plants had no antifungal activities. References: 1-Berahou A, Auhmani A, Fdil N, Benharref A, Jana M, Gadhi CA (2007) J Ethnopharmacol 112: 426 – 429 2-Warnock DW (1991) Methods with antifungal drugs In: Evans EG and Richardson MD. (Eds.) Medical Mycology: A Practical Approach. IRL Press, Oxford University Press 179 – 200.

One of the Korean mistletoe species, Loranthus yadoriki Sieb. exhibited potent inhibitory activities against monooamine oxidases Hwang K1, Kim J1, Choi Y1, Choi K2, Park K2
1Plant Resources Research Institute, Dukssung Women’s University, Seoul, Korea, 132-714.; 2Korea National Arboretum, Pocheon-si, Gyeonggi-do, Korea, 487 – 821

It is well known that Viscum album L var. coloratum Ohwi has antihypertensive, hypotensive and antiviral activities. The European mistletoe (V. album var. album) white fruit has been studied for its antiviral activity as it is widely used for the treatment of various malignant tumors and their supplement. Lectin and viscosotoxin are the active antiviral ingredients. Homoflavoydoralirin-B was reported as an antioxidant component of the mistletoe, and oleoscopic acid was also reported as an antioxidant component. There are five kinds of Mistletoe species in Korea. Most of them have above constituents evenly besides only one species. One of the Korean mistletoe, Loranthus yadoriki was very different constituent pattern. There were no main indicational bioactive ingredients, such as lectin, homoflavoydoralirin-B, and oleanolic acid was also smaller amount other than species. To investigate the bioactivities of Loranthus yadoriki several bioassays were applied. Loranthus yadoriki showed potent inhibitory activity against both types of monooamine oxidases and dopamine beta hydroxylase. But Loranthus yadoriki did not inhibit NO production in the cell. We are isolating the bioactive compounds from this plant with MDA inhibitory activity as a guide assay. Acknowledgement: This work was supported by grants from Scientific research (KNA1 – 2-11,10 – 2) of Korea National Arboretum.

Contents of ephedrine-like alkaloid synephrine in traditional Chinese decoctions Spriano D, Meier B
Zurich University of Applied Sciences, Life Sciences, Wädenswil, 8820, Switzerland

The FDA’s ban on ephedra has led to an increase in the use of the ephedrine-like alkaloid synephrine, in dietary supplements for the purpose of body loss. Synephrine naturally occurs in bitter-orange (Citrus aurantium L.) and other Citrus species. Concerns have been raised about the safety of products containing synephrine. Tangerine peel (Citrus reticulata Blanco; Cheri) is a herbal drug used in traditional Chinese medicine (TCM) and also contains small amounts of synephrine [1]. Traditional decoctions [2] of this drug are evaluated: i.e. the extraction yields for synephrine in dependence to extraction time. Thereof, an assumed daily intake is calculated for synephrine. Results showed a content of synephrine of 3.0 mg/g in the herbal drug (batch 1). Traditional decoction resulted in extraction yields of 65% synephrine, referred to dried drug. An extraction profile over time showed similar yields (about 67%) also after 3 hours of decoction. Maceration in cold water was about the same effective, yielding up to 71% of synephrine after 3 h. The analysis of a second herbal drug batch showed a synephrine content of 1.7 mg/g. Traditional decoction of it resulted in an extraction yield of 75% synephrine. A longer decoction lasting 2 hours lead to extraction yields up to 93%. It can be concluded that, assuming a daily dose of Chenpi of 3 – 9 g, there could result a daily intake of up to 19 mg synephrine. Such doses are below the levels exhibiting pharmacological effects, which are reported to be of 100 to 150 mg [3]. Acknowledgement: We thank Jian Chinaher, Switzerland, for the supply of herbal drug material.

Antioxidant activity of Rhodomyrtus tomentosa (L.) Hassk. From Terengganu coastal area Analii A, Abdul Manaf A
Faculty of Agriculture and Biotechnology, Universiti Sultan Zainal Abidin, Terengganu, Malaysia

Rhodomyrtus tomentosa (L.) Hassk. has shown interesting capability to scavenge free radicals and hydrogen peroxide as well as play effective role inhibition of lipid peroxidation. Results of DPPH scavenging assay shows the IC50 of 30 µg/mL and the 80% maximum inhibition at the concentration of 100 µg/mL. The 50% inhibition against hydrogen peroxide is at the concentration of 0.17 µg/mL and the maximum inhibition of 98% at the concentration of 0.25 µg/mL FTC and TBA assay shows the 77.11% and 95.88% inhibition, respectively. Acknowledgement: The authors are thankful to the Faculty of Agricultural and Biotechnology, University of Sultan Zainal Abidin for the funding and research facilities

Antinociceptive Activity of Eryngium kotschyi Boiss. Root Extracts Aslan Erdem S1, Arban G2, Mitaine Offer A3, Iskit A4, Miyamoto T5, Kartal M6, Scalice Dubois M6
1Department of Pharmacognosy, Faculty of Pharmacy, University of Ankara, Tandogan, 06100-Ankara, Turkey; 2Department of Physiology, Faculty of Medicine, Hacettepe University, Ankara 06100, Turkey; 3Laboratoire de Pharmacognosie, Unité UIMR, UPSRES EA 3660, Faculté de Pharmacie, Université de Bourgogne, 7, Bd Jeanne d’Arc, BP 87900, 21079 Dijon Cedex, France; 4Department of Pharmacology, Faculty of Medicine, Hacettepe University, Ankara 06100, Turkey; 5Graduate School of Pharmaceutical Sciences, Kyushu University, Fukuoka, Japan

Eryngium species, belonging to Apiaceae family are well known plants in ethnobotanical culture in the world and also in Turkey. They are used as antitussive, diuretic as well as for analgesic and antiinflammatory purposes. Anticancer: Res 21(4A): 2687 – 2691. Kim YK, Kim J
In African and Asian countries with low incomes up to 80% of the populations depend on traditional medicine for primary health care (WHO, 2008 World Health Report). In West-African countries and especially in the northern region of the Republic of Benin patients with pain-associated diseases used traditionally some plants like Entada africana Guill. & Perr., Ficus thonningii Guill. & Perr., Ficus conyzoides M. Ma., Ageratum conyzoides L., F. conyzoides M. Ma., Ageratum conyzoides L., Piliostigma thonningii (Schumach.) Milne-Redh. and Chasmanthera sp. Primary goal of this project was to test a potential analgesic effect of novel gel formulations containing a defined combination of ethanolic or aqueous extracts of these plants on pain associated with irritation provoked by arthritis or musculoskeletal trauma. Second objective was to compare effectiveness of the novel gel-formulations with diclofenac gel. The analgesic effect was assessed using criteria of evaluation within 10 days. 81.8% of the patients (n = 11) responded with reduced pain score after topical application twice daily of the gel formulation containing the ethanolic extracts, whereas the water-based formulation was less effective (57.1%; n = 7). Diclofenac gel (25 mg) reduced the pain by 58.3% (n = 12) of the patients within two weeks. The results demonstrated that the ethanolic extract was more effective than the aqueous extract and the well established diclofenac gel. Our study involving pain-associated patients explained the importance of an adequate formulation for extracts used in the traditional medicine and pointed out that a combination of plant extracts could be an alternative to typically applied synthetic analgesics. Further studies are necessary to examine the mechanisms contributing to the analgesic effect of the plant extracts.

PF68

Anti-inflammatory activity from Limonium brasiliense (Boiss.) Kunzte

Rodriguez SA, Viña MD, Murray AP, Leiro JM

1 INQUISUR, Departamento de Química, Universidad Nacional del Sur, Av. Alem 1253, 8000, B. Bca. (Pcia. B.A.), Argentina; 2 Laboratorio de Parasitología, Instituto de Investigación y Análisis Alimentario, Universidad de Santiago de Compostela, c/Constantino Candeira s/n, 15782, Santiago de Compostela, Spain; 3 Departamento de Farmacología, Facultad de Farmacia, Universidad de Santiago de Compostela, 15782, Santiago de Compostela, Spain

Limonium brasiliense Kunzte (Plumbaginaceae) is a medicinal plant, known as “Guaycuru” from southern Argentina. Infusion from the roots is popularly used in the treatment of hemorrhage, menstrual disorders, rheumatism and it is believed to have cardioprotective properties [1] The aim of this work was to evaluate the anti-inflammatory activity of methanolic extract from roots of *L. brasiliense* and its major constituent, myricetin 3-O-rhamnoside, in vitro. [2, 3] This extract was partitioned with different solvents of increasing polarity to obtain sub-extracts that were fractionated by silica gel column chromatography, for isolation and purification of the active compounds. The fractions and isolated compounds have been tested in cultures cell lines. These were not cytotoxic against RAW 264.7 and HL60 cell lines. Thus, these fractions and the isolated compound have been tested on inhibition of nitric oxide (NO) production on LPS-stimulated RAW 264.7 cells. The best anti-inflammatory potency (40 μg/ml = 63% inhibition) was provided by a fraction coming from the ethyl acetate sub-extract. This fraction contains myricetin 3-O-rhamnoside. (IC50 = 13.29 μM/ml) Also, we investigated the antioxidant effects of these fractions and the isolated compound on inhibition of intracellular and extracellular production of reactive oxygen species (ROS). These have inhibited both ROS production. The results presented demonstrate that myricetin 3-O-rhamnoside displayed a typical antioxidant activity; it markedly inhibited intracellular and extracellular ROS production. These results also support the claims of traditional medicine about the use of *L. brasiliense* roots in the treatment of inflammatory diseases. Therefore antidepressive research should also be extended to in vivo models. Acknowledgement: This work was supported financially by Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET, PIP N°0655), Universidad Nacional del Sur (UNS, PGI.

PF69

Anti-inflammatory Evaluation of Agaratum conyzoides L. Leaves

Awad NE, Kassem HA, Elkhabyt Z, Elfeky AM

1 Pharmacognosy Department, National Research Center, Dokki, Cairo, Egypt; 2 Pharmacognosy Department, Faculty of Pharmacy, Cairo University, Alkayr Alani, Cairo, Egypt; 3 Medical biochemistry Department, National Research Center, Dokki, Cairo, Egypt

Alcohol extract (70%) as well as successive extracts of the air dried powdered *Agaratum conyzoides* leaves have been evaluated for their anti-inflammatory and chemical constitution. All extracts showed high significant anti-inflammatory activities against carrageenan induce edema. Moreover, crude extract and ethyl acetate exhibited good anti-inflammatory activity against cytokine interleukin (1L)-6 than the other extracts. Quantitative and qualitative estimation of the total flavonoid, steroidal, triterpenoidal, protein and carbohydrate contents of 70% alcohol extract were determined. The mucilage of crude extract was isolated, identified using TLC and evaluated for their anti-inflammatory activity. The free and glycosidal flavonoids of ethyl acetate extract were isolated. Kaempferol, p- hydroxyl benzoic acid, quercetin-3-O-rhamnoside and quercetin-3, 7- diglucopyranoside were isolated from ethyl acetate extract. The isolated flavonoidal fractions and compounds are evaluated for anti-inflammatory activity. The glycosidal flavonoid fractions proved greater anti-inflammatory effect than the isolated compounds.

PF70

Folk medicines of Çamlıdere (Ankara)

Günbatan F, Gürbayıs F, Gençler Özkaz M

1 Pharmacognosy Department, National Research Center, Dokki, Cairo, Egypt; 2 Medical biochemistry Department, National Research Center, Dokki, Cairo, Egypt

In this study, folk medicines which are being currently used in Çamlıdere district (Ankara) were determined. For this purpose, scientific trips were organized to the Çamlıdere. During these trips, interviews were conducted with those who have knowledge on folk medicines. When the plants that collected during the trips and constituted the majority of folk medicines were evaluated systematically, it was determined that 65 species belonging to 61 genera of 29 families are being used in the treatment of various diseases. Besides, 19 medicines of animal origin and 34 medicines were isolated from the air dried leaves in the district. The plants of the Asteraceae family were determined to be used mostly, Lamiaceae and Rosaceae were the other frequently used families. The folk medicines were mainly used for respiratory tract diseases, dermatologic problems, gastrointestinal system diseases and rheumatic complaints in study area. Best of our knowledge, eight plant taxa were determined at the first time to be used as folk medicine in Turkey. Additionally, different usages of 41 predefined folk medicines were designated in this study. Finally, striking erosion observed in ethnobotanical heritage and the urgent need of systematic ethnobotanical researches to record this precious knowledge before it is completely lost were also emphasized.
Evaluation of acute and sub chronic hepatotoxicity of hydroalcoholic extract of \textit{Teucrium polium} L. in non-diabetic rats

Kiyani M$^1$, Ostad SN$^2$, Arbabi S$^3$
$^1$Traditional Pharmacology Department, Traditional Medicine Faculty, Tehran University, Tehran, Iran; $^2$Toxicology and Pharmacology Department, School of pharmacy, Tehran University, Tehran, Iran; $^3$Islamic Azad University, Pharmaceutical Sciences Branch (IAUPS), School of Pharmacy, Dept. of Toxicology & Pharmacology, Tehran, Iran

While it is well-known and widely used for its hypoglycemic and anti spasmodic properties in traditional medicine of many countries (1, 2), probable side effects of \textit{Teucrium polium} L. (Tp), especially hepatotoxicity in diabetics, needs more investigation. The purpose of this study is to determine the acute and subchronic hepatotoxicity of Tp hydroalcoholic extract in non-diabetic rats. In acute phase, rats were given doses from 50 – 7000 mg/kg of the solution by gastric gavages. However, our paraclinic and histopathologic studies were focused on the dose of 3000 mg/kg. In sub chronic phase, 1000 mg/kg of the solution was given through drinking water once daily. On the day 45, liver damage was again evaluated through blood samples and biopsy. (3) There was no mortality seen. AST and ALT rose, more in females, but not to a significant level in either sex. Histopathologic examination revealed signs compatible with non specific reversible hepatic inflammation. The results were the same in both phases. Our study suggests that hydroalcoholic extract of \textit{Teucrium polium} L:1 is non-toxic in vivo and does not induce hepatotoxicity. However, the same result may not be seen in diabetic rats and that entails more investigation. Additionally, the growth place of Tp may have some effects on the results. References: 1. Hasani-Ranjbar S, Nayebi N, Larjani Band Abdollahi M (2010) International Journal of Pharmacology 6(4); 315 – 325 J.Lubuncic P, Azaiezh H, Portnaya I, Cogan U, Said O, Saleh KA, Bonzon A (2005) Ethnopharmacol 99(1): 43 – 7 3. Ecobichon DJ (1997) The basis of toxicity testing. CRC press II c (Second edition), Boca Raton, New York

Antioxidant and hepatoprotective activity of \textit{Tragopogon porrifolius} melon extract

Mroueh M$^1$, Daher C$^2$, El Sibili M$^3$, Tenkerian C$^2$
$^1$School of Pharmacy, Lebanese American University, PO Box 36, Byblos, Lebanon; $^2$School of Arts and Sciences, Natural Sciences Department, Lebanese American University, PO Box 36, Byblos, Lebanon

\textit{Tragopogon porrifolius} L. (Asteraceae), commonly known as purple salsify, is cultivated for its edible root and shoot. The present study investigates the in vitro and in vivo antioxidant activity of the methanolic extract of the aerial part of \textit{T. porrifolius} as well as its protection against CCl$\textsubscript{4}$-induced hepatotoxicity in rats. Total phenolic and flavonoid contents, were assessed using the Folin-Ciocalteu and the aluminum chloride colorimetric methods and found to be 37.1 mg/g GAE and 16.6 mg/g QE respectively per gram dry weight of the extract. The FRAP assay showed a probable side effects of \textit{Phyllanthus amarus} methanolic extract in non-diabetic rats. In acute phase, rats were given doses from 50 – 7000 mg/kg of the solution by gastric gavages. However, our paraclinic and histopathologic studies were focused on the dose of 3000 mg/kg. In sub chronic phase, 1000 mg/kg of the solution was given through drinking water once daily. On the day 45, liver damage was again evaluated through blood samples and biopsy. (3) There was no mortality seen. AST and ALT rose, more in females, but not to a significant level in either sex. Histopathologic examination revealed signs compatible with non specific reversible hepatic inflammation. The results were the same in both phases. Our study suggests that hydroalcoholic extract of \textit{Teucrium polium} L:1 is non-toxic in vivo and does not induce hepatotoxicity. However, the same result may not be seen in diabetic rats and that entails more investigation. Additionally, the growth place of Tp may have some effects on the results. References: 1. Hasani-Ranjbar S, Nayebi N, Larjani Band Abdollahi M (2010) International Journal of Pharmacology 6(4); 315 – 325 J.Lubuncic P, Azaiezh H, Portnaya I, Cogan U, Said O, Saleh KA, Bonzon A (2005) Ethnopharmacol 99(1): 43 – 7 3. Ecobichon DJ (1997) The basis of toxicity testing. CRC press II c (Second edition), Boca Raton, New York

In vitro schistosomicidal activity of triterpenoids from the African plant \textit{Monordica balsamina}

Ramalhete C$^1$, Magalhães L$^2$, Rodrigues V$^3$, Mulhovo S$^4$, Filho AS$^5$, Ferreira MU$^1$
$^1$Research Institute for Medicines and Pharmaceutical Sciences (Med.UF), Faculdade de Farmácia, Universidade de Lisboa, Av. das Forças Armadas, 1600 – 083, Lisboa, Portugal; $^2$Núcleo de Ciências Exatas e Tecnológicas, Universidade de Franca, São Paulo, Brazil; $^3$Faculdade de Medicina de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto, Brazil; $^4$Departamento de Ciências Agro-Pecuárias, Universidade Pedagógica, Moçambique; $^5$Departamento de Ciências Farmacêuticas, Faculdade de Farmácia, Universidade Federal de Juiz de Fora, 36036 – 330, Juiz de Fora, MG, Brazil

Schistosomiasis, also known as bilharziasis, is a chronic liver and intestinal parasitic disease caused by trematode worms of the genus Schistosoma. Praziquantel is the only available drug against all species of this disease. The development of praziquantel resistance is a great concern and new drugs are urgently needed [1]. \textit{Monordica balsamina} L. (Cucurbitaceae), commonly known as African pumpkin, is a vegetable widespread in tropical and subtropical regions that has been used as food, mainly in sub-Saharan Africa. It has also been widely used in traditional medicine in Africa to treat various disease symptoms, mostly diabetes and malaria. In previous work, bioassay-guided fractionation of the methanol extract of the aerial parts of \textit{M. balsamina} led to the isolation of several cucurbitane-type triterpenoids. Most of the isolated compounds as well as their acylated derivatives displayed antimalarial activity [2, 3]. Continuing our search for antiparasitic compounds, the aim of this work was to evaluate the in vitro schistosomicidal activity of several triterpenoids isolated from \textit{M. balsamina} against Schistosoma mansoni adult worms [4, 5]. Praziquantel was used as positive control. A remarkable schistosomicidal activity was observed for two of the ten compounds tested (at 50 and 100 μM), which caused the death of all S. mansoni adult worms after 24 h of incubation. Both compounds, at 10 – 50 μM, induced significant reductions in the motor activity of the worms and significantly decreased the egg production. Furthermore, they were able (at 100 μM) to separate the adult worm pairs into male and female after 24h. Acknowledgement: \textit{This study was supported by FCT, Portugal (SRH(BD/22321/2005) as well as Fapesp (2006/00132 – 4) and CNPq, Brazil References: 1. WHO (2010). Fact sheet n° 115. 3. Ramalhete C et al. (2011) Bioorg Med Chem 18: 5254 – 60. 4. Ramalhete C et al. (2011) Bioorg Med Chem 19: 330 – 8. 3. Magalhães LG et al. (2010) Parasitol Res 106: 395 – 401. 4. Magalhães LG et al. (2009) Parasitol Res 104: 1197 – 120

Phytochemical screening and the effects of aqueous extracts of \textit{Phyllanthus amarus} leaves on the lipid profile and cardiac muscle cyclic guanosine monophosphate of male Guinea pigs

Samuel TA, Okonkwen EK, Akande IS, Megbagbole OA

Extracts of the leaves of \textit{Phyllanthus amarus} Schumach. (Euphorbiaceae) are used as folk medicine for the treatment of jaundice and other diseases in Nigeria and other countries. Recently the extract is becoming popular for increasing and or improving libido and reproductive functions in men. The effects of several aqueous extracts of the leaves of \textit{Phyllanthus amarus} on lipid profile and the cardiac muscle cyclic guanosine monophosphate (cGMP) in male Guinea pigs was investigated and compared to the effects of sildenafil citrate on the same parameters. The phytochemical screening was also carried out. The results showed that the administration of aqueous extract of the \textit{Phyllanthus amarus} leaves to the animals (100, 200 and 400 mg/kg body weight) caused a statistically non significant (p > 0.05) increase in cholesterol, triacylglycerol, low density lipoprotein and high density lipoprotein level while the administration of 100 mg/kg body weight of sildenafil citrate caused a non significant (p > 0.05) decrease in lipid profile levels but a non significant increase in the level of triacylglycerol. However the administration of aqueous extract of \textit{Phyllanthus amarus} (100 and 200 mg/kg body weight) caused a non significant (p > 0.05) decrease in the level of cardiac cGMP, while the administration of 100 mg/kg body weight of sildenafil citrate and 400 mg/kg body of the aqueous extract cause a non significant increase p > 0.05 in the levels of cGMP. Furthermore, the phyto-
The effects of Cucurbita pepo seeds on testosterone induced benign prostatic hyperplasia
Newfla T1, Daher C2, Mroueh M2, Baroody K1, Nasser S3, Baroody G1

1School of Arts and Sciences, Natural Sciences Department, Lebanese American University, PO Box 36, Byblos, Lebanon; 2School of Pharmacy, Lebanese American University, PO Box 36, Byblos, Lebanon; 3School of Medicine, Lebanese American University, PO Box 36, Byblos, Lebanon

The Cucurbita pepo (pumpkin) seeds are considered a snack food in most social gatherings in Lebanon and the Middle East. Many herbal combinations containing pumpkin seeds are used to treat symptoms of benign prostatic hyperplasia (BPH). Because the seeds are the most commonly consumed and not the seed oil, this study was carried out to examine the effects of pumpkin seeds on testosterone (3.57 mg/kg body weight) induced BPH in rats (1). After achieving hyperplasia (30 days), treatment with pumpkin seeds (10, 20, 30 and 60% w/w of chow) or finasteride (5 mg/kg body weight) was initiated for 12, 24, and 36 days. Results showed that pumpkin seeds exerted maximum inhibition (86.7, 98.0 and 98.4%) of hyperplasia at 30% w/w dose after 12, 24 and 36 days respectively. They were comparable to finasteride (78.1, 89.5 and 96.4%). There was no significant effect on weight gain in rats treated with testosterone and pumpkin seeds. Additionally, no significant effects were observed on levels of sGOT-AST enzyme and ALP, while slight increase was observed on sGPT-AST. The findings on prostatic hyperplasia were confirmed by histopathological studies where tissue showed abundant stoma between glandular cells and lack papillary projections into the lumen of the glands. In conclusion, pumpkin seeds inhibit prostate hyperplasia induced by testosterone, and improve the histology of the prostate. Acknowledgement: Mr. Jean Karam. References: 1. Gonzales G (2007) Asian J Androl 9(2): 245 – 251.
Effect of *Garcinia kola* Heckel seeds on bioavailability of two commonly used drugs in Nigeria (sulphamethazine and paracetamol)

Ezekwesili Ojiffo J, Ozie OC, Felicia ON

1Nnamdi Azikiwe University, Awka, Nigeria; 2University of Nigeria, Enugu Campus, Enugu, Nigeria

*Garcinia kola* Heckel (Guttiferae), is a large forest tree found throughout Western and Central Africa. Widely known in commerce as ‘bitter kola’, the seeds are used extensively in African traditional medicine as a social masticatory agent and for the treatment of various diseases, especially cough, mouth and respiratory tract infections, and are also claimed to reduce the effectiveness of drugs and toxic substances in general. They are thus used locally as antidote to poisons and in cases of drug overdose. The effects of concurrent administration of *G. kola* whole seed suspension were investigated on the bioavailability of two commonly used drugs in Nigeria, namely, sulphamethazine, a sulphonamide antibiotic, and paracetamol, an analgesic acetaminophen. Two specimens of g. kola, each weighing 500 g, were extracted by boiling with a concentration of 0.5 g/kg body weight of seed suspension given concurrently with 150 mg/kg body weight of sulphamethazine and paracetamol respectively. Control groups were given equivalent doses of either drug alone. Blood was withdrawn from the left ear at one hour intervals for five hours. Results showed that *G. kola* seeds decreased significantly (p<0.05) the bioavailability of the two drugs. Relative bioavailability was calculated to be 77.56% for sulphamethazine and 75.39% for paracetamol. The time of peak and peak concentrations were also reduced, while the concentration at one hour was only significantly different for paracetamol at p<0.05. These results suggest that *G. kola* seeds may reduce bioavailability by interfering with drug absorption across the gastrointestinal mucosa.

Acknowledgement: The Management and Staff of Emmanuel Research Laboratory, Enugu Nigeria for allowing the use of their facilities.


Inhibitory Effect of Crude Aqueous *Brucia amarissima* Extract on the Growth Profile of Oral *Candida albicans*

Augustyn WA, Combrinck S, Botha BM

Department of Chemistry, Tshwane University of Technology, Pretoria, South Africa

*Mangiferina indica* L. has high concentrations of mangiferin content in the pulp, peel, stem and leaves. Honeybush (*Cyclopa genistoides* Vent.), an indigenous South African herbal tea, used for its antioxidant property and other health benefits, also contains high levels of mangiferin (2). The aim of this study was to determine the levels of mangiferin in the leaves of three mango cultivars to investigate the possibility of using mango leaves as a health beverage. Mangiferin was extracted by infusing the dried leaves in boiling water and the extract was subsequently quantified using UV-Vis (3), HPLC and HPTLC (4). Spectroscopic methods were used to analyse the powdered leaf materials. Chromometric analysis (O-PLS) was used to develop a predictive model for mangiferin. Extracts of the mango leaves were added to fruit juices and the stability of mangiferin determined regarding time, light and pH. The levels of mangiferin in mango were compared to those found in honeybush teas. Mangiferin levels found in a leaf infusion of mango indicated that mango leaves may have more health benefits than honeybush tea.


Purification of verbascoside from plant extracts using column and countercurrent chromatography

Combrinck S, Oyounou JN, Regnier T, Marston A

1Department of Chemistry, Tshwane University of Technology, Private Bag X680, Pretoria, 0001, South Africa; 2Department of Chemistry, University of Free State, Bloemfontein, 9300, South Africa

Verbascoside, a phenylethanoid glycoside, displays diverse biological activities. The antifungal activity of *L. camara* polar extracts of *Lippia* species against *Penicillium digitatum*, a common pathogen of citrus, was attributed to the presence of verbascoside. Partial purification of such plant extracts to increase the verbascoside content could provide natural mycobiocides for postharvest control of pathogens on fruit. Verbascoside was extracted from dried *Lippia welwitschii* H.Pearson plant material using aqueous methanol. The extracts were purified using silica gel column chromatography and the verbascoside concentrations were compared to that obtained by countercurrent chromatography (CCC). The crude extract contained 4.8 g/l verbascoside, corresponding to 12.5% of the extract. After column chromatography, the verbascoside content of the purified extract was substantially increased, but was lower than that obtained by CCC (40.8%). High performance liquid chromatography was used for the analyses of the target compound. The widespread in-vader weed *Lantana camara* L., was investigated as an alternative source of verbascoside. The verbascoside content of extracts from 13 populations in South Africa were analysed by HPLC. Both intra- and interpopulation variability was observed in the verbascoside content. Combinations of flowers and leaves from *L. camara* could serve as a good source of verbascoside. Several stability tests were conducted to evaluate the stability of the compound under different conditions. The shelf life stability study proved that the compound is stable in a dry form when stored in the dark, but decomposes rapidly when exposed to light. Verbascoside also proved to be reasonably stable under steam distillation conditions.

References: 1 Shikenga et al. (2009) Fruits 64:3 – 12

The prevalence of oral *Candida* infections has increased, due to immunosuppressive effect of antifungal agents on resistant hosts [1,2]. Growth rate is a key attribute of virulence among infectious microorganisms including *Candida* species. The aim of this study was to evaluate the growth inhibitory effect of *Brucia amarissima* (Lour.) Merr. leaves extract based on changes in the pattern of growth profile of *Candida* sp. *Candida albicans*, *Candida tropicalis* and *Candida dubliniensis* were used in this study. Crude extract of *B. amarissima* was prepared and the minimal inhibitory concentrations (MICs) towards *Candida* sp. were determined. The growth responses were recorded based on changes in the doubling time (g-values) and specific growth rates (μ-values). The values in the presence of extract were computed as percentage in the optical density (OD) relative to the total cells suspension in the absence of extract. 0.12% w/v chlorhexidine (CHX)-containing mouth rinse and sterile distilled water were used as controls. *C. tropicalis* was found to have the highest growth rates indicating high bioactivities and reproducibilities. *C. dubliniensis* and *C. tropicalis* showed the highest reduction of μ-values at a minimal concentration with 87.04% and 57.28%, respectively. At higher concentration (6 mg/mL), the extract exhibited significant reduction towards the growth (p<0.05). Also, was able to reduce the μ-values of all Candida strains with more than 90% reduction. The extract
exhibited fungistatic (< 6 mg/mL) and fungicidal (≥ 6 mg/mL) activities towards oral Candida sp. hence, may be considered as a promising candidate for the development of antifungal agent of natural products.


PF84

Plants known as “Node-to-dog” (Heterotepa assms aphrodisiaca O. Mach. (Malpighiaceae) and its medicinal use in southwestern Mato Grosso, Brazil

Rieder A1, Arruda PC2

1University of Mato Grosso states – UNEMAT, Av. São João, s/n, Bairro Cavalhada, CEP 78200 – 000 Cárceis (MT), Brazil; 2Cárceis, Mato Grosso, Brasil

There are several species of medicinal plants known as “Node-to-dog”. The most used is the Heterotepa assms aphrodisiaca O. Mach. (Malpighiaceae). We conducted field and laboratory studies and also review on popular usage, occurrence and bioactivity of H. assms aphrodisiaca in southwestern Mato Grosso (MT-sw), Brazil from 2003 – 2009. The plant occurs in savannah and field areas. It is widely used as an aphrodisiac by local communities. Its beautiful flowers are ornamental, yellow, often visited by wild bees. The plant has long, expanded and nodulated roots, hence the name of “node-to-dog”. It spreads easily by seeds, adapts well in adverse environments and resists fires. Experi-

ments on more appropriate systems of cultivation, developed in Cárceis (MT-sw), revealed that the species adapts equally well in mono and polyculture. The root of the adult plant is used in folk medicine to treat nervous disorders, sexual problems, high cholesterol, and is physical invigorating. The root is macerated in wine for consumption as an appetite stimulant. There are substances in the H. assms aphrodisiaca promising to treat fatigue, memory loss and Alzheimer’s disease. Phytochemical analysis revealed the presence, in root extracts, of polyphenols, tannins, alkaloids; cardiotonic, aromatic and flavonoid glycosides; and of saponins. In MT-sw it is a commonly used medicinal plant and the root, bark, and sap are used in boxes.

Acknowledgement: Fapemat – financial support, and for UNEMAT – institutional support; to the collaborators colleagues from the research group FLOBIO – (Plants carrying Bioactive substances)

PF85

Plant known as “Sangra d’água” (Croton urucurana Baill. (Euphorbiaceae) and its medicinal use in southwestern Mato Grosso state, Brazil

Rieder A1, Figueiredo GC2, Pereira ES3

1University of Mato Grosso states – UNEMAT, Av. São João, s/n, Bairro Cavalhada, CEP 78200 – 000 Cárceis (MT), Brazil; 2University of Mato Grosso-UNEMAT, Cárceis (MT), Brazil

“Sangra d’água” is a medicinal tree plant (genus Croton). There are several species, including: C. urucurana Baill., C. salutaris Casar., C. lecithi Müll.Arg., C. planostigma Klotzch. (Croton urucurana (Euphorbiaceae) is the best known. We conducted “filed, lab studies” and a literature review on popular usage, occurrence and bioactivity of C. urucurana in southwestern Mato Grosso (MT-sw), Brazil 2003 – 2007. In MT-sw it grows spontaneously and quickly in watercourses margins. The additive light plant exposure accelerated its reproduction. The inflores-

ments: For Fapemat – financial support, and for UNEMAT – institutional support; To the collaborators colleagues from the research group FLOBIO – (Plants carrying Bioactive substances)

PF86

Plant known as “Leather hat” (Echinodus spp. (Alismataceae) and its medicinal use in southwestern Mato Grosso, Brazil

Rieder A, Figueiredo GC, Bonilla MG

Universidade do Estado de Mato Grosso –UNEMAT, Av. São João, s/n, Bairro Cavalhada, CEP 78200 – 000, Cárceis (MT), Brazil

Several plant species (> 10) known as “Leather hat” (Echinodus spp. – Alismataceae) used in traditional medicine. We made field and laboratory studies and review on popular usage, occurrence and bioactivity of “Leather hat” in Mato Grosso (MT), Brazil, from 2003 – 2007. The following species have been known to occur in MT: E. grandiflorus Mitch., E. lanceolatus Ratay, E. macrophyllus Kunth and E. teretocapitus Haynes. They prefer humid tropical conditions. The species E. grandiflorus and E. macrophyllus are also used as tea. Studies of ethnic knowledge in southwestern MT revealed that leaves of “Leather hat” are used as anti-inflammatory, antiseptic, diuretic, laxative, astringent, for gargling, washing sores, treating kidney, liver, arthritis, rheumatism, high blood pressure; roots – to treat atherosclerosis, hernia, boils; leaves and flowers – to treat syphilis, purgative; roots, leaves and flowers – for skin disorders. The healers collect the plant in the dry season, and indicate that E. grandiflorus and E. macrophyllus are used as tea, poultice, and tincture. Some scientific studies and review on popular usage, occurrence and bioactivity of “Leather hat” reveal the presence in the root extracts, of polyphenols, tannins, alkaloids; cardiotonic, aromatic and flavonoid glycosides; and of saponins. In MT-sw it is a commonly used medicinal plant and the root, bark, and sap are used in boxes.

Acknowledgement: Fapemat team – for financial support; UNEMAT team – by institutional support; Colleagues of the research group FLOBIO – (Plants carrier of bioactive substances) who collaborated in this study.

PF87

Antidiabetic effects of Allium ascalonicum methanolic extract in experimental diabetes

Montasser Khouarsi S, Fehresti Sani M

Department of Cellular and Molecular Biology, School of Biology, University of Tehran, Iran

In the present study we have investigated the antidiabetic effects of bulbs of Persian shallot Allium ascalonicum L., (Alliaceae) methanolic extract (AAE) at doses of 500 and 250 mg/kg bw, on Allancon-induced diabetic male Wistar rats in comparison with Acarbose (as a reference drug), by measuring postprandial blood glucose (PBG), oral glucose tolerance test (OGTT), inhibition of rat intestinal α-glucosidases activities, changes in the levels of plasma lipids profiles antioxidant enzymes activities, including superoxide dismutase (SOD), glutathione peroxidase (Gpx) and catalase (CAT), pancreatic Insulin and cardiac Glut-4 mRNAs expression. In diabetic Wistar rats, in short term period, effects of AAE on PBG showed significant reduction after 24h of oral administration. After 3 weeks of treatment, significant chronic decrease in the PBG was observed. For OGTT, the increase in PBG levels reduced mildly in AAE treated diabetic rats, at 1hour. Intestinal sucrose and maltase activities were inhibited by AAE, 17.41% and 14.62% respectively. In diabetic rats, AAE also increased the activities of SOD by 65%, Gpx by 43% and CAT by 55%, showing strong antioxidant effects. AAE demonstrated antihyperlipidemic properties by reducing serum TG, LDL, VLDL and TC. In addition, we have observed increased expression of Ins and Glut-4 genes in diabetic rats treated with methanolic extract of Allium ascalonicum, compared to control group. Acknowledgement: The authors would like to thank the Cellular and Molecular Department of University of Tehran for financial support. References: Vincent AM, Russell JW, Low P, Feldman EL (2004) Endocr Rev 25: 612 – 28. Marcus AO (2001) Postgrad Med110: 113 – 23. Day C (1998) Br J Nutr 80: 5 – 6. Augusti KT & Sheela CG (1996) Experientia 52: 115 – 120. Feshani AM, Khouarsi SM, Mohammadi S (2010)J Ethnopharmacol 133: 67 – 74.

Planta Med 2011; 77: 1229–1472 Georg Thieme Verlag KG Stuttgart · New York · ISSN 0032-0943
Molecular and biochemical effects of the methanolic extracts of the leaves of Salvia officinalis on diabetic male wistar rats

Mohammad Kourosh S. Motamed Abdollahi
Department of Cellular and Molecular Biology, School of Biology, University College of Science, University of Tehran, Iran.

Common sage (Salvia officinalis L.) is among the plants that are claimed to be beneficial to diabetic patients and previous studies have suggested that this plant has hypoglycaemic effects in normal and diabetic rats. In the current study, we have investigated the effects of methanolic extract of S. officinalis leaves on blood glucose, plasma biochemical parameters, pancreatic insulin and cardiac Glut-4 mRNAs expression, inhibition of rat intestinal α-glucosidases activities and erythrocyte antioxidant enzymes. Treatment of Alloxan monohydrate-induced diabetic rats with oral administration of sage leaves methanolic extract for 3 weeks, resulted in a significant reduction in blood glucose (Glucose oxidase assay). Total cholesterol (TC), triglycerides (TG), LDL/HDL ratio and VLDL were mildly decreased after treatment of diabetic rats by plant extract. We have also observed significant enhancement in activity of blood antioxidant enzymes including superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx), we have observed increased expression of Ins and Glut-4 genes in diabetic rats treated with methanolic extract of S. officinalis, compared to control group. The extract showed strong inhibition effect on intestinal α-glucosidases enzymes activities. Acknowledgement: The authors would like to thank the Cellular and Molecular Department of University of Tehran for financial support. References: Lin YF, Tsai HL, Lee YC, Chang SJ (2005) Journal of Nutrition 135: 2457 – 61. Lu YR and Foo LY (2001) Tetrahedron Lett 42: 8223 – 8225. Wang M et al. (1998) Agric Food Chem 46: 4869 – 4873.

New Approaches in Characterisation of Herbal Preparations

Orland A1, Knopp K2, Krämer E1, Wiesner J1, Kehrhaus S2, Frötschl R1, König GM1, Knöss W1
1Federal Institute for Drugs and Medical Devices, Bonn, Germany; 2Institute of Pharmaceutical Biology, University of Bonn, Bonn, Germany

New techniques have recently been established in instrumental analytics and molecular biology, which are also applicable to medicinal plants. These can demonstrate the suitability of such methods for the identification of herbal preparations by PCR-based methods and for characterisation of herbal preparations by NMR-fingerprinting in combination with principal component analysis [1, 2]. The next step is characterisation of biological activities and correlation with fingerprint profiling. Extracts with solvents of different polarity (Ethanol, Ethanol/Water, Dichloromethane) were obtained from herbal substance of Cedronium majus L. and characterised by means of HPLC and NMR-fingerprinting. Extracts were applied to human liver cells (HepG2) and cell proliferation was monitored in a real time cellular monitoring system, xCELLigence (Roche). Growth of HepG2 cells was drastically inhibited compared to control by ethanolic extracts and partially by ethanolic/aqueous extracts. For aqueous extracts and dichloromethane extracts only minor growth inhibition was observed. Toxic effects of the extracts will be further investigated with expression profiling using DNA microarray technology to check if there is a correlation with known toxic effects which have been reported to herbal preparations containing Cedronium majus L. References: 1. Daniel C et al. (2008) Zeitschrift für Phytotherapie 29: 270 – 274 2. Kersten T et al. (2008) Zeitschrift für Phytotherapie 29: 122 – 128

Assessing the in vitro gastric stability and gastrointestinal transport of selected natural molecules Chan W, Mbadanga B, Hamman J, Viljoen A
Department of Pharmaceutical Sciences, Faculty of Sciences, Tshwane University of Technology, Pretoria 0001, South Africa

Natural antioxidants in foods and plants play a major role in helping the body’s defense system to fight the destruction caused by reactive oxygen species. They may act by decreasing oxygen concentration and as metal inactivators, hydroperoxide decomposers, oxygen scavengers and synths (Shahidi, 1997). Several studies have revealed the possible beneficial effects of natural antioxidants in the human body, without considering the influence the gastrointestinal tract may have on the composition, activity and absorption of these compounds (Cao et al., 1998; Serrano et al., 2007). This study aimed at assessing the in vitro gastrointestinal stability and intestinal transport of orally ingested anti-oxidants in food and plants. Curcumin, epicatechin, ferulic acid, gallic acid, quercetin, resveratrol, rosmarinic acid, and rutin were selected. Compounds were incubated in simulated gastric fluid (SGF, pH 1.2) for 1 hour and in simulated intestinal fluid (SIF, pH 6.8) for 3 hours. Concentrations were detected before and after incubation. The results indicated that all the compounds were stable in SGF, only epicatechin and rutin were unstable in SIF (with 6.27% and 5.16% degradation, respectively). The in vitro transport experiment was conducted across porcine intestinal tissue in the apical-to-basolateral direction in a Sweetana-Grass

Assessing the in vitro gastric stability and gastrointestinal transport of selected natural molecules

Combrinck S1, Gercke N1
1Department of Chemistry, Tshwane University of Technology, Pretoria, South Africa; 2Department of Pharmaceutical Sciences, Tshwane University of Technology, Pretoria, South Africa; 3Sun Valley, 7985, South Africa

Sceletium tortuosum (L.) N.E.Br. is indigenous to South Africa and has traditionally been used for its mood enhancing properties. Recently, products containing S. tortuosum have become increasingly popular and are commonly administered as tablets, capsules, teas, decoctions or tinctures, while the dried plant material has traditionally been masticated and the resulting extracts swallowed. In this study, the in vitro transport of four major S. tortuosum alkaloids across porcine intestinal, sublingual and buccal mucosa in their pure form as well as in the form of three different crude extracts; water, methanol and an acid-base alkaloid-enriched extract, was evaluated. The permeability of mesentery across intestinal tissue was higher than that of the highly permeable reference compound, caffeine, in its pure form as well as in the form of crude extracts. The intestinal permeability of mesentery was similar to that of caffeine, while that of mesembranol and mesembrenone was lower than that of caffeine but much higher than that of the poorly permeable reference compound, atenolol. In general, the permeability of the alkaloids was lower across the sublingual and the buccal tissues than across the intestinal tissue. However, comparing the transport of the alkaloids with that of the reference compounds there are indications that absorption from the oral cavity may contribute considerably to the overall bioavailability of the alkaloids when the plant material is chewed. The results from this study predict relatively good bioavailability of the alkaloids of S. tortuosum in purified or crude extract form when administered orally.

New Approaches in Characterisation of Herbal Preparations

Assessing the in vitro gastric stability and gastrointestinal transport of selected natural molecules

An investigation of the in vitro transport of Sceletium tortuosum alkaloids across porcine buccal, sublingual and intestinal membranes Shikango EA1, Viljoen AM2, Chen W3, Hamman JF4, Combrinck S5
1Department of Chemistry, Tshwane University of Technology, Pretoria, South Africa; 2Department of Pharmaceutical Sciences, Tshwane University of Technology, Pretoria, South Africa; 3Sun Valley, 7985, South Africa

Sceletium tortuosum (L.) N.E.Br. is indigenous to South Africa and has traditionally been used for its mood enhancing properties. Recently, products containing S. tortuosum have become increasingly popular and are commonly administered as tablets, capsules, teas, decoctions or tinctures, while the dried plant material has traditionally been masticated and the resulting extracts swallowed. In this study, the in vitro transport of four major S. tortuosum alkaloids across porcine intestinal, sublingual and buccal mucosa in their pure form as well as in the form of three different crude extracts; water, methanol and an acid-base alkaloid-enriched extract, was evaluated. The permeability of mesentery across intestinal tissue was higher than that of the highly permeable reference compound, caffeine, in its pure form as well as in the form of crude extracts. The intestinal permeability of mesentery was similar to that of caffeine, while that of mesembranol and mesembrenone was lower than that of caffeine but much higher than that of the poorly permeable reference compound, atenolol. In general, the permeability of the alkaloids was lower across the sublingual and the buccal tissues than across the intestinal tissue. However, comparing the transport of the alkaloids with that of the reference compounds there are indications that absorption from the oral cavity may contribute considerably to the overall bioavailability of the alkaloids when the plant material is chewed. The results from this study predict relatively good bioavailability of the alkaloids of S. tortuosum in purified or crude extract form when administered orally.

An investigation of the in vitro transport of Sceletium tortuosum alkaloids across porcine buccal, sublingual and intestinal membranes

Assessing the in vitro gastric stability and gastrointestinal transport of selected natural molecules

Assessing the in vitro gastric stability and gastrointestinal transport of selected natural molecules

Assessing the in vitro gastric stability and gastrointestinal transport of selected natural molecules
Antihyperalgesic and antidiabetic effects of Pinus koraiensis leaf oil
Kim S1, Lee H', Jeong S', Lee E', Lee M'
1College of Oriental Medicine, Kyung Hee University, Seoul 130 – 701, South Korea; 2College of Life Sciences and Biotechnology, Kyung Hee University Yongin 446 – 701, South Korea

Metabolic disease is a complex syndrome to develop cardiometabolic risk factors, including central obesity, insulin resistance, glucose intolerance, dyslipidemia and hypertension. In the present study, anti-diabetic and hyperalgesic activities of essential oil from leaves of Pinus koraiensis Siebold & Zucc. (EOPK) were evaluated. EOPK significantly reduced the blood glucose concentration in streptozotocin (STZ)-induced diabetic mice without weight loss, while significant weight loss was observed in STZ treated mice. Furthermore, EOPK significantly suppressed the production of α-amylase, an enzyme that catalyzes the breakdown of carbohydrate into glucose, in a dose-dependent manner and also prevented the STZ-induced cytotoxicity and nitric oxide (NO) production in HIT-T15 pancreatic β-cells. In addition, EOPK significantly inhibited the level of human cholesterol acyltransferase (hCAT)-1 and -2 and reduced low-density lipoprotein (LDL) oxidation in a dose-dependent manner with an IC50 value of 40 μg/mL. Also, Gas chromatography-mass spectrometry (GC-MS) revealed that EOPK contains alpha-pinene (21.3%), alpha-terpineol (11.0%), 8-3-carene (10.2%), terpinolene (7.2%), camphene (6.2%) and limonene (5.2%). Taken together, our findings suggest EOPK could be a potent pharmaceutical agent for prevention and treatment of metabolic syndrome including diabetes and hyperalgesic effects.


An ethnobotanical survey of medicinal plants in Valopei district of Savadkouh (Mazandaran-Iran)
Cholpour A1, Samadi S1, Isazadeh Araz M1, Sonboli A2
1Department of Biology, Payame Noor University (PNU), Sari, Iran; 2Department of Biology, Medicinal Plants and Drugs Res. Inst., Shahid Beheshti University, Tehran, Iran

This study was aimed to identify wild plants collected for medical purposes by the local people of Valopei County, located in the Northern of Iran, and to establish the uses and local names of these plants. Field study was carried out over a period of two years (2009 – 2011). Valopei district with over thousand years oldness, 52 villages and high rich knowledge about useful plants is situated in central Elburz region of Iran. A total of 66 plant species belonging to 61 genera and 39 families have been reported from the study area [1]. The most encountered medicinal plant family was Lamiaceae with seven species. The plants are used as medicinal, food and vegetable, provender, treatment of animal diseases, hunting, building construction, manure, weaving and dyeing, respectively based on significance. The use of several species such as Ligularia persica Boiss., Albizia julibrissin Durazz., Ruscus hyrcanus Woronow, Gedetschia caspica Desf., Tamus communis L., Hyoscyamus niger L., Quercus castaneifolia C.A.Mey., Punica granatum L., Calystegia sepium (L.) R.Br. and Potentilla reptans L. are reported for the first time. Mode of preparation and administration are discussed along with the family and local names of plants and plant parts used [2]. References: 1. Rechinger KH (1963 – 1999) Flora Iranica, Vol. 1 – 174. Akademische Druck und Verlagsanstalt Graz. Austria. 2. Martin GJ (1995) Ethnobotany. A methods manual. London: Chapman & Hall.

Antioxidant activity, total phenolic and flavonoid content of Ficus deltoida Jack varieties
Afnani A, Nazhiyeh M, Halimatun Saadiah O, Abdul Manaf A
Faculty of Agriculture and Biotechnology, Universiti Sultan Zainal Abidin, Terengganu, Malaysia
Six varieties of Ficus deltoida Jack namely F. deltoida Jack var. deltoida, F. deltoida Jack var. angustifolia (Miq.) Corner, F. deltoida Jack var. intermedius Corner, F. deltoida Jack var. biloba Corner, F. deltoida Jack var. renguannuensis Corner and F. deltoida Jack var. kunstleri (King) Corner were collected from various locations in Peninsular Malaysia. The total phenolic content in Ficus deltoida var. intermedius was remarkably high, followed by varieties kunstleri, renguannuensis, deltoida and biloba. The lowest flavonoid content was observed in the angustifolia variety. Antioxidant activity of aqueous extracts was determined by measuring DPPH and H2O2 scavenging activities. Very strong antioxidant activity was observed for the extract of intermedius variety with IC50 of 40 μg/mL, whereas moderate activities were recorded in the extracts of biloba and kunstleri varieties with IC50 of 150 and 200 μg/mL respectively. Low DPPH scavenging activities were observed in the extracts of renguannuensis, deltoida and angustifolia varieties; with IC50 of 325, 380 and more than 500 μg/mL respectively. Higher H2O2 scavenging activity was observed in all varieties studied, when measured at 500 μg/mL as compared to vitamin C.

Ethnobotanical and ethnopharmacological study in the practice of midwifery in pastoral Iran
Kazemi E1, Kazemi N2
1Social Security Organization, Kermanshah, Iran; 2Payam Noor University, Islam Abad, Iran

In this study the ethnobotanical and ethnopharmacological features of twenty medicinal plants native to Iran were investigated. Midwives in pastoral communities across the world are very important as main health care providers, but few researches have recognized the therapeutic plants engaged in this age-old practice. Semi-structured interviews were carried out with 5 midwives in 12 pastoral communities near Kermanshah, Iran, concerning the plants they utilize during child delivery as well as pregnancy. Twenty different plant species used to treat 5 conditions happening during the pregnancy, birth and postpartum stages were recorded. Most plants and uses were reported by only one or two midwives. It is interesting to mention that most midwives in this area had emigrated from different parts of the country. Approximately all the midwives used or knew of plant remedies for the treatment of miscarriages, postpartum abdominal pain and hemorrhages, retained placenta, and for speeding up contractions during labor. The most commonly cited plants as well as those for which there was greatest consensus tended to be widespread cultivated or wild species. Although use of medicinal plants by midwives has been reduced as a result of retraining programs by government health centers, midwives’ knowledge of medicinal plants may provide an important resource for improving maternal-infant health in Iran and elsewhere. References: 1. Tahraoui A, El-Hilaly J, Isralhi ZH, Lyoussi B (2007) Ethnopharmacol. 110: 105 – 117. 2. WHO (2000), www.who.int/diabetes/facts/world-figures (World Health Organization, Switzerland).
Phytochemicals, especially the secondary metabolites synthesized by plants, play key roles in human nutrition, health, wellness and disease prevention. Preliminary studies using LC-ESI provides a simple, rapid technique for the analysis, that is suitable for routine screening of plant materials. Two glucosinolates were identified in the aqueous extract of *Brassica oleracea* L var. *italica* Plenck, glucobrassicin and 4-c-(a-L-rhamnopyranosyl) benzyl glucosinolate, they were identified by liquid chromatography–negative ion electrospray mass spectrometry (LC-ESI) [1]. Two compounds were isolated after enzymatic hydrolysis of the aqueous extract by myrosinase, one of them was identified as 4-vinyl-3-pyrrolidinone. The second compound (sulforaphane) 1-isothiocyanate-4-methyl-sulphinyl butane, converted to the most stable form of thiourea, (sulfurphane thiourea) [2]. The crude extract (80% alcohol extract) of broccoli florets was examined for cytotoxic activity against different cell lines [3], it showed good inhibition to colon tumor (IC50 3.88 µg/mL). But when the same test was repeated on each successive extract no significant cytotoxic activity produced with any of them. When myrosinase hydrolyase was tested for cytotoxic activity on colon tumor cell line, it showed very high activity 95% lethality up to 0.78 µg/mL.


**Investigation of Flavonoid Constituents and Hepatoprotective Activity of Myoporum laetum**

**Hassan EM**1, **Mohamed SM**1, **El Tahawy KA**2, **Mohamed AN**3

1Medical and Aromatic Plants Dept., National Research Centre, Tahrir St., 12311, Dokki, Cairo, Egypt; 2Chemistry of Medicinal Plants Dept., National Research Centre, Tahrir St., 12311, Dokki, Cairo, Egypt; 3Medical Chemistry Dept., National Research Centre, Tahrir St., 12311, Dokki, Cairo, Egypt

Myoporum laetum G.Forst. (Myoporaceae) is an evergreen ornamental shrub and it flowers from May to June [1]. Fractionation and isolation of the butanol extract of *Myoporum laetum* yielded five major flavonoids, luteolin 4‘-O-α-L-rhamnose, 5,7,3'-trihydroxy-3,5'-dihydroxy-28-O-d-glucopyranoside and 3-O-β-D-glucopyranosyl-28-O-β-D-glucopyranoside, 7-O-β-D-glucopyranoside and 3-O-β-D-glucopyranoside.

PG6

Previous phytochemical investigation of Ormosiara kirkii S.Moore (Papilionaceae) has led to the isolation and identification of 3-β-D-β-hydroxy-7,8-dihydroxyflavonoids, some of them with antiproliferative activity (Dhooghe et al., 2010). The major compound of the total extract (80% MeOH) was (+)-chamaejasmin. However, a series of minor constituents remained unidentified. The aim of this work was to further characterise the 80% MeOH extract of O. kirkii and to identify the minor constituents using an integrated platform of LC-MS and LC-SPE-NMR. The crude 80% MeOH extract of O. kirkii was partitioned into four fractions by means of liquid-liquid extraction: n-hexane, chloroform, ethyl acetate and water. The chloroform and ethyl acetate fractions were used for the LC-SPE-NMR study. By using the multiple trapping technique several components were enriched on the SPE cartridges. After drying and eluting with deuterated methanol into 3 mm NMR tubes, high-resolution NMR spectra were recorded. The structure elucidation of these compounds was based on 1D and 2D NMR, and MS data. A total of sixteen compounds proved a significant antioxidant effect. References: [1] Sharma OP, Makar HPS, Dawra RK (1988) Toxicol 26: 975. [2] Kirtikar KR, Basu BD (1935) Indian medicinal plants, vol. III. Dehra Dun: Bishen Singh and Mahendra Pal Singh.;1914. [3] Chopra RN, Nayar SL, Chopra IC (1956) Glossary of Indian medicinal plants. New Delhi: CSIR,149.

PG7

Lantana camara L., Family Verbenaceae commonly known as wild or red sage, is the most widespread species of this genus, growing luxuriantly in tropical, sub-tropical and temperate regions [1]. L. camara is used in folk medicine as vulnerary, diaphoretic, carminative, antiseptic, anti-spasmodic and tonic and [2-3]. A phytochemical investigation of the aqueous-methanolic extract of its flowers had led to isolation of a new flavonoidal compound, apigenin-7-O-β-D-galacturonopyranosyl-(2′-1)-O-β-D-galacturonopyranosyl with the eleven flavonoids, luteolin-7-O-β-D-glucuronopyranosyl-(2′-1)-O-β-D-galactopyranosyl, apigenin-7-O-β-D-glucuronopyranosyl-(2′-1)-O-β-D-galactopyranosyl, vitexin, isovitexin, apigenin-7-O-β-D-glucuronopyranosyl, luteolin-7-O-β-D-glucopyranosyl, luteolin-7-O-β-D-galactopyranosyl, luteolin-4′-O-β-D-glucopyranosyl, apigenin-7-O-β-D-galactopyranosyl, luteolin and api-genin. The aqueous methanolic of L. camara flowers and some compounds proved a significant antioxidant effect. References: [1] Pretorius SJ et al. (1991) J Trop Med Hyg 94: 159 – 165. [2] Balde AM et al. (2005) Turk J Chem 29: 177 – 186. [3] Ansari A 1, Hawas UW 3, Mahmoud K 1 1Pharmacognosy Department, National Research Centre, Cairo, Egypt; 2Chemistry of Natural Products Department, National Research Centre, Dokki, Cairo, Egypt; 3Phytochemistry and Plant Systematic Department, National Research Centre, Dokki, Cairo, Egypt

Flavonoids from the flowers of Lantana camara L. with in vitro antioxidant activity

Abou El Kassem L1*, Mohammed R2*, Salah El Din S1, El Ansari A1, Hawaii UV1, Mahmoud K31

1Pharmacy Department, National Research Centre, Cairo, Egypt; 2Chemistry of Natural Products Department, National Research Centre, Dokki, Cairo, Egypt; 3Phytochemistry and Plant Systematic Department, National Research Centre, Dokki, Cairo, Egypt

PG8

Phytochemical constituents of the root of Combretum micranthum G. Don (family: Combretaceae)

Umar HD, Abdurahman EM, Ilyas N, Agunu A

Department of Pharmacognosy and Drug Development, Ahmadu Bello University, Zaria-Nigeria


PG9

Oligostilbenoids from the stem bark of Dryobalanops aromatica

Wibowo A, Abmat N, Hamzah A

Faculty of Applied Sciences, Universiti Teknologi MARA, 40450 Shah Alam, Selangor Darul Ehsan, Malaysia

Dryobalanops is one of the Dipterocarpaceae families which contain oligostilbenoid that show various biological activities [1]. The oligostil benoid in Dryobalanops genera is very unique, as some compounds such as cis- and trans-diptoindonesin B and malaysianol A have difference oxidative pattern compared to other oligostilbenoids in Dipterocarpaceae [2, 3]. The aim of this study is to isolate the oligostilbenoid constituents in Malaysian D. aromatica C.F.Gaertn. and to determine their cytotoxic activity. The dried powder of the stem bark of D. aromatica was macerated with acetone and evaporated under reduced pressure. The crude of acetone extract was subjected to vacuum liquid chromatography (VLC) to give 10 major fractions. Purification of the sixth fraction with radial chromatography and am (13) gave laevifolin (1) (93 mg) and amplexosin E (2) (397 mg) while the fifth fraction afforded α-viniferin (3) (91 mg) and ε-viniferin (4) (20 mg) and the tenth fraction yielded diptodionisin A (5) (30 mg). The effect of the isolated compounds (1 – 3) against HL-60, MCF-7, HepG2, A-549 and WR1-68 cell lines were evaluated by using


Abstracts | 59th International Congress of the GA | 4th-9th September 2011, Antalya, Turkey
A novel sesquiterpene acid and an alkaloid from leaves of the Eastern Nigerian mistletoe with potent immunostimulatory activity on C57BL/6 splenocytes

Omeje EO1, Osadebe PO1, Kawamura A2, Esimone CO1, Proksch P1, Nwodo JN1
1Department of Pharmaceutical and Medicinal Chemistry, Faculty of Pharmaceutical Sciences, University of Nigeria, Nsukka, 410001, Nsukka; 2Department of Chemistry, Hunter College of CUNY, The City University of New York, U.S.A.; 3Department of Pharmaceutical Microbiology and Biotechnology, Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University; 4Department of Pharmaceutical Biology, Heinrich-Hein University, Düsseldorf, Gebade, Germany

In our continued efforts to identify the immunomodulating constituents of a local mistletoe species in Eastern Nigeria, a novel sesquiterpenoid acid, 2, 3-dimethoxy-benzo [a, b] cyclopentenyl-3', 3', 5'-trimethyl pyran-4-one (1), and a known alkaloid, lupinine (2) [1] were isolated from a bioactive chloroform fraction of the leaf extract of the mistletoe parasitic on kola tree, Cola acuminata (P.Beauv.) Schott & Endl. These compounds were screened for immunostimulatory activities on isolated C57BL/6 mice splenocytes at concentrations of 10, 25 and 100 µg/ml. Their effects on the expression of CD69, an early immune cells activation marker [2], were determined using flow cytometry techniques and compared to Lipopolysaccharide (LPS: 10 µg/ml) and Concanavalin A (Con A: 2 µg/ml) as standards. The compounds (1 and 2) at a concentration of 25 µg/ml showed statistically significantly (p < 0.05) stimulatory activity on the isolated splenocytes compared to the non-stimulated control with values of 56.34 ± 2.26% and 69.84 ± 0.19% respectively compared to 1.69 ± 0.05% for the non-stimulated control cells. The compounds were characterized using a combination of UV/visible, IR, NMR (13C-NMR and 1H-NMR) and DEPT, MS and 2-dimensional correlation (H-H COSY, HSQC, HMBC, NOE, and NOESY) studies. These compounds may be responsible for the immunomodulatory activities already established for the Eastern Nigerian mistletoes. Acknowledgement: The authors wish to thank Mr Alfred Ozioko of BDCP Nsukka for identifying the plant material and Associate Professor Akira Kawamura of CUNY for the detailed spectral identification.


PG11

Flavonoids from Centaurea gloriosa and antioxidant activity of its extract

El Toumy SA1, Omar EA2, Brouard P1, Bermejo P3
1Chemistry of Tannins Department, National Research Centre, 12622 Dokki, Cairo, Egypt; 2Pathology Department, National Research Center, 12622 Dokki, Cairo, Egypt; 3Instituto de Productos Naturales y Agrobiologia, Av. Astrofisico F. Sanchez 3, 38206 La Laguna, Tenerife, Spain

In the early nineties the presence of flavonoids in herbal begin to attract the attention of a number of researchers, as a result of their biological and physiological importance [1]. The present study deals with the isolation and identification of flavonoids from Centaurea gloriosa Vahl. and evaluation of antioxidant activity of the extract. The aqueous alcoholic extract (MeOH:H2O:7:3) of Centaurea gloriosa aerial parts was subjected to extensive repeated column chromatography on polyamide, and Sephadex LH-20 resulted in two new flavonoids named quercetin 6-methoxy-7-O-galactoside (1) and quercetin 6,4'-dimethoxy-7-O-galactoside (2) as well as apigenin 8-G-c-glucoside, apigenin 6-C-glucoside, quercetin 6-methoxy, quercetin and apigenin. Structures of the isolated compounds were established by chromatography, UV, HRESI-MS and 1D/2D 1H/13C NMR spectroscopy. The radical scavenging activity of the extract was quantified spectrophotometrically, using DPPH radical. The effective dose 50 (ED50) of the extract was compared with that of standard antioxidants as vitamin C.

radial chromatography (CHCl\textsubscript{3}-MeOH system) to yield compound 1. Fraction 5 was subjected to VLC (Hex:EtOAc system) and purified by radial chromatography repeatedly (CHCl\textsubscript{3}:EtOAc:MeOH system) to give compound 2, 3 and 4. The structures of compounds 1-4 were determined based on spectroscopic data including UV, IR, 1D and 2D NMR and comparison with those previously reported. The similarity to these published data from Tanaka et al., 2000 [1], Ito et al., 1997 [2] and Tanaka et al., 2001 [3] suggested compound 1 as daviol A, a trimer resveratrol and compound 2, 3 and 4 as tetramer resveratrols namely hepaneohol, isohepaneohol and hesmleyanol D, respectively. Acknowledgement: Scholarship of one of the authors was financed by the National Science Fellowship (NSF) from Ministry of Science, Technology and Innovation Malaysia (MoSTI). References: [1] Tanaka T et al. (2000) Phytochemistry 53: 1009 – 1014. [2] Tuikiran AS et al. (2005) Biochemical Systematics & Ecology 33: 631 – 634. [3] Tanaka T et al. (2001) Heterocycles 55:729 – 740.

PG14 Isolation of Trimer stilbenoids from the bark of Shorea maxwelliana
Nik Abdullah Zawawi N, Ahmad N, Ahmad R
Faculty of Applied Sciences, Universiti Teknologi MARA (UiTM), 40450 Shah Alam, Selangor, Malaysia.

The stem bark of Shorea maxwelliana King (2.85 kg) was collected from reserved forest Jenga, Pahang, Malaysia. Three major trimer stilbenoids have been isolated from acetone extract of the stem bark of Shorea maxwelliana. Sample of S. maxwelliana was macerated in acetone for five times, and the solvent evaporated using rotary evaporator to produce crude acetone extract. The acetone extract (178.90 g) was fractionated by a series of solvent partitions into an EtOH soluble phenolic fraction (25.78 g). This fraction was repeatedly chromatographed on silica gel columns with Hex-EtOH (1:1) and EtOH, and then with a CHCl\textsubscript{3}-MeOH gradient of increasing MeOH to provide four fractions (A-D). Fraction A was the n-hexane soluble fraction (12.6 g). Fraction B (2.26 g) was subjected to silica gel chromatography eluted with CHCl\textsubscript{3}-MeOH (95:10 to 80:20) to give compound 1 (500 mg). Fraction C (9.8 g) was subjected to silica gel chromatography eluted with CHCl\textsubscript{3}-EtOAc-MeOH (85:10:0.5) to afford compound 2 (250 mg). Compound 3 (185 mg) was obtained from fraction D (1.12 g) by silica gel chromatography eluted with CHCl\textsubscript{3}-MeOH (8:2) [2]. The molecular structures of 1, 2 and 3 were determined based on spectroscopic data, including UV, IR, \textsuperscript{1}H NMR, \textsuperscript{13}C NMR, 2D NMR and comparison with that reported in the literature. Spectral data of compounds 1, 2 and 3 showed very close similarity to \textsuperscript{1}H-viniferin [1], vaticanol A [1] and copalliferol A [2].

Acknowledgement: The authors would like to thank the Ministry of Science, Technology and Innovation, Malaysia (MoSTI) for funding the research grant, Fundamental Research Grant Scheme (FRGS), 600-BMI/FRGS 5/3 (Ph) (2009) and National Science Fellowships (NSF) for financing the study of one of the authors. References: [1] Sahidin EHH et al. (2007) Journal of Chemical Society Perkin Transactions (1): 659.

PG15 A study on exudates and micromorphology of Primula Blattia TD\textsuperscript{1}, Adlassnig W\textsuperscript{2}, Brecker L\textsuperscript{3}, Vetschera KV\textsuperscript{4}
\textsuperscript{1}Department of systematic and evolutionary botany, University of Vienna, Vienna, Austria; \textsuperscript{2}Institute of Cell Imaging and Ultrastructure Research, University of Vienna, Austria; \textsuperscript{3}Institute of Organic Chemistry, University of Vienna, Vienna, Austria; \textsuperscript{4}Institute of Chemical Systematics, University of Vienna, Vienna, Austria

The genus Primula L. comprises more than 400 species, grouped in 6 subgenera and 37 sections [1]. Especially the production of oily or farinaceous exudates on aerial surfaces of leaves, stems, calyces and flowers is a conspicuous character of this genus. These exudates consist primarily of un-substituted flavones and other flavones with unusual substitution patterns, which are probably derived from a still unidentified biogenous pathway [2]. Exudate profiles were monitored by HPLC and TLC, and known structures were identified by comparison of their UV-spectra and retention times with those of reference compounds of our spectral library. New structures were elucidated additionally by NMR spectroscopy. The auto-fluorescent property of some of these flavonoids was studied by measurement of fluorescence intensity. Different colors of fluorescence were observed within a single leaf of P. vulgaris Huds., while leaves of P. vialii Delavay ex Franch. showed uniform fluorescence. The significance of our findings in relation to chemodiversity, morphology, and micromorphological character differentiation will be discussed. Acknowledgement: Hochschuljubiläumsstiftung der Stadt Wien, Gesellschaft zur Förderung der Pflanzenwissenschaften. References: [1] Richards J (2002) Primula. J.R. Batsford Ltd. London. 2. Valant-Vetschera KM et al. (2009) NPC 4: 365 – 370.

PG16 New Lanostane Triterpenoids from Antrodia camphorata
Kuo Y
Tsuzuki Institute for Traditional Medicine, China Medical University, No.51 Huhe-Shih Road, Taichung, Taiwan, R.O.C

Many polyynes are used for medicinal purposes in traditional Chinese medicine. Antrodia camphorata (M. Zang et C.H. Su) Sheng H. Wu, Rywarden et T.T. Chang, known as “niu-chang-chhi”, is restricted to the endemic tree, Cinnamomum kanehira Hayata. Traditionally the fungus has been used for the treatment of food and drug intoxication, diarrhea, abdominal pain, hypertension, skin itching, and liver cancer. The components of this fungi have shown activities such as anti-inflammatory, immune-modulation, anti-Helicobacter pylori, and neuroprotection from A\textsubscript{β} damage. Here, we present our chemical studies on the mixture of flowering body and mycelia of solid cultures of A. camphorata. As a result, eight new lanostane triterpenoids, 3,7,11-tri-3oxo-5a-lanosta-8,24-(E)-diene-26-oic acid (1), methyl 11\textsuperscript{a}-hydroxy-3,7-dioxo-5u-lanosta-8,24-(E)-diene-26-oic acid (2), methyl 3,7,11,12,15,23-hexa-oxo-5u-27\textsuperscript{z}-lactone (3), ethyl 11\textsuperscript{a}-hydroxy-3,7-oxo-26-oic acid (4), ethyl lucidenate A (5), ethyl lichenolate F (6), acetyl ganolucidic acid A (7), 3,11,15,23-tetra-oxo-5u-27\textsuperscript{z}-lactone-8-b-dienoic acid (8), were isolated and elucidated. These compounds were evaluated for their cytotoxicity against several human tumor cell lines.

PG17 New type of polyacetylene sesquiterpenoid conjugates from Nototergyrium incisum
Liu X\textsuperscript{1}, Kunert G\textsuperscript{2}, Schinkovitz A\textsuperscript{3}, Fakhrudin N\textsuperscript{2}, Heiss E\textsuperscript{3}, Atanasov A\textsuperscript{1}, Dirsch V\textsuperscript{1}, Bauer R\textsuperscript{2}
\textsuperscript{1}Institute of Pharmaceutical Sciences, Department of Pharmacognosy, Karl-Franzens-Universität Graz, 8010 Graz, Austria; \textsuperscript{2}Institute of Pharmaceutical Sciences, Department of Pharmaceutical Chemistry, Karl-Franzens-Universität Graz, 8010 Graz, Austria; \textsuperscript{3}College of Pharmacy, University of Vienna, 1090 Vienna, Austria

Qiang Huo, the dried rhizome and root of both Nototergyrium incisum Ting ex H. T. Chang and N. forbesi Boiss. (Umbelliferae) are used widely in China for treating cold, and inflammatory diseases. Peroxisome Proliferator Activated Receptor gamma (PPAR-gamma) is involved in inflammatory processes, and has become an important pharmacological target. From the dichloromethane extracts of the underground parts of Nototergyrium incisum, we have obtained through bio-guided isolation, a series of falcarienol derivatives with significant PPAR-gamma agonistic activity. In addition, we have isolated eight polyacetylene derivatives (1-8), which were identified by NMR as unique polyacetylenes fused with sesquiterpenoids. They are a second type of polyacetylene adducts connected through an ether bond, besides previously reported coumarin adducts. The sesquiterpenoid moiety of 5 and 6 is reported for the first time.
New Pyrones from the Mangrove Endophytic Fungus Pestalotiopsis sp
Xu J, Lin Q*,Proksch P*, Wray V*  

*Key Laboratory of Protection and Development Utilization of Tropical Crop Germplasm Resources, Ministry of Education, College of Material and Chemical Engineering, Hainan University, Haikou 570228, China;  
*Key Laboratory of Tropical Medicinal Plant Chemistry, Ministry of Education, Hainan Normal University, Haikou 571158, China;  
*Institut für Pharmazeutische Biologie und Biotechnologie, Heinrich-Heine-Universität, Geb. 26.23, Universitätsstrasse 1, D-40225 Düsseldorf, Germany;  
Department of Structural Biology, Helmholtz Centre for Infection Research, Inhoffenstrasse 7, D-38124 Braunschweig, Germany

Pestalotiopsis is generally found as endophytes of tropical plants and as prolific producers of structurally unusual natural products [1]. Our previous chemical investigations on fungus species Pestalotiopsis JCM2A4 isolated from the Chinese Mangrove plant Rhizophora mucronata Lam. have led to the isolation of a series of natural products and yielded over twenty different compounds with seventeen of them being new natural products including chromones, cytosporones and coumarins [2–3]. Following cultivation of the fungus now yielded eight new pyrone derivatives (1) as well as a known compound. The structures of all compounds were unambiguously established from their spectroscopic data, that included HR-ESIMS and 1- and 2-dimensional NMR spectroscopy, and by comparison with the literature [4]. Our findings proved endophyte genus Pestalotiopsis to be particularly productive.
tree is up to 50 m in height and the timber is light hardwood. The study was undertaken to extract and isolate the chemical constituents from the stembark of Shorea species namely Shorea bracteolata and to elucidate the structures of the chemical constituents isolated by using modern spectroscopic methods. The stembark of S. bracteolata (4 kg) was dried and cut into small pieces and ground to powder about 1 mm mesh size using grinder. The sample was extracted with acetone, filtered and evaporated in vacuo at 40°C to yield crude extract (320 g). Diethyl ether was added to crude extract to remove the tannin. The tannin free crude extract was fractionated using vacuum liquid chromatography (VLC) to give six fractions. Fraction 2 was subjected to radial chromatography 1 [Hex:EtOAc: 5:5] and 2 [CHCl3:MeOH (9.5:0.5) to yield e-viniferin (1) (Mohr et al., 2005) and a-viniferin (2) (Sutopo, 2009). Double purification of fraction 5 using radial chromatography [EtOAc: MeOH (9:1)] and [CHCl3:Acetone:MeOH (7:2:1)] afforded homopaphenol (3) (Guebalia et al., 2006). The structure of the isolated compounds was determined based on analysis of spectroscopic data, including NMR, UV, IR and comparison with previous reported studies.


Figure 1: Structure of compound 1 – 3

Synthesis and Evaluation of Gamgogic Acid Analogs as Cytotoxic Agents

Jin L1, Kyoko N2, Kenneth FB1, Wu Y1, Lee K1
1Graduate Institute of Natural Products, Kaohsiung Medical University, Kaohsiung 80708, Taiwan; 2Natural Products Research Laboratories, UNC Eshelman School of Pharmacy, University of North Carolina at Chapel Hill, NC 27599 – 7568, USA; 3Division of Medicinal Chemistry and Natural Products, UNC Eshelman School of Pharmacy, University of North Carolina, Chapel Hill, NC 27599 – 7568, USA; 4Graduate Institute of Integrated Medicine, College of Chinese Medicine, China Medical University, Taichung 40402, Taiwan; Natural Medicinal Products Research Center, China Medical University Hospital, Taichung 40402, Taiwan.

Gamboic acid (1, GA) was isolated from the resin of Garcinia hanburyi Hook. f. (Clusiaceae), a tree growing in Southeast Asia. The resin is used in folk medicine and as coloring agent in China (1,2). The chemical structure of GA shows a unique 4-oxatricyclo[4.3.1.0]decan-2-one ring system attached to a xanthone backbone. In order to analyze structure-activity relationships (SAR) of GA, we converted it into xanthone derivatives (Figure 1) and performed the synthesis of some structurally related prenylated derivatives, using as building blocks 1,3,6-trihydroxysterophane (2) and tested them for cytotoxic activity. All newly synthesized compounds were assayed for in vitro cytotoxicity against 4 human cancer cell lines: KB (nasopharyngeal), KBvin (multidrug-resistant nasopharyngeal over-expressing P-gp), A549 (lung) and DU-145 (prostate). Among them, compounds 9 and 10 showed remarkable IC50 values of 0.91 and 0.82 µg/mL, respectively, against KBvin cells.

Figure 1: The structures of compounds 9 and 10


PG21

Linalool Effect on HepG2 cells: Structure Function Relation

Usta J, Racha K, Boushra K, Shatha S, Yolla B, Omar R, Karim E
1Department of Biochemistry and Molecular Genetics, Faculty of Medicine, American University of Beirut, Beirut, Lebanon; 2Department of Biology, Balamand University, Faculty of Science-Deir-el-Balamand Elkoura, Lebanon

Linalool is a monoterpene that is widely used in fragrance industry and cosmetics. The effect of linalool on cultured cells (HepG2, MCF7, HeK293, Caco2 and NIH3T3) was investigated. We have recently reported a significant decrease of 50% and 100% in the viability of HepG2 by 0.4 µM and 2 μM linalool, respectively (1). Other studies reported the cytotoxicity of linalool on hematopoietic malignancies but not on normal human cells (2). These findings identify linalool as a potential anticancerogenic molecule. The aim of this study was to investigate the importance of the structural features of linalool in exerting the effect on HepG2 cells. Eleven chemicals with were tested. HepG2 cells were treated with various chemicals at 2 – 500 µM for 24 hours and the viability was estimated using MTT. None of the screened compound had the same potent effect of linalool (2 μM). No effect was demonstrated at concentrations lower than 50 µM. We obtained cell death in HepG2 74% with myrcene & nerolidol (100 µM); 55% with trans-2-nonenal, Decanal (100 µM); 20% with Nonyl aldehyde, citronellal, citral, Perillaldehyde, trans-2-octen1-ol, and 1-octen-3-ol at (500 µM). Our findings suggest that the effect of linalool is specific to 1–ene 3-ol moiety. The hydroxyl group needs to be tertiary. Hydrogen of myrcene and L-perillaldehyde did not have any significant effect on HepG2 viability. This may be attributed to: favorability of the hydration reaction and to possible steric effect. Alternative metabolism of linalool into other products may not be ruled out. Acknowledgement: Medical Practice plan and University Research Board of the American University of Beirut References: 1: Usta J et al. (2009) Chem-Biol Interact 180: 39 – 46. 2: Gu Y et al. (2010) Toxicology 268: 19 – 24

PG22

New Alkaloids, Sessilifoliamides K and L from the Roots of Stemona sessilifolia

Takeya K, Hitotsuyanagi Y, Uemura G
Tokyo University of Pharmacy & Life Sciences, 1432 – 1 Horinouchi, Hachioji, 192 – 0392 Tokyo, Japan

Plants belonging to the genus Stemona (family Stemonaceae) are noted for producing a series of alkaloids with unique structures, most of which are characterized by incorporating a pyrrolo[1,2-a]azepine core. Of the genus Stemona plants, Stemona japonica (Blume) Miq., S. tuberosa Lour,
and S. sessilifolia Franch. & Sav. have been used in China and Japan as an insecticide and also as a remedy for cough, and their biological activities are considered to be related to their alkaloid components. In our studies on the chemical constituents of S. sessilifolia, we isolated eleven new alkaloids, sessilifluomides A–I and sessilifluoline A, with novel alkaloid skeletons. In this meeting, the isolation and structure determination of further new Stemona alkaloids, sessilifluomides K and L are represented.

The genus Pavetta have long being used in ethnomedicine as anti-malarial, remedy for tuberculosis and for relieve of stomach pain (1). The literature does not report any phytochemical studies on Pavetta corymbosa (DC.) F.N. Williams. The dichloromethane and the ethyl acetate extracts were investigated for phytochemical constituents. Fractionation of the dichloromethane extract by Flash column chromatography, sephadex LH-20 and Preparative TLC afforded the trieterpenes: α-sitosterol and stigmasterol, while the ethylacetate extract was fractionated over sephadex LH-20 to give the known flavonoids; quercetin, quercetin 7-O-rhamnoside and kaempferol. The structures were elucidated by NMR spectroscopy and compared with literature (2, 3, 4) and are reported here for the first time. References: 1. Dalziel J.M and Hutchinson J (1955) Useful plants of West Africa. Crown Agents for Oversea Publication, London 2. Reynold WF et al. (1999) |Braz Chem Soc 10(3): 237–240 3. Ahmad SH and Nordin HL (1998) ARBEC II: 1–6 4. Mabry T.J et al. (1970) The Systematic Identification of Flavonoids Springer-Verlag Publication, New York.

Chemodiversity of Pentadesma grandifolia (Clusiaceae) from Cameroon
Valent Vetschera KM1, Djoufack NWboulou Gl2
Brecker L2
1Department of Systematic and Evolutionary Botany, University of Vienna, Vienna, Austria; 2Institute of Organic Chemistry, University of Vienna, Vienna, Austria

Within the family Clusiaceae, the genus Pentadesma is represented by three species only, which are distributed in the tropical regions of Africa and America. Pentadesma grandifolia Baker f. is used in African folk medicine, and the roots and stem bark are applied to treating fever and malaria in the western part of Cameroon [1]. Xanthones, biflavonoids and triterpenoids are the major secondary metabolites of this genus as reported recently [1]. A new glycosidic biflavonoid and a further xanthone derivative were identified now from the same accession originating from Cameroon [1]. Profiling of different plant organs was done both by TLC and HPLC, and isolated structures were identified on basis of 13C NMR, 1H NMR, HRMS and ESI-MS. Structure elucidation and the distribution of isolated compounds in different parts of the plant will be presented. Tests for antifungal activity were performed by bio-autography on TLC against spore suspensions of Cladosporium sphaerospermum. The significance of bioactivity results and reported bioactivities of Pentadesma compounds are shortly discussed. Acknowledgement: The authors are grateful to the financial support of O.E.A.D. for G. L. Djoufack.


PG24

Triterpenoids and Flavonoids from Pavetta corymbosa
Ahmadu AA1, Agunu A3, Magiatis P3
1Delta University, Wilberforce Island, Yenagoa-Nigeria, Department of Pharmaceutical and Medicinal Chemistry, Nigeria; 2Ahmadu Bello University, Department of Pharmacognosy and Drug Development, Zaria, Nigeria; 3University of Athens, Panepistimioiopolis-Zografou, Greece, Department of Pharmacognosy and Natural Products Chemistry, Athens, Greece

The genus Pavetta have long been used in ethnomedicine as anti-malarial, remedy for tuberculosis and for relieve of stomach pain (1). The literature does not report any phytochemical studies on Pavetta corymbosa (DC.) F.N. Williams. The dichloromethane and the ethyl acetate extracts were investigated for phytochemical constituents. Fractionation of the dichloromethane extract by Flash column chromatography, sephadex LH-20 and Preparative TLC afforded the trieterpenes: α-sitosterol and stigmasterol, while the ethylacetate extract was fractionated over sephadex LH-20 to give the known flavonoids; quercetin, quercetin 7-O-rhamnoside and kaempferol. The structures were elucidated by NMR spectroscopy and compared with literature (2, 3, 4) and are reported here for the first time. References: 1. Dalziel J.M and Hutchinson J (1955) Useful plants of West Africa. Crown Agents for Oversea Publication, London 2. Reynold WF et al. (1999) |Braz Chem Soc 10(3): 237–240 3. Ahmad SH and Nordin HL (1998) ARBEC II: 1–6 4. Mabry T.J et al. (1970) The Systematic Identification of Flavonoids Springer-Verlag Publication, New York.

PG25

Chemodiversity of Pentadesma grandifolia (Clusiaceae) from Cameroon
Valent Vetschera KM1, Djoufack NWboulou Gl2, Brecker L2
1Department of Systematic and Evolutionary Botany, University of Vienna, Vienna, Austria; 2Institute of Organic Chemistry, University of Vienna, Vienna, Austria

Within the family Clusiaceae, the genus Pentadesma is represented by three species only, which are distributed in the tropical regions of Africa and America. Pentadesma grandifolia Baker f. is used in African folk medicine, and the roots and stem bark are applied to treating fever and malaria in the western part of Cameroon [1]. Xanthones, biflavonoids and triterpenoids are the major secondary metabolites of this genus as reported recently [1]. A new glycosidic biflavonoid and a further xanthone derivative were identified now from the same accession originating from Cameroon [1]. Profiling of different plant organs was done both by TLC and HPLC, and isolated structures were identified on basis of 13C NMR, 1H NMR, HRMS and ESI-MS. Structure elucidation and the distribution of isolated compounds in different parts of the plant will be presented. Tests for antifungal activity were performed by bio-autography on TLC against spore suspensions of Cladosporium sphaerospermum. The significance of bioactivity results and reported bioactivities of Pentadesma compounds are shortly discussed. Acknowledgement: The authors are grateful to the financial support of O.E.A.D. for G. L. Djoufack.


PG26

Seeds from South African plants as a source of bioactive metabolites
Marston A1, Du R1, Van Vuuren SP2, Van Zyl R1, Zeitzman P3
1Chemistry Department, University of the Free State, Bloemfontein, South Africa; 2National Museum, Bloemfontein, South Africa; 3Department of Pharmacy and Pharmacology, University of Witwatersrand, Parktown, South Africa

South Africa has a rich diversity of plant species which contain various classes of bioactive compounds [1]. Seeds of these plants have been little studied from a chemical viewpoint and a survey of their constituents is of high interest. The source plants of these seeds may grow in areas with extreme climatic conditions, thus increasing the chance of finding original metabolites. Seeds from plants (mainly trees) growing in different areas of South Africa were extracted with methanol and screened for radical scavenging activity in a TLC assay with the 2,2-diphenyl-1-picrylhydrazyl radical. Inhibition of acetylcholinesterase by TLC bioautography [2] was also performed, as compounds which inhibit this enzyme may have some application in the management of Alzheimer’s disease. The extracts were also screened for antimicrobial and antimarialar activities. Two species were selected for further study: Schotia brachypetala Sond. (Fabaceae) and Colophospermum mopane (J. Kirk ex Bent.) J. Kirk ex J. Leonard (Fabaceae). The bioactive constituents of the seeds, including two new 4-coumaroyl derivatives of flavonoids (1, 2), were isolated by a combination of high-speed counter-current chromatography and classical chromatographic techniques. References: 1 Mul-holland DA and Drewes SE (2004) Phytochemistry 65: 769 – 782. 2. Marston A, Kissling J and Hostettmann K (2002) Phytochemical Analysis 31: 51 – 54.

PG27

Chemical constituents of Artocarpus xanthocarpus and their inhibitory effects on melanin biosynthesis
Ko H1, Lin C2, Yen Y1, Chen Y1, Li J2
1Department of Cosmetic and Fragrance Science, College of Pharmacy, Kaohsiung Medical University, Kaohsiung, Taiwan; 2School of Pharmacy, College of Pharmacy, Kaohsiung Medical University, 807 Kaohsiung, Taiwan; 3Graduate Institute of Natural Products, College of Pharmacy, Kaohsiung Medical University, 807 Kaohsiung, Taiwan

Artocarpus xanthocarpus Merr. is an evergreen meconoid tree with milky juice, distributed in Philippines, Borneo and Taiwan (only on Lan-yu) [1]. Various phenolic compounds, including isoprenylated flavonoids, stilbenoids, and 2-arylbenzofuranos are widely distributed in plants of the genus Artocarpus. Many of these compounds exhibit cytotoxic, anti-inflammatory, anti-platelet, antibacterial, antimalarial, anti-tubercular, antiviral and antioxidant activities [2, 3]. In continuation of our research on natural whitening agents, the present study aims to characterize the chemical constituents of A. xanthocarpus and demonstrate their potential whitening potency. Three new compounds, artoxanthocarpuones A–C (1–3), and twelve known compounds (4–15) have been isolated from the root of A. xanthocarpus. Their structures were determined on the basis of spectroscopic evidence. These compounds were evaluated for their antioxidative property, tyrosinase inhibitory potential and cytotoxicity. In B16F10 melanoma cells, compound 1, norartecarpetin (4), oxyreseratervatol (5), albanin A (8) and steppogenin (9) reduced tyrosinase activity and also inhibited the α-MSH induced melanin production. We may conclude that isolates of A. xanthocarpus with antioxidant and tyrosinase reducing activities may be considered as depigmenting agents. Acknowledgement: The authors are thank to the financial support from the National Science Council of Taiwan (NSC-99-2320-B-037-021). References: 1. Liao JC (1996) Moraceae in Flora of Taiwan, 2nd edition. Vol. 2: 136 – 137. Editorial Committee of the Flora of Taiwan. Taipei 2. Hakim EH et al. (2006) Nat Med 60: 161 – 184. 3. Jagtap UB, Bapat VA (2010) Ethnopharmacol 129: 142 – 166.
PG28 Evaluation of effects of climate condition on the quality of sugar beet production in Iran
Honarvar M1, Hamedi S2
1College of Food Sciences/Technology, Science and Research branch, Islamic Azad University, Tehran, Iran; 2Department of Traditional Medicine, Tehran University of Medical Sciences, Tehran, Iran

This research has been carried out during the 9 years (1998 – 2008) and in some provinces (Chehar Mahal Bakhtiari, Isfahan, Qazvin, Zanjan, Azarbeyejan Gharbi) in 5 sugar factories. Our aim was the evaluation of qualitative specificiations of sugar beet produced in different climates and their effects on the amounts of sugar produced. Conclusions of this research were based on the analysis of 13811 samples of sugar beet consignments received during 9 years. The results have shown that amounts of sugar changed from min. 15.38% to max. 62.2%. K+ contents varied between min. 5.06 and max. 8.1; while Na+ varied from min. 1.8 to max. 5.49 meq., and harmful nitrogen ranged between 1.96 to 4.37. These results showed the annual variations in the quality of sugar produced in Iran with respect to geographical variations.

PG29 Structural analysis of arabinogalactan-proteins from suspension cultures of Pelargonium sidoides DC.
Duchow S, Blaschek W, Classen B
Pharmaceutical Institute, Dept of Pharmaceutical Biology, University of Kiel, Gutenbergstrasse 76, 24118 Kiel, Germany

Pelargonium sidoides DC. is a traditional medicinal plant from South Africa. An aequous-ethanolic formulation of the roots is approved for the treatment of acute bronchitis. The main effects could be related to antibacterial activities and the stimulation of the non-specific immune system by the main components of Pelargonium sidoides: coumarins, phenols and tannins [1]. Due to wild harvesting, Pelargonium sidoides is an endangered species. Therefore the propagation of the plant materi- al by cell cultures and the extraction of ingredients are interesting tasks. From suspension cultures of Pelargonium sidoides high amounts of pure Arabinogalactan-proteins (AGPs) could be isolated by precipitation with β-glucosyl Yariv reagent. These AGPs have been investigated with regard to their structure. Quantification of neutral sugars by acetylation pointed out arabinose (Ara) and galactose (Gal) as dominating monosaccharide residues in a ratio of 1:2. Colourimetric determination of uronic acids revealed an amount of 6 – 8%. Linkage type analysis in combination with the reduction of the uronic acids showed that the main components are 1,3-Gal(p), 1,3-Gal(p) and 1-Ara(f) as well as major amounts of 1,6-Gal(p), 1-Glc(p), 1,4-Gal(p), 1-Gal(p), 1,5-Ara(f) and 1,2-Ara(f). Molecular weight of AGPs has been determined by size exclusion chromatography with laser light scattering detection and found to range between 80 and 85 kDa. The characterisation of the AGP-protein moiety pointed out an untypical low protein content for AGPs with 1%. According to the amino acid analysis the protein moiety consists of high amounts of Hyp (42.8 – 51.1%) as well as Pro, Gly, Glx, Arg, Ser, Ala, Leu and Thr. References: 1. Kolodziej H (2008) Planta Med 74: 661 – 666

PG30 Thymol, benzofuranoid, and phenylpropanoid derivatives: anti-inflammatory constituents from Eupatorium cannabinum subsp. asiaticum
Chen J1, Tsai Y2, Hwang Y2
1Department of Pharmacy & Graduate Institute of Pharmaceutical Technology, Tajen University, Pingtung 907, Taiwan; 2Graduate Institute of Natural Products, Chang Gung University, Taoyuan 333, Taiwan

Eupatorium cannabinum L. subsp. asiaticum Kitam. (Compositae) [1] is a perennial herb distributed in Himalaya mountain range, China, and Taiwan. E. cannabinum, locally called 'Taiwan ze-lan' or 'liu-yue-xue', has been used as a folk medicine to treat hepatitis, headache, diarrhea, hypertension, and Diabetes mellitus in Taiwan. Sesquiterpene lactones, diterpenoids, flavonoids, pyrrolizidine alkaloids, thymols, benzofurans, and their derivatives are widely distributed in plants of the genus Eupatorium. Many of these compounds were found to exhibit cytotoxic, antimicrobial, and anti-inflammatory activities. In our studies on the anti-inflammatory constituents of Formosan plants, many species have been screened for in vitro inhibitory activity on neutrophil pro-inflammatory responses, and E. cannabinum has been found to be an active species. Five new compounds, 9-O-angeloyl-8,10-dehydrothymol (1), 9-(3-methylbutanoyl)-8,10-dehydrothymol (2), eutapobenzofuran (3), 2-hydroxy-2,6-dimethylbenzofuran-3(2H)-one (4), and 1-(2-hydroxy-4-methylphenyl)propan-1,2-dione (5) and 16 known compounds have been isolated and identified from the aerial part of E. cannabinum subsp. asiaticum. Compounds 6 – 8, 11, 13, and 15 exhibited inhibition (IC50 values < 18.4μM) of superoxide anion generation by human neutrophils in response to formyl-L-methionyl-L-leucyl-L-phenylalanine/cytochala- sin B (FMLP/CB). Compounds 2, 5, 10, 13, and 15 inhibited FMLP/CB-induced elastase release with IC50 values < 18.3μM.

PG31 Value-added products from Pinus banksiana wood
Si CL1,2, Zhang Y1, Liu SC1, Ni YH1
1Tianjin Key Laboratory of Pulp and Paper, Tianjin University of Science and Technology, Tianjin 300457, China; 2State Key Laboratory of Pulp and Paper Engineering, South China University of Technology, Guangzhou 510640, China

Pinus banksiana Lambert (Pinaceae) is a boreal conifer species, with a sparse, variable crown and spreading branches at maturity. The species is widely available in Canada, and an important timber species for pulp and lumber [1]. In this work, the chemical composition, such as ash, lignin, cellulose and hemicellulose contents, of the P. banksiana wood chips from Eastern Canada, was determined. In addition to timber and pulp, P. banksiana wood may be a rich source of unexploited potentially novel bioactive compounds. A previous study showed that P. banksiana wood extracts possessed strong antioxidant and anti-tumor activities [2]. In this work, the chemical constituents of the extracts from P. banksiana wood were further investigated. The GC-MS results of essential oils from P. banksiana wood showed that there were 76 volatile compounds presented, including phenolic acids, phenylpropanoids, alkali-oids and terpenoids. Among those determined, 1-Naphthalene-2-carboxylic acid was the most abundant (29.06%). Based on the successive Sephadex LH-20 column chromatographic separation of P. banksiana wood aided by Thin Layer Chromatography, 5 yellowish low-molecular eight natural compounds, including 2 flavan-3-ols ([+]-Catechin (I) and (-)-Epicatechin (II)), a phenolic acid [Caffeic acid (III)], a phenylpropanoid [Isoconiferin (IV)] as well as a lignan [Cedrusin (V)], were isolated. Structure elucidation of the isolates was based on their physiochemical and spectroscopic data. To the best of our knowledge, this was
the first time of isolation low-molecular-weight natural compounds from P. banksiana wood. The results in the study might lead to the further development of high value-added products from this pine species. Acknowledgement: This work was financially supported by National Natural Science Foundation of China (NSFC, No. 31000279), Program for New Century Excellent Talents in University (NCET 2010) and Natural Science Foundation of Tianjin City (No. 09JCYBJC15800). References: 1. Poncsak S et al. (2009) J Wood Chem Technol 29: 251 – 264. 2. Phelan M et al. (2009) J Med Food 12: 1245 – 1251.

Galanthus transcaucasicus Fomin, as a source of isoquinoline alkaloids

Yousef Beyk F, Azadi B, Amin G, Salehi Sormagh M, Ammi M, Sharifzadeh M

1Department of Pharmacognosy, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran; 2Department of Medicinal Chemistry, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran; 3Department of Toxicology and Pharmacology, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran

Galanthus transcaucasicus Fomin (Amaryllidaceae) is an endemic species of the Caucasus region and the Alborz mountains in Iran and is used in folk medicine to recover paralysis and nerve pain (1, 2). All species of Galanthus are famous for their bioactive alkaloids such as galanthamine, an acetylcholinesterase inhibitor, which is used for the treatment of Alzheimer’s disease (2). This study is designed to identify major constituents of the alkaloid fraction of the plant prepared. Major constituents of alkaloid fraction were purified using different chromatographic methods. Finally, five isoquinoline type alkaloids involving galanthamine, narwedine, lycorine, caranine and tazettine were identified with spectroscopic methods. The results showed that this species can be considered as a source of isoquinoline type alkaloids especially galanthamine which is a long acting, competitive and reversible acetylcholinesterase inhibitor (2). References: 1. Bastida J et al. (2000) The alkaloids. Elsevier Scientific Publishing, Amsterdam 63: 177 – 179. 2. Heinrich M et al. (2004) J Ethnopharmacol 92: 147 – 162.

Activity-guided supercritical CO2 isolation of antioxidative constituents from Eucommia ulmoides

Si C1, Qin PP2, Liu Z2

1State Key Laboratory of Pulp and Paper Engineering, South China University of Technology, Guangzhou 510640, China; 2Tianjin Key Laboratory of Pulp and Paper, Tianjin University of Science and Technology, Tianjin 300457, China; 3Tianjin University of Science and Technology, Tianjin 300457, China

Increasing evidence exhibited that antioxidants played very important role in protecting against various diseases like cancer, atherosclerosis, diabetes, cataracts, neurodegenerative disorders [1]. Eucommia ulmoides Oliv., the sole species in Eucommia genus and Eucommiaceae family, is a large medicinal hardwood native to China and widely cultured in East-ern Asia and has widely been used as a tonic to strengthen the kidney in addition to previously isolated polyene-yne derived compounds. 1, 2

Figure 1: structures of polyacetylene ferulic acid conjugates from N. incisum.

New skeleton polyacetylene ferulic acid conjugates from Nototerygium incisum

Liu Xi1, Kunert O2, Schinkovitz A1, Fakhrudin N1, Heiss E3, Atanassov A1, Dirsch V3, Bauer R1

1Institute of Pharmaceutical Sciences, Department of Pharmacognosy, Karl-Franzens-Universität Graz, 8010 Graz, Austria; 2Institute of Pharmaceutical Sciences, Department of Pharmaceutical Chemistry, Karl-Franzens-Universität Graz, 8010 Graz, Austria; 3Department of Pharmacognosy, University of Vienna, 1090 Vienna, Austria

In our search for natural products activating PPAR-gamma we have isolated three polyacetylene ferulic acid conjugates (1-3) from the underground parts of Nototerygium incisum Ting ex H. T. Chang (Qiang Huo), in addition to previously isolated polyene-yne derived compounds. 1, 2 Their structures were elucidated by NMR and MS. Compound 1 is formed by an ester bond, while compound 2 and 3 represent two new skeletons. Pharmacological evaluation of the isolated compounds is in progress.
Isolation of anthocyanins with identified qualitative-quantitative properties

Labun P1, Salamon P2, Marichuk R3, Fejer J1

1Department of Ecology, Presov University, 01, 17th November St., SK-081 16 Presov, Slovakia; 2Excellence Centre of Human and Animal Ecology, Presov University in Presov, 01, 17th November St., SK-081 16 Presov, Slovakia; 3Department of Ecology and Environmental Protection, Faculty of Chemistry, Uzhhorod National University, Pidgirna 46, UA – 88000, Uzhhorod, Ukraine

Technology of natural substances isolation is substantial element affecting on the quality of natural preparations. The isolation of the plant substances from a raw material is carried out by distillation and extractions with different solvents. The methods used for anthocyanin separation due to high temperatures, etc. destroy their structures and decrease their therapeutic effects. The present study is aimed to establish optimal procedures for anthocyanins after their extraction to secure the stability of substances by lyophilisation. The freeze drying technology seems to be an appropriate method to keep the structure and qualitative-quantitative properties of these natural compounds. The focus of lyophilisation technology is vacuum sublimation of ice crystals, i.e. phase transition from solid to gas state, which is created during freezing the water solution. The process is carried out in three phases: freezing, primary drying and secondary drying. LC/MS/IT/TOF equipment is used for the determination of anthocyanin qualitative-quantitative characteristics before and after lyophilisation. The biological and antimicrobial properties of these natural compounds are tested as well. The freeze drying technology was established by Mediprodukt Company in Lipany, the East Slovakia. Keywords: Hydrolyzation, lyophilisation, plant material, secondary metabolites Acknowledgement: The participation is supported by the Ministry of Education, Science, Research and Sport of the Slovak Republic, the project: 00162 – 0001 (MS SR-3634/2010–11).

Secondary metabolites from the Root of Raphiopelis indica var. tashiroi and Their Anti-inflammatory Activity

Chun Hung L1, Hsun Shuo C1, Chang Hui I2, Ih Sheng C1, Ian Lih T1

1Kaohsiung Medical University, No.100, Shi-Chuan 1st Rd., Kaohsiung 807, Taiwan; 2Chang Gung University, No. 259, Wen-Hwa 1st Rd., Kwei-Shan Tiaoouyuan 333, Taiwan

Raphiopelis indica (L.) Lindl. ex Ker var. tashiroi Hay, ex Matsum. (Rosacea) is an evergreen shrub or small tree which distributes from India to southern China, the Ryukyus, Japan, Korea, and Taiwan at low altitudes. The methanolic extract of the root of this species showed anti-inflammatory activity using N-formylmethionylleucylphenylalanine (fMLP)-induced production of superoxide anion, an inflammatory mediator produced by neutrophils in vitro. Previously, we reported seven compounds including four new dibenzofuran derivatives, raphiopelins A – D (1–4), two new biphenyl derivatives, raphiopeliphylen A – B (5–6) along with 3-hydroxy-5-methoxybiphenyl (7) from a supercritical CO2–methanol extract of the root of this plant. Continuing investigation of the active EtOAc-soluble layer of this plant’s root led to the isolation of three new triterpenoids, namely 2α,3β,5β-trihydroxyolean-11,13(18)-dien-28,19-olide (1), (2R,7αR)-methyl-3-hydroxy-4'-((S)-1-hydroxyethyl)-5'-oxo-3,4,4a,7a-tetrahydro-1H,5'H-spiro[cyclopenta[c]pyran-7,2'-furan]-4-carboxylate (2), and 3β,5β,6α-trihydroxy-18-carboxylic acid (3). The structures of these isolates were elucidated by spectral analyses. Among the isolates, 3, 6, and 7 exhibited potent inhibition against FMLP-induced superoxide production with IC50 values less than 8.36 μM. Acknowledgement: National Science Council of the Republic of China (NSC 97 – 2320-B-037 – 010-MY3)

Secondary Metabolites from the Root of Neolitsea daibuensis and Their Anti-inflammatory Activity

Ih Sheng C1, Su Ling W1, Hsun Shuo C1, Guei Jane W2, Michael YC3, Hung Yi H1, Chu Hung L1

1Kaohsiung Medical University, No. 100, Shi-Chuan 1st Rd., Kaohsiung 807, Taiwan; 2China Medical University Hospital, No. 91, Hushe-Shih Rd., Taichung 404, Taiwan; 3Institute of Pharmacy, Pharmacy and Pharmacognosy and Center for Molecular Biosciences, University of Innsbruck, Innrain 52c, 6020 Innsbruck, Austria; 4Institute of Vascular Biology and Thrombosis Research, Medical University of Vienna, Schwarzenbergstr. 17, 1090 Wien, Austria; 5Institute of Pharmacy, Pharmaceutical Technology, University of Innsbruck, Innrain 52c, 6020 Innsbruck, Austria

Neolitsea daibuensis Kamikoti (Lauraceae) is a small semideciduous trees, endemic to Taiwan, confined to broad-leaved forests from 800 to 1000 m in the southern part. Recently, approximately 40 species of Mosman Lauraceous plants have been screened for anti-inflammatory activity using an inducible nitric oxide synthase (iNOS) assay, and the methanolic extract of the root of this species has been shown with potent inhibition of NO production without any cytotoxicity on RAW 264.7 cells. Besides, the chemical constituents of its root have not extensively been studied yet. Bioassay-guided fractionation of the ethyl acetate soluble layer of the root of this species led to the isolation of three new alkaloids: daibucarbolines A – C (1–3), three new sesquiterpenoids: daibulactones A-B (4-5), and daibudioxide (6), together with twenty known compounds. The structures of these compounds were determined by spectroscopic analysis. Among the isolates, daibucarbole A (1), hiranlactone B, isolinderalactone, 7-O-methylprunetin, and prunetin showed iNOS inhibitory activity with IC50 values as 18.41 ± 0.07, 29.30 ± 0.92, 3.70 ± 0.01, 19.55 ± 0.25, and 10.20 ± 0.35 μM, respectively. Acknowledgement: National Science Council of the Republic of China (NSC 99 – 2300-B-037 – 009)

Phytochemical Investigation of Himatanthus sucubu bark leading to the identification of novel and anti-inflammatory compounds

Walunberger B1, Midhly Bison J2, Gelibrich T3, Grieser UJ1, Biochillov V1, Binder BE2, Röllinger JM1, Stepka S2,3,7a2,7a2R2-methyl-3-hydroxy-4'-(5'-1-hydroxyethyl)-5'-oxo,3,4,4a,7a-tetrahydro-1,5'H-spirocyclopenta[c]pyran-7,2'-furan]-4-carboxylate, flavonoids (biochanin A, dihydrobiochanin A, daibazol, naringenin, ferrein, and dihydrocarjanin), and the lignan pinoresinol. Except for plumericin and pinoresinol this is the first time these compounds are reported to be isolated from Himatanthus sucubu. The structure of the new iridoid was determined using X-ray crystallography. Interestingly, NMR experiments showed the presence of two compounds indicating stereochromatic conversion. The isolated constituents were analyzed for their anti-inflammatory activity using an inducible nitric oxide synthase (iNOS) assay, and the new compound in this general anti-inflammatory assay. The elucidation of the molecular mechanism of action is currently under evaluation. Acknowledgement: This work was granted by the Austrian Science Fund (S 10703). References: 1. Amaral ACB et al. (2007) Pharmacogn Rev 1: 305 – 313.

New isoflavones and bioactive constituents from the fruits of Psoralea corylifolia

Chen I1, Chen C1, Lai R1, Chen H2, Kuo W1, Liao T1

1Graduate Institute of Pharmacy, National Taiwan University, Taipei 106, Taiwan; 2Graduate Institute of Pharmacology & Department of Pharmacy, Tajen University, Pingtung 907, Taiwan; 3Chung Jen College of Nursing, Health Science and Management, Chiayi 600, Taiwan

Psoralea corylifolia (Chinese name Buguzhi), dry fruits of leguminous plant P. corylifolia L., is one of the most popular traditional Chinese medicines. This crude drug has used for the treatment of pollakiuria, enuresis, osteoporosis, depression, and various kidney problems. It is believed that the iridoid (plumericin and pinoresinol this is the first time these compounds are reported to be isolated from Himatanthus sucubu. The structure of the new iridoid was determined using X-ray crystallography. Interestingly, NMR experiments showed the presence of two compounds indicating stereochromatic conversion. The isolated constituents were analyzed for their anti-inflammatory activity using an inducible nitric oxide synthase (iNOS) assay, and the new compound in this general anti-inflammatory assay. The elucidation of the molecular mechanism of action is currently under evaluation. Acknowledgement: This work was granted by the Austrian Science Fund (S 10703). References: 1. Amaral ACB et al. (2007) Pharmacogn Rev 1: 305 – 313.
known compounds, including angelicin (3), psoralen (4), bavachalcone (5), bakuchiol (6), 12,13-dihydro-12,13-epoxybakuchiol (7), p-hydroxybenzaldehyde (8), b-sitosterol (9), and stigmasterol (10). The structure of new compounds 1 and 2 was determined through spectroscopic and MS analyses. Among the isolated compounds, psoralen (4) exhibited inhibition (IC$_{50}$ value $= 1.10 \pm 0.60 \mu$g/ml) of superoxide anion generation by human neutrophils in response to formyl-L-methionyl-L-leucyl-L-phenylalanine/cytochalasin B (fMLP/CB).

**PG41**

A new benzylophloroglucinol derivative with an adamantyl skeleton and other constituents from *Garcinia multiflora*: Effects on neutrophil pro-inflammatory responses

Chen J$^1$, Ting C$^2$, Yen M$^2$, Hwang T$^1$, Chen I$^2$
$^1$Graduate Institute of Pharmaceutical Technology & Department of Pharmacy, Tajen University, Pingtung 907, Taiwan; $^2$Faculty of Pharmacy, Kaohsiung Medical University, Kaohsiung 807, Taiwan

*Garcinia multiflora* Champ. is a small evergreen tree, distributed in South China, Hong Kong, and Taiwan [1]. The genus *Garcinia* (Guttiferae) comprises about 400 species with pantropical distribution. In Taiwan, the genus *Garcinia* is represented by three species, viz., *G. linii*, *G. multiflora*, and *G. subelliptica*. Plants of this genus contain a variety of secondary metabolites including xanthones, benzophenones, phloroglucinols, terpenoids, biflavonoids, and their derivatives. Many of these compounds exhibit antioxidant, trypanocidal, cytotoxic, antitubercular, anti-inflammatory, and anti-HIV activities. As part of our studies on the anti-inflammatory constituents of Formosan plants, many species have been screened for in vitro inhibitory activity on neutrophil pro-inflammatory responses. In the course of this screening, an ACN-soluble fraction of the fruit of *G. multiflora* exhibited inhibitory activities with IC$_{50}$ values of 7.21 $\pm$ 1.07 and 6.01 $\pm$ 0.37 $\mu$g/ml, respectively, against fMLP/CB-induced superoxide anion generation and elastase release.

Investigation of the active fraction afforded a novel benzylphloroglucinol derivative, garmicultiflorone D (1), with an unusual adamantly caged skeleton and four known compounds. The structure of 1 was determined through extensive 1D/2D NMR and mass-spectrometric analyses. Garmicultiflorone D (1) exhibited inhibitory activities with IC$_{50}$ values of 7.21 $\pm$ 1.07 and 6.01 $\pm$ 0.37 $\mu$g/ml, respectively, against fMLP/CB-induced superoxide anion generation and elastase release.

**Figure 1:** Structure of new compound 1

Acknowledgement: This research was supported by grants from the National Science Council of the Republic of China (No. NSC 95–2320-B–127–001-MY3 and NSC 98–2320-B–127–001-MY3), awarded to Prof. J.-J. Chen.


**PG42**

New biphenyl derivatives and anti-inflammatory constituents from the stem bark of Magnolia officinalis

Chen J$^1$, Kuo W$^1$, Chung C$^2$, Hwang T$^1$
$^1$Graduate Institute of Pharmaceutical Technology & Department of Pharmacy, Tajen University, Pingtung 907, Taiwan; $^2$Chung Jen College of Nursing, Health Science and Management, Chiayi 600, Taiwan

The stem bark of *Magnolia officinalis* Rehd. et Wils. (*Magnoliaceae*) has been used as a traditional medicine for the treatment of gastrointestinal disorders, bronchitis, and emphysema, in China, Taiwan, Japan, and Korea [1]. Chemical studies have revealed a variety of neo-lignans and...

**Acknowledgement:** This research was supported by grants from the National Science Council of the Republic of China (No. NSC 95–2320-B–127–001-MY3 and NSC 98–2320-B–127–001-MY3), awarded to Prof. J.-J. Chen.


**Figure 1:** Structures of new compounds 1 and 2

Acknowledgement: This research was supported by grants from the National Science Council of the Republic of China (No. NSC 95–2320-B–127–001-MY3 and NSC 98–2320-B–127–001-MY3), awarded to Prof. J.-J. Chen.


**Figure 1:** Structures of new compounds 1 and 2

Chen J$^1$, Cho J$^1$, Lee T$^2$, Hwang T$^1$, Chen I$^4$
$^1$Graduate Institute of Pharmaceutical Technology & Department of Pharmacy, Tajen University, Pingtung 907, Taiwan; $^2$School of Pharmacy, Taipei Medical University, Taipei 110, Taiwan; $^3$Graduate Institute of Natural Products, Chang Gung University, Taoyuan 333, Taiwan; $^4$Faculty of Pharmacy, Kaohsiung Medical University, Kaohsiung 807, Taiwan

*Pachycentria formosana* Hayata (Melastomataceae) is an endemic creeping shrub distributed in 300–2100 m forests throughout Taiwan [1]. Various tannins, flavonoids, and triterpenoids are widely distributed in plants of the family Melastomataceae. Many of these compounds exhibit antioxidant, anti-inflammatory, cytotoxic, antifungal, antiprotozoal, and antimicrobial activities. In our studies of Formosan plants for in vitro anti-inflammatory activity, *P. formosana* was found to be an active species. Investigation on the CH$_2$Cl$_2$-soluble fraction of the leaves and twigs of *P. formosana* led to the isolation of a new ferulic acid ester, 1,24-tetracosadiol di-($Z$)-ferulate (1) and a new ellagic acid derivative, 3,4,3',4'-dimethylenedioxyellagic acid (2), along with eight known compounds. Among the isolated compounds, oleanolic acid (6), ursolic acid (7), and 3-epi-betulinic acid (9) exhibited potent inhibition (IC$_{50}$ values $< 21.8 \mu$M) of $O_2^-$ generation by human neutrophils in response to N-formyl-L-methionyl-L-leucyl-L-phenylalanine/cytochalasin B (fMLP/CB). In addition, oleanolic acid (6), 3-O-($E$)-feruloylursolic acid (8), 3-epi-betulinic acid (9), and lawsonic acid (10) also inhibited fMLP/CB-induced elastase release with IC$_{50}$ values $< 18.6 \mu$M.

**Figure 1:** Structures of new compounds 1 and 2

Acknowledgement: This research was supported by grants from the National Science Council of the Republic of China (No. NSC 95–2320-B–127–001-MY3 and NSC 98–2320-B–127–001-MY3), awarded to Prof. J.-J. Chen.

alkaloids as constituents of this plant. Many of these compounds exhibit central depressant effect, muscle relaxation, and antiagastic ulcer, antibacterial, antiallergic, vasorelaxant, and neurotrophic activities. Investigation on ETOAc-soluble fraction of the stem bark of *M. officinalis* has led to the isolation of three new biphenyls, 5-allyl-5'-(1-hydroxyallyloxy)biphenyl-2,2-di01 (1), 5.5'-diallyl-2-(allyloxy)biphenyl-2-ol (2), and 5.5'-diallyl-2'(3-methylbut-2-enyloxy)biphenyl-2-ol (3), together with 12 known compounds, including four neolignans, magnolol (4), honokiol (5), (-)-monoterpymagnolol (6), and randainal (7), two norlignans, maenaldehyde D (8) and randainal (9), and six steroids, β-sitosteneone (10), stigmastera-4,22-dien-3-one (11), β-sitosterol (12), stigmasteral (13), 3β-hydroxystigmaster-5-en-7-one (14), and 3β-hydroxystigmasta-5,22-dien-7-one (15). The structure of new compounds (1-3) were determined through spectroscopic and MS analyses. Among the isolates, magnolol (4) and honokiol (5) exhibited potent inhibition against FMLP-induced superoxide production with IC50 values of 4.42 ± 0.24 and 0.68 ± 0.20 μM, respectively. In addition, magnolol (4) inhibited fMLP/CB-induced elastase release with IC50 values of 1.45 ± 0.20 μM.

**Figure 1:** Structures of new compounds 1 – 3


**New Coumarin Derivative and Bioactive Constituents from the fruits of Cnidium monnieri**

Cnidium monnieri (L) Cusson (Apiaceae) is a crude drug “Fructus Cnidi” (Chinese name Shechaungzi) used in traditional Chinese to treat impotence, frigidity, and skin-related disease. Coumarins, chromosomes, and their derivatives were isolated from this plant in previous studies. Many of these coumarins were found to exhibit antiadrenal, antiallergic, antifungal, and antibacterial activities. Investigation on ETOAc-soluble fraction of the fruits of *C. monnieri* has led to the isolation of a new coumarin, 5-0-methylmurraol (1), together with 12 known compounds, including 11 coumarins, meranzin hydrate (2), peroxymurraol (3), auraptenol (4), demethylauraptenol (5), peroxymurraol (6), murraol (7), osthol (8), bergapten (9), isopimpinellin (10), xanthotoxin (11), and xanthotoxol (12), and a chromone, cinodinol (13). The structure of new compound 1 was determined through spectroscopic and MS analyses. Among the isolates, osthol (8) completely inhibited ADP-induced platelet aggregation at 100 μg/ml. Xanthotoxin (11) showed complete inhibitory activity on platelet aggregation at 100 μg/ml induced by arachidonic acid.

**Figure 1:** Structure of new compound 1


**PG43 Anti-HIV activity of dial-oleane triterpenoids from Cassine xylocarpa**

Oxaro AA1, Torres D1, Bedoya LM2, Muñoz A2, Alcamí J2, Bazzocchi IL1

1 Instituto Universitario de Bio-Orgánica “Antonio González” Universidad de La Laguna, La Laguna, Tenerife, Spain; 2Unidad de Inmunopatología, Centro Nacional de Microbiología, Instituto de Salud Carlos III, Majadahonda, Madrid, Spain

The species of the Celastraceae have a long history in traditional medicine, and they produce an extraordinary variety of bioactive metabolites, including pentacarbonyl triterpenoids. Thus, a diversity of oleanane-type triterpenoids have been isolated from this family, mainly those corresponding to the Δ12-oleane skeleton, however, only a few examples of Δ18-oleane-type have been reported[1]. The human immunodeficiency virus (HIV) is the causative agent of acquired immunodeficiency syndrome (AIDS). Although antiretroviral therapy (ART) is still the mainstay of the progression of the disease, drug toxicity and viral resistance are important limitations[2]. Therefore, an unprecedented effort is being performed to find new anti-HIV drugs with acceptable toxicity, good resistance profiles and novel mechanisms of action. Thus, bezuvirat, a derivative of betulinic acid represents a unique first-in-class of anti-HIV compounds known as maturation inhibitors (MIs), which has succeeded in phase Ib clinical trials. In addition, moronic acid, a Δ18-oleane triterpene, also exhibits promising anti-HIV activity as a lead compound with an EC50 of 0.1 μg/ml and a good therapeutic index (TI > 186) relative to its cytotoxicity[3]. As a part of our research for bioactive compounds from Celastraceae species, herein we report the isolation of eleven new Δ18-oleane triterpenes, along with two known ones, from the stem of *Cassine xylocarpa* Vent. Their structures were determined by spectroscopic methods, including 1D and 2D NMR techniques. The compounds were tested for their activity as inhibitors of HIV replication. Ten of the compounds showed significant inhibitory effects, ranging from 66 to 99% at a concentration of 10μM. Acknowledgement: We are indebted to the Agencia Canaria de Investigación, Innovación y Sociedad de la Información (C200801000049) project for financial support.Osorio AA. thanks to MAEC-AECID for the fellowship. References: 1. Alcaraz L2, Colapietro ED, Ferro E (2005) Studies in Natural Products Chemistry (Part K). Elsevier. 30: 635 – 702. Amsterdam. 2. Mehlholz Y, De Clercq E (2010) J Med Chem 53: 521 – 538. 3. Qiu K et al. (2010) J Med Chem 53: 3133 – 3141.
Bioactivity-guided isolation of leishmanicidal chalcones from Piper delineatum

Ticco J.1, Flores N.2, Salamanca E2, Giménez A2, Macedo JR2, Jiménez IA2, Bazzocchi IL1, Lucci M3, Kabouche Z1, Bilia A2

1Laboratoire d’Obtention de Substances Thérapeutiques (LOST), Faculté des Sciences, Université Mentouri – Constantine, Campus Chabert Ersas, 25000 Constantine, Algeria; 2Department of Pharmaceutical Sciences, University of Florence, Via Ugo Schiff 6, 50019, Sesto Fiorentino (FI), Italy; 3Magnetic Resonance Center, Via Luigi Sacco 6, 20139 Sesto Fiorentino (FI), Italy

Astragalus armatus Willd. is an endemic shrub of the Northern Africa (Algeria, Morocco, Tunisia), is distributed in the pre-Saharan zone and is associated with the desertification in arid areas due to overgrazing [1]. In Tunisia it is used as tonic, stimulant and in cases of anaemia [2]. From the aerial parts of A. armatus a new acylated flavonoid triglycoside, isorhamnetin-3-O-[(4”-p-hydroxybenzoyl)-α-apiosyluronaryl]-[1-2]→(6)-β-galactopyranosyl (1) and one new flavonoid triglycoside, tamarixetin-3-O-α-apiosyluronaryl-[1-2]→(6)-β-galactopyranosyl (2), have been isolated together with six known flavonoids: isorhamnetin-3-O-α-apiosyluronaryl-[1-2]→(6)-β-galactopyranosyl, kaempferol-3-O-α-apiosyluronaryl-[1-2]→(6)-β-galactopyranosyl, nikotirolin and narcisin. The structures of the isolated compounds were established by means of 2D NMR, HPLC-DAD-MS, HR-MS, and UV spectral analyses. Pivotal role in the structure elucidation and in particular in the determination of the sugar sequence, played ROESY and HSQC-TOSY experiments.

Figure 1: Structures of the new naturally occurring compounds

Acknowledgement: The authors are grateful to ANDRS and DG-RSDT (MERSYS, Algeria) for financial support and to Prof. Gérard De Béhér (Faculty of Sciences, University Badjji-Mokhtar-Annaba) for the identification of the plant material and to Dr. Eleonora Castoldi (Mass Spectrometry Center, University of Florence – CISM) for recording the HRESI mass spectra. The authors would like to thank Stefano Rocchi for technical assistance.

References:

Leishmaniasis affects approximately 12 million people worldwide, primarily in developing regions [1]. Conventional chemotherapy with pentavalent antimonials is considerably toxic and prone to induce resistance. Second-line drugs, such as amphotericin B and its liposomal formulations, are either too toxic or expensive for routine use in developing countries. At the same time, the efficacy of miltefosine against cutaneous leishmaniasis remains to be ascertained [2,3]. Therefore, there is an urgent need to search for novel, effective and safe drugs for the treatment of these diseases [4]. In our ongoing study of potential leishmanial agents from Piper species [5], we have carried out a bioguided fractionation of the chalcone extract of the leaves of Piper delineatum Trel. This extract was subsequently partitioned between water and organic solvents of increasing polarity, giving CH2Cl2, EtOAc and fractions. These extracts and the remaining aqueous layer were tested for their leishmanial activity against promastigote forms of two strains of Leishmania (L. amazonensis and L. braziliensis). The bioactive CH2Cl2 fraction was subjected to column chromatography, yielding thirteen fractions. The most active fraction (IC50 1.1 and 1.3 µg/mL against L. amazonensis and L. braziliensis, respectively) was further subjected to repeat chromatography, affording two new bioactive trans-chalcones: 2’3,4’-tri-hydroxy-6’-methoxy-chalcone and 2’3,4’-tri-hydroxy-5,6-dimethoxy-chalcone, whose structures were elucidated by means of spectrometric and spectroscopic techniques. These results support the use of the Piper species as a traditional remedy in the treatment of parasitic diseases.

of two megastigmane glycosides, premannosioide (1), and salvinosioide B (2), an aliphatic alcohol glycolide, (3R)-1-octen-3-ol-3-O-D-xylopyranosyl-(1→6)-O-D-glucopyranosyl (3), a flavonoid, 5-hydroxy-3,7,4'-trimethoxyflavone, two hydroxycinnamic acid derivatives, rosmarinic acid, and 3-O-methyl-rosmarinic acid and sucrose. The structures of the compounds were established by means of 1D- and 2D-NMR experiments and MS. To the best of our knowledge, compound 1 is being reported for the first time from Lamiaceae, while compounds 2 and 3 are new for the genus Salvia. This work also constitutes the first phytochemical work on the aerial parts of S. dichroantha. References: 1. Hedge IC (1982) Salvia L. In: Davis PH (ed.) Flora of Turkey and East Aegean Islands. Edinburgh University Press. Edinburgh. 2. Baytop T (1999) Therapy with Medicinal Plants in Turkey, Nobel Tip Kitapleri, Istanbul. 3. Kawazo K et al. (1999) Phytochemistry 50: 493 – 497.

Iridoid, phenylethanoid and flavonoid glycosides from Sideritis trojana

Kirmizibekmez H1, Anthuru E1, Masullo M2, Yesilada E1, Piantoni S1

1Yeditepe University, Faculty of Pharmacy, Department of Pharmacognosy, TR-34755, Kayzadag, Istanbul, Turkey; 2University of Salerno, Department of Pharmaceutical and Biomedical Sciences, Via Ponte Don Mellilo, 84084 Fisciano, Salerno, Italy

The genus Sideritis is represented by 45 species in the flora of Turkey [1]. S. trojana Bornm., a perennial herb endemic to Kazdaglari (Ida Mountains), is utilized as an herbal tea for various purposes. Previously, several diterpenes were reported from S. trojana [2]. However, there is no report on its iridoid and phenolic constituents. In the continuation of our work on the bioactive secondary metabolites from Lamiaceae family, we now describe the isolation and structure elucidation of the second-ary metabolites from the roots of S. trojana as well as their antioxidant activity. From the H2O-soluble portion of the MeOH extract, a new iridoid glycoside, 10-O-(E)-feruloylmethylitoliside (1) was obtained in additi-on to four known iridoid glycosides (melittoside,10-O-(E)-p-coumaroylmethylisolitoside, stachyosydioside E and G). Moreover, five phenylethanoid glycosides (verbascoside, isoacteoside, lamalboside, leonoside A, isola-vandulifolioside), three flavone glycosides (isoscutellarein 7-O-β-D-glucopyranoside, 3′-hydroxy-4′-O-acetyl-glucopyranoside, 3′-(1→2)-β-glucopyranoside), four-O-methylosuccul- larein 7-O-β-acetyl-β-glucopyranosyl-(1→2)-β-glucopyranoside, 3′-hydroxy-4′-O-methylosucculentarein 7-O-β-acetyl-β-glucopyranosyl-(1→2)-β-glucopyranoside and a benzyl alcohol derivative (di-O-methylcreatin) were obtained and identified. Characterization of the isolates was carried out by using NMR experiments and HR-MS. All compounds were tested for their antioxidant activity by in vitro TEAC assay and some of them exhibited moderate activity (0.97 – 1.44 mM) if compared with the reference compound (quercetin, 1.86 mM). Refer-ences: 1. Aytac Z and Aksoy N (2000) Flora Mediterranea 10: 181 – 184. 2. Topcu G et al. (2001) Nat Prod Let 16: 33 – 37.

Searching for iridoids from tropical plants: detection, isolation and antibacterial activity

Litudon M1, Le Borgne E1, Teres P1, Deguin B1, Lecoc Borret M1, Guéritte F1

1Centre de Recherche de Gif, Institut de Chimie des Substances Naturelles, CNRS, UPR2301, Gif-sur-Yvette, France; 2Laboratoire de Pharmacognosie UMR 8638, Université de Paris-Descartes, Paris, France; 3Laboratoire de Microbiologie, EA 4065, Université Paris Descartes, Faculté des Sciences Pharmaceutiques et Biologiques, Paris, France

Iridoid glycosides, which form an important group of cycloartenone monoterpeneoïds, are biosynthesized by a large number of plant species belonging to approximately twenty important botanical families. Although they possess a wide range of pharmacological and biological properties, such as anti-allergic, anti-inflammatory, antibacterial, anti-fungal, antiviral, anti-oxidative, immunomodulatory, neuroprotective, etc. [1,2], no molecule is currently used as a drug. However, iridoids, which possess a highly functionalized aglycon, may be regarded as starting material for the synthesis of a new of number of new chiral molecules. In this context, we have launched a research project aimed at searching for new chiral scaffold of iridoid-type from higher plants of the tropical biodiversity. This project, part of an ANR program called IRNA-CHIR, focuses on species, which contain high iridoids content. For this study, approximately 500 species were selected from iridoid-containing families, and were subjected to a methanolic extraction followed by flash chromatography into fractions and semipreparative HPLC column into individual compounds. Their characteristically related compounds can be targeted. Certain levels of compounds with immunoreactivity similar to isoflavonoids were identified. The extract was subsequently separated by flash chromatography into fractions and subjected to detailed analysis of 1H and 13C NMR spectra. According to previous literary data describing various biological activities of rotenoids, we suppose that future research on this new rotenoid may lead to new findings in the phytochemistry of these bioactive compounds.

Figure 1: 12α-hydroxyrotenone

Phenylated acetophenone derivatives constitute a characteristic chemical group of constituents of *Acronychia pedunculata* (L.) Miq. [1]. In continuation of a previous study, seven acetophenone dimers were isolated among them five structural isomers [2,3]. Such acetophenones exhibit particular structural characteristics as fully substituted and polyhydroylated aromatic rings. The presence of inter- and intra-molecular hydrogen bonds and their conformational behavior due to the occurrence of rotamers complicates their structure elucidation. In the present study, NMR spectroscopy was used in order to determine the structures and the different rotamers of all isolated acetophenones. The developed methodology included variation of different solvents (DMSO-d<sub>6</sub>, CDCl<sub>3</sub>, CD<sub>3</sub>D<sub>2</sub>) as well as acquisition of NMR spectra in a broad range of temperatures (0 to 47°C) where acrovestone was used as a model compound. Two principal rotamers of acrovestone are the most populated in CDCl<sub>3</sub> solution at 0°C, while at 47°C their representative NMR signals are not resolved due to fast inter-conversion between the rotamers. According to our study, *Acronychia* acetophenone rotamers' determination can be accomplished with NMR spectroscopy, by changing the polarity of solvent used as well as by altering temperature conditions of measurements. In parallel, an LC-APC(+)HRMS and MS/MS method was developed for the analysis of acetophenone derivatives using a LTQ-Orbitrap mass analyzer. A characteristic ion corresponding to the major fragment at m/z 319 was defined and used as diagnostic peak of the isolated phenylated acetophenone dimers. This novel developed LC-MS/MS method could be applied for the detection and identification of acrovestone-type prenylated acetophenone dimers in other substrates. References: 1. Adsersen A et al. (2007) Biochem Syst Ecol 17: 447 – 453. 2. Kouloura E et al. (2008) Planta Med 74: 1051 – 1052. 3. Kouloura E et al. (2009) Planta Med 75: 914.

**Figure 1: Structure of new compounds 1 and 2**


---

**PG65**

**Sesquiterpenoids from the root of Solanum erianthum**

Chen Y<sup>1</sup>, Lee H<sup>2</sup>

<sup>1</sup>University of Science and Technology of China, School of Pharmacy, Hefei 230026, China. 2School of Life Sciences, Fudan University, Shanghai 200433, People’s Republic of China.

Solanum erianthum D. Don (Solanaceae) is an evergreen shrub or small tree which is native to South America, widespread in tropical Asia and Oceania [1]. It is a traditional folk medicine used for the treatment of mouth, gingivitis, edema, fever, cough, ulcers, eczema, toothache and dermatitis [2]. In a screening program of Formosan plants, the MeOH extract of the root of this plant showed significant cytotoxic activities and was partitioned into n-hexane, EtOAc, n-ButOH and H<sub>2</sub>O-soluble layers. Investigation of the active EtOAc-soluble layer led to the isolation of 4 known compounds, including solanetin, anhydro-β-sitosterol, solafuranone, lycifuranone A; 1 phenylalanik acid: acetovalinone, and 2 steroids: β-sitosterol and stigmastol. Solavetivone, the major constituent, was reported oving cytotoxicity against OVCAR-3 (IC<sub>50</sub> = 0.1 mM) [3]. The structure of the new sesquiterpoid was determined by spectral analyses. Acknowledgement: This work was kindly supported by a grant (NSC 98 – 2320-B-039 – 015-MY3) from the National Science Council of the Republic of China. References: 1. William GD, Peng CI (1998) Solanaceae in Flora of Taiwan, 2nd edition. Editorial Committee of the Flora
The fruits and seeds (star anise) of *Illicium* plants are used traditionally as spices and folk medicines in Southern China. Those are one of the most important natural resources of shikimic acid (SA), which is the raw material of the antiviral drug Tamiflu. China is the largest star anise supplier in the world and 80% of raw resources are from Guangxi province. A simple and rapid HPLC method was established to analyze the content of SA in the fruits and leaves of 22 samples from different species and habitats, and SA with high content was found in the fruits of three species (> 8%) and leaves of eight species (> 5%). Thus these materials can be used as the raw resources of SA. Researches found that even the trace amount of anisatin and its analogs could arouse toxic effects. The mechanism studies revealed they are non-competitive antagonists of GABA receptor. Chemical investigations on three *Illicium* plants resulted in 14 sesquiterpenoids including anisatin and its analogs to be obtained. Based on the summarized MS behaviors of anisatin and its analogues, a qualitative analytical method was developed to detect anisatin in the fruits of the above species, the results turned out that most species contained anisatin and its analogues including the edible species (*I. verum* Hook. f.) from Rongshui County, while three edible species (*I. verum* and *I. majus* Hook. f. & Thomson in Jinxiu County, *I. jadinii* Hook. f. Chang in Lingyun County) did not contain anisatin or its analogues and they are safe to use as spices and folk medicines.

**Pharmacognostic study and safety evaluation on *Illicium* plants**

**Sheng Y, Ping TC, Qiang KC, Zin Q, Yang Y**

Department of Natural Products Chemistry, State Key Laboratory of Drug Research, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, 555 Zu-Chong-Zhi Road, Zhangjiang Hi-Tech Park, Shanghai 201203, P. R. China,

Abstracts | 59th International Congress of the GA | 4th–9th September 2011, Antalya, Turkey

The fruits and seeds (star anise) of *Illicium* plants are used traditionally as spices and folk medicines in Southern China. Those are one of the most important natural resources of shikimic acid (SA), which is the raw material of the antiviral drug Tamiflu. China is the largest star anise supplier in the world and 80% of raw resources are from Guangxi province. A simple and rapid HPLC method was established to analyze the content of SA in the fruits and leaves of 22 samples from different species and habitats, and SA with high content was found in the fruits of three species (> 8%) and leaves of eight species (> 5%). Thus these materials can be used as the raw resources of SA. Researches found that even the trace amount of anisatin and its analogs could arouse toxic effects. The mechanism studies revealed they are non-competitive antagonists of GABA receptor. Chemical investigations on three *Illicium* plants resulted in 14 sesquiterpenoids including anisatin and its analogs to be obtained. Based on the summarized MS behaviors of anisatin and its analogues, a qualitative analytical method was developed to detect anisatin in the fruits of the above species, the results turned out that most species contained anisatin and its analogues including the edible species (*I. verum* Hook. f.) from Rongshui County, while three edible species (*I. verum* and *I. majus* Hook. f. & Thomson in Jinxiu County, *I. jadinii* Hook. f. Chang in Lingyun County) did not contain anisatin or its analogues and they are safe to use as spices and folk medicines.

**New sesquiterpene derivatives and anti-inflammatory constituents from *Pachira aquatica***

Chen J, Cheng L, Liao C, Chung M

Graduate Institute of Pharmaceutical Technology, Tajen University, Pingtung 907, Taiwan; 2Faculty of Pharmacy, College of Pharmacy, Kaohsiung Medical University, Kaohsiung 807, Taiwan; 3Graduate Institute of Natural Products, Chang Gung University, Taoyuan 333, Taiwan

*Pachira aquatica* Aulett (Bombacaceae) is an evergreen tree, distributed in tropical America and introduced to Guangdong, Yunnan, and Taiwan as a cultivated plant [1]. Cadinane sesquiterpenoids, sesquiterpene lactones, and triterpenes are widely distributed in plants of the family Bombacaceae, and many of these compounds exhibit antiangiogenic, hypotensive, and antimicrobial activities. Investigation of n-hexane-soluble fraction of the stem of *P. aquatica* has led to the isolation of two new sesquiterpene derivatives, 11-hydroxy-2-O-methylhibiscolactone A (1) and O-methylhibiscone D (2), together with 18 known compounds, including 5-hydroxysauranetin (3), kaempferol-3,7,4’-trimethyl ether (4), santi-7-methyl ether (5), 3,5,6,7,8,9’-4’-heptamethoxyflavone (6), calycocerin (7), retusin (8), 5,4’-diidihydroxy-3,7-dimethoxyflavone (9), isohemigossylic acid lactone-7-methyl ether (10), hibiscolactone A (11), hibiscone C (12), hibiscone D (13), 2-O-methylisohemigossypolone (14), scopoletin (15), benzophenone (16), 2α,3β-dihydroxyrupene (17), lupenone (18), 24-methylencyclocaertanol (19), and (23E)-cyloact-23-ene-3β,25-diol (20). The structures of new compounds 1 and 2 were determined through spectroscopic and MS analyses. Among the isolates, 5-hydroxysauranetin (3) and isohemigossylic acid lactone-7-methyl ether (10) exhibited potent inhibition against N-formyl-L-methionyl-L-leucyl-L-phenylalanine-induced superoxide production with IC_{50} values of 28.84 ± 2.26 and 12.77 ± 2.48 µM, respectively.

**Figure 1:** Structure of new compounds 1 and 2


**Figure 1:** Star anise and the structures of shikimic acid (SA) and anisatin.


**A new natural Pepstatin from Kitasatospora (Actinomycetales)**


Fundación MEDINA, Centro de Excelencia en Investigación de Medicamentos Innovadores en Andalucía, Parque Tecnológico de Ciencias de la Salud, Avenida del Conocimiento 3, 18100 Armilla (Granada), SPAIN.

The pepstatins are linear peptides biosynthesized and excreted into culture filtrates by several species of Actinomycetes and are well known inhibitors of aspartic proteinases, such as pepsin and cathepsins D and E. Apart from their role as proteinase inhibitors, their other pharmacological and cellular activities remain unclear. Our lab has isolated Pepstatin A [1] and a new member of this family from an actinomycete strain belonging to the genus *Kitasatospora*. BLAST searches and sequence alignments of partial ribosomal DNA sequences revealed that this strain is closely related to strains of the species *Kitasatospora mediocidica*. The producing organism was isolated from the rhizosphere of a juniper-tree (*Juniperus communis* L.) after plating and incubation of a soil suspension on a selective glycerol arginine agar isolation medium. How was it fermented? Fermentation discovery strategy? A 1L fermentation of the strain was extracted with acetone and, after evaporation of the organic solvent, fractionated on a SPE resin (SP207ss) on reverse phase mode. Acetonitrile/water semi-preparative HPLC gradient of one of these fractions led us to the detection (LC/MS) of two components related to the pepstatin family. Both secondary metabolites were isolated by preparative HPLC under similar conditions. Structural elucidation of both components was based on NMR (1 H, 13C, COSY, HSQC, HMBC) and low and high resolution Mass Spectrometry (LC/MS-ESI) data. The isolated compounds were finally identified as pepstatin A and the new derivative we designate as pepstatin K. Data on the isolation, structural characterization and biological properties of both compounds will be presented.

**Figure 1:** Pepstatin Family

Isolation and structural elucidation of coumarine from *Micromelum falcatum* (Rutaceae)  
Danika E., Koulaoura E., Sothea K., Halabalaki M.*  
1Laboratory of Pharmacognosy & Natural Products Chemistry, School of Pharmacy, Panepistimio Ioupoli, Zografou, 15771, Athens, Greece; 2Joint Laboratory of Phytochemistry, Faculty of Pharmacy, University of Health Sciences 73, Bd Monivong, Phnom Penh, Cambodia

*Micromelum falcatum* Tanaka (Rutaceae) is a small tree growing in Southeastern Asia [1] showing protective and therapeutic effects against cold and rheumatoid arthritis according to the traditional medicine of China [2]. The leaves of the plant, collected in Cambodia, after drying cold and rheumatoid arthritis according to the traditional medicine of China [2]. The leaves of the plant, collected in Cambodia, after drying cold and heating, were extracted with EtOAc and MeOH was followed for focused isolation of alkaloids [3]. The resulting extracts were evaluated qualitatively using a novel HPLC-DAD method. The analytical profiling revealed the presence of coumarins as the major class of constituents in both CH$_2$Cl$_2$ and EtOAc extracts before and after the alkylation. The HPLC-DAD method was transferred to semi-preparative scale and was used for the isolation of the detected coumarins. Nine known and two new coumarins, microalpinin (1) and microcoumarin (2) were isolated. Three among them, micromarin A, B and C were isolated for the first time from *M. falcatum*. The identification of the isolated compounds was performed by HRMS and NMR (1 & 2D) spectroscopy.

**Figure 1:** Microcoumarin

**Figure 2:** Microalpinin


---

**PG62**  
Four new kaemperol glycosides from the leaves of *Brugmansia suaveolens*  
1Department of Pharmaceutical/Medicinal Chemistry, University of Tübingen, Germany; 2Escuela de Química and CIPRONA, University of Costa Rica, Costa Rica; 3Department of Organic Chemistry, University of Tübingen, Germany; 4Department of Pharmaceutical Industry, Federal University of Santa Maria, Brazil; 5Department of Pharmaceutical Biology Biotechnology, University Freiberg, Germany

*Brugmansia suaveolens* (Humb. & Bonpl. ex Willd.) Bercht. & C. Presl (Syn. *Datura suaveolens*; Common name: Angel’s trumpet) is a flowering shrub of Solanaceae family and it is native from coastal regions of the rainforest in Southeast Brazil. This plant has been investigated due to its anti-inflammatory and wound healing activities [1] and mainly due to the presence of alkaloids [2]. Nevertheless, only few studies related the characterization of flavonoid glycosides [3]. This prompted us to investigate the ethanolic extract prepared from its leaves. In order to have pure compounds, the plant material was submitted to successive chromatographic separations using open column chromatography and HPLC on RP-18. Up to now, four new flavonol glycosides, namely, kaemperol 3-O-$\beta$-$D$-glucopyranosyl-(1’’’’-2’’)-O-$a$-$L$-arabinopyranoside (1), kaemperol 3-O-$\beta$-$D$-[6’’’’-O-(3,4-dihydroxy-cinnamoyl)]-glucopyranosyl-(1’’’’-2’’)-O-$a$-$L$-arabinopyranoside (2), kaemperol 3-O-$\beta$-$D$-[2’’’’-O-(3,4-dihydroxy-cinnamoyl)]-glucopyranosyl-(1’’’’-2’’)-O-$a$-$L$-arabinopyranoside (3), and kaemperol 3-O-$\beta$-$D$-[2’’’’-O-(3,4-dihydroxy-cinnamoyl)]-glucopyranosyl-(1’’’’-2’’)-O-$a$-$L$-arabinopyranoside (4) were isolated and identified by means of extensive spectroscopic methods including 1D-(1H and 13C) and 2D NMR experiments (COSY, HSQC and HMBC) as well as ESI-MS.

**Figure 1:** Isolated flavonol glycosides

The genus Alyssum belonging to family of Cruciferae is represented by 89 species in Turkey, 52 of them being endemic [1]. Although there are no reports of the medicinal uses of Alyssum corsicum Duby, the aerial flowered part, flowered stems and inflorescences of A. maritimum (L.) Lam. are employed as renal lixiviant in infusion and decoction in the Iberian Peninsula. It is also claimed to be a hepatic lithotripter, and to have other benefits associated to the hepatic function (hepatoprotective, antiinflammatory, the last use in veterinary) [2]. In Iran, seeds of A. minutum Patrin ex DC. are used as a treatment for fevers and other ailments. Glucosinolates, hydrocarbons, fatty acids and flavonol 7-glucuronides were isolated from the genus Alyssum previously [3]. Glucosinolate profiles of the seeds of the various Alyssum species were also screened by Ion-Pair LC-MS method [4]. This is the first phytochemical report on Alyssum corsicum. In this study three known compounds (Tamarixin 3,7-diglucoside, Tamarixin 3-O-D-glucopyranoside-7-O-a-rhamnopyranoside, Tamarixin 3-O-D-glucoside) were isolated from the MeOH extract of Alyssum corsicum by using preparative chromatographic methods. The structure elucidation of the isolated compounds was based on analyses of their spectroscopic data (1D and 2D NMR). References: 1. Davis PH (1965) Flora of Turkey and East Aegean Islands. University Press. Edinburg. 2. Parada et al. (2009) J Ethnopharmacol 124: 609 – 618. 3. Afsharypuor S, Lockwood GB (1986) J Nat Prod 49: 944 – 945. 4. Bennett RN et al. (2004) J Agric Food Chem 52: 428 – 438.

The genus Verbascum (Scrophulariaceae) is represented by 228 species, of which 185 are endemic in the flora of Turkey and East Aegean Islands [1]. Verbascum species contain biologically active compounds, such as flavonoids, phenylethanoid and neolignan glycosides, saponins, and iridoid and monoterpenic glycosides [2]. The leaves and flowers of Verbascum are reported to have expectorant, mucolytic and demulcent properties [3], 3,7-diglucoside, Tamarixetin 3-O-D-glucopyranoside, Tamarixetin 3-O-D-glucoside were isolated from Verbascum reeseanum (Karayil) DC. It is also claimed to be a hepatic lithotripter, and to have other benefits associated to the hepatic function (hepatoprotective, antiinflammatory, the last use in veterinary) [2]. In Iran, seeds of A. minutum Patrin ex DC. are used as a treatment for fevers and other ailments. Glucosinolates, hydrocarbons, fatty acids and flavonol 7-glucuronides were isolated from the genus Verbascum previously [3]. Glucosinolate profiles of the seeds of the various Verbascum species were also screened by Ion-Pair LC-MS method [4]. This is the first phytochemical report on Verbascum reeseanum. In this study three known compounds (Tamarixin 3,7-diglucoside, Tamarixin 3-O-D-glucopyranoside-7-O-a-rhamnopyranoside, Tamarixin 3-O-D-glucoside) were isolated from the MeOH extract of Verbascum reeseanum by using preparative chromatographic methods. The structure elucidation of the isolated compounds was based on analyses of their spectroscopic data (1D and 2D NMR). References: 1. Davis PH (1965) Flora of Turkey and East Aegean Islands. University Press. Edinburg. 2. Parada et al. (2009) J Ethnopharmacol 124: 609 – 618. 3. Afsharypuor S, Lockwood GB (1986) J Nat Prod 49: 944 – 945. 4. Bennett RN et al. (2004) J Agric Food Chem 52: 428 – 438.

The family Rubiaceae is represented by about 500 genera and 6000 species, with about 200 known Asperula species [1]. Asperula species are traditionally used by Lebanese people to reduce blood pressure and inflammation/edema, and for diabetes [2]. Moreover they are rich sources of antimicrobial agents [3]. The chemical composition of genus Asperula characterized by the presence of iridoids, flavonoids and anthraquinone glycosides [4,5,6]. During our search on the aerial parts of Asperula cypria Ehrend., an endemic plant of Cyprus, a number of secoiridoids, flavonoids and phenolic acids was achieved from the complex extracts using dual mode or gradient mode CCC. The purity and identity of isolated compounds was confirmed by NMR spectroscopy. It is worth noting that the phytochemical analysis of L. siliquosus, T. purpureus and C. hassertiana is presented for the first time. In conclusion, it is clearly indicated that counter-current chromatography is a valuable technique and can be successfully employed for rapid and effective separation of natural compounds from crude active extracts of Fabaceae species. References: 1. Spanou C et al. (2008) J Agric Food Chem 56: 6967 – 6976. 2. Bertoth A et al. (2009) Pure Appl Chem 81(2): 355 – 367.
Centaurea is a complex genus of about 500 species belonging to the Asteraceae family [1]. Sesquiterpene lactones are the main chemical taxonomic markers of the genus [2,3]. Some members of this genus are used in folk medicine [4]. In the present study, we report the main compounds isolated from Centaurea pannonica (Heufel) Simionak, a taxon belonging to the section Jacea [6, 7]. The plant was collected in Sumadija region-Serbia, on September 2008. The aerial parts were extracted according to the Bohlmann isolation method, slightly modified [5]. One newly occurring sesquiterpene lactone (Fig.1), six known guaianolides, namely babylin A, chlorohyssopifolin C, chlororepdiolide, repdiolide, janerin, 19-deoxyjanerin and three known lignans arctigenin, matairesinol, arctiin were isolated by repeated CC and RP18-HPLC. The structure of the isolated compounds were elucidated by spectroscopic methods, particularly high-field NMR spectroscopy (1H NMR, 13C NMR/DEPT, 1H-1H COSY, NOESY, HSQC, HMBC). So far, the presence of guaianolides is characteristic for the taxa of the section Jacea [6, 7].

Figure 1

the possibility of rapid in vivo bioactivity analysis at the microgram scale, an attractive feature when combined with high-resolution fractionation technologies and microgram-scale analytical methods such as UHPLC-TOF-MS and microflow NMR. Using this platform, we have performed high-resolution in vivo bioactivity profiling of Solanum torvum Schidl., one of several Solanaceae species used as medicinal plants for the treatment of epilepsy.


UHPLC-TOF-MS and microflow NMR 3. Using this platform, we have performed a continuing investigation for biologically active molecules, the methanol extract of E. boetica aerial parts has been studied. The crude methanolic residue was suspended on a methanol-water mixture and extracted with ethyl acetate. Repeated column chromatographic fractionation and further purification by HPLC of the ethyl acetate soluble part afforded six diterpenes with the lathryane skeleton that have a new acylation pattern. In addition, a cytochrome triterpene was also isolated and identified. The chemical structures of the isolated compounds, including stereochemical features were deduced from their physical and spectroscopic data, which include: Infrared Spectroscopy, low and high resolution Mass Spectrometry (MS), and extensive one- and two-dimensional Nuclear Magnetic Resonance studies (1D and 2D-NMR). Acknowledgement: This work was supported by Fundação para a Ciência e Tecnologia (FCT) (Project PTDC/QUI-QUI/099815/2008). References: 1. Hartwell J (1965) Lloydia 22: 153–205. 2. Lage H et al. (2010) Phytomedicine 17: 441–448. 3. Duarte N et al. (2008) Bioorg Med Chem 16: 9323–9330. 4. Duarte N. et al. (2008) Bioorg Med Chem 16: 9323–9330.

Multidrug-resistance phenomenon (MDR) to anti-cancer drugs is one of the most serious obstacles in the success of a chemotherapy treatment. P-glycoprotein (P-gp) is often implied in the efflux of drugs as anti-cancer drugs, vinca alkaloids, and other related drugs, lowering the effective concentration of such drugs in the cytoplasmic compartment [1]. One of the most promising approaches to overcome MDR is the development of molecules that can effectively modulate the activity of P-gp, thus inhibiting the drug efflux. In the past decades, several natural and synthetic compounds have been reported as MDR modulators, but none is currently available for the clinical practice. In previous works, we have isolated, from Euphorbia species (Euphorbiaceae), several macrocyclic jatrophane [2,3] and lathyrane-type [3,4,5] diterpenes with strong P-gp modulation activity. In order to obtain a library of bioactive lathyrane and jatrophane diterpenes, required for QSAR studies and further refinement of an in-house P-gp modulators pharmacophore model, the phytochemical study of Euphorbia piscatoria Ait., an endemic species from Madeira island traditionally used in fishing activities, has been carried out. Fractionation by chromatographic methods of the crude methanolic extract of the aerial parts of Euphorbia piscatoria yielded a large amount of a lathyrane-type diterpenoid that was acylated, using different alkanyl and aroyl chlorides/anhydrides. Several new esters were obtained whose structures were assigned based on spectroscopic methods namely 1D NMR ([H, 13C, DEPT] and 2D NMR (COSY, HMBC, HMQC) data. Acknowledgement: This work was supported by Fundação para a Ciência e Tecnologia (FCT) (Project PTDC/QUI-QUI/099815/2008 and grant SFRH/BD/72915/2010). References: 1. Teodori E et al. (2002) II Farmaco 57: 385–415. 2. Valente C et al. (2004) Planta Med 70: 81–84. 3. Duarte N et al. (2006) Planta Med 72: 162–168. 4. Duarte N et al. (2008) Bioorg Med Chem 15: 546–554. 5. Duarte N. et al. (2008) Bioorg Med Chem 16: 9323–9330.
Antimicrobial constituents from the African medicinal plant Zanthoxylum capense
Luo X, Pedro 1, Milic V, Rameira C, Mulhovo S, Duarte A, Duarte N, Ferreira M
1Research Institute for Medicines and Pharmaceutical Sciences (iMed.UL), Faculdade de Farmácia, Universidade de Lisboa, Av. das Forças Armadas, 1600 – 083, Lisboa, Portugal; 2Research Institute for Medicines and Pharmaceutical Sciences (iMed.UL), Faculdade de Farmácia, Universidade de Lisboa, Av. das Forças Armadas 1600 – 083, Lisboa, Portugal; The School of Pharmacy, University of London, 29 – 39 Brunswick Square, London, WC1N 1AX, United Kingdom; 3Departamento de Ciências Agropecuárias, Escola Superior Técnica de Agricultura, Pedagogía, Campus de Lhanguene, Av. de Moçambique, 21402616 Maputo, Mozambique

The genus Zanthoxylum, comprising approximately 250 species, is well known for its ethnobotanical uses among the Rutaaceae family [1]. Previous studies have demonstrated that plants belonging to this genus are rich sources of biologically active compounds, such as alkaloids, aliphatic and aromatic amides, coumarins, as well as lignans [2]. Zanthoxylum capense (Thunb.) Harv. is a medicinal plant indigenous to Zimbabwe, South Africa, and Mozambique. Traditional healers use the decoction of its roots for snakebites, and the decoction of its root barks to treat tuberculosis, paralysis, and relief of toothache. However, until date there have been relatively few phytochemical studies on this species [3, 4]. During our search for bioactive compounds from the methanolic extract of Z. capense roots, we have isolated a new benzophenanthridine alkaloid and two new 2-arylenzofuran neolignans. In addition, several known compounds were also isolated, including six alkaloids with the benzophenanthridine scaffold, one furoquinoline-type alkaloid and a lignan. The structures of the compounds were elucidated by means of MS, extensive 1D and 2D-NMR analyses by comparing their spectral data with those reported in the literature. All the isolated compounds were evaluated for their in vitro antibacterial activity against Gram-positive and Gram-negative bacteria. Some compounds showed inhibitory activity mainly against Staphylococcus aureus ATCC 6538 with MIC values ranging from 12.5 to 50 μg/ml. These compounds might be promising leads for the development of new antimicrobials. Acknowledgement: This study was supported by a fellowship from FCT, Portugal (reference number SFRH/BPD/37179/2007). References: 1. Sun KW et al. (1996) Acta Pharma Sin 31: 231 – 240. 2. Chen JJ et al. (2008) Nat Prod 71: 212 – 217. 3. Calaerood JM et al. (1970) Phytochemistry 9: 675. 4. Fish F et al. (1973) Phytochemistry 12: 2553 – 2554.
endemic to the country [1]. *Salvia chassanica* Bunge is one of the Iranian endemic species of *Salvia* that only grows in Iran that belongs to the Lamiaceae family. There is not any reported literature on *S. chassanica* so the present project set to start search for finding various diterpenoids from this plant. Air-dried and powdered roots of *S. chassanica* were extracted with EtOAc (3 x 3L), for about 24h at ambient temperature. After filtration, the combined extracts were concentrated yielding 20 g of total extract. Two diterpenoids, Taxodione (1) [2] and Ferruginol (2) [3], were isolated by means of chromatographic methods mainly column chromatography checked by TLC, and purified by preparative RP-HPLC. The structures of compounds 1 – 2 were determined on the basis of spectroscopic data [4,5] using 1H NMR, 13C NMR, DEPT-135, HMBC, HSQC, COSY and NOESY experiments. In conclusion, based on the result obtained from our study *S. chassanica* can be considered a rich source of different abietane diterpenoids. This is the first report of compounds isolated from this plant. References: 1. Emami A et al. (2008) de l’Universite d’Iran des Sciences Medicales pp. 362 – 391. 2. G/252ner A, Ozhatay N, Ekim T, Bas/252r

**PG79**

A new cycloartane-type glycoside from *Astragalus schottianus* Boiss

Karah/252y F, Bedir E

Department of Bioengineering, Faculty of Engineering, Ege University, Bornova, 35100 I/252zmir, Turkey

*Astragalus* L., the largest genus in the family Leguminosae, is represented by 445 species, of which 224 are endemic. They can be attributed to 62 sections in the flora of Turkey [1,2,3]. The roots of *Astragalus* species represent a very old and well-known drug in traditional medicine for its usage as an antiperspirant, diuretic and tonic drug [4]. In the district of Anatto, located in South Eastern Turkey, an aqueous extract of the roots of *Astragalus* is traditionally used against leukemia and for its wound-healing properties. Known biologically active constituents of *Astragalus* roots represent two major classes of chemical compounds, poly saccharides and saponins [4]. In our continuing search on Turkish *Astragalus* species, we have isolated a new cycloartane-type triterpene glycoside from methanolic extract of *A. schottianus* by combined chromatography on reverse phase C-18 and silica gel. The structure of the new compound was determined as 3-O-b-D-xylopyranosyl-3β,6α,16β,20S,25S,25-hydroxy-cycloartane by the extensive use of 1D and 2D-NMR techniques and mass spectrometry. This compound represents the first entry of the series of cycloartane-type compound possessing a 20-OH functional group in *Astragalus* genus.

**PG80**

Semi-synthesis of Cytotoxic Molecules From Cycloartane Type Sapogenols

T/252g D/252, Akgun IH/252, Kocbabas F/252, Korkmaz KS/252, Bedir E/252

Ege University, Faculty of Science, Chemistry Department, 35100, Izmir, TURKEY; 2Ege University, Faculty of Engineering, Bioengineering Department, 35100, Izmir, TURKEY

Semi-synthetic anticancer drug-discovery programs focusing on saponins mainly engaged with commercially available triterpenoids such as oleanolic acid and ursolic acid (1,2,3), not including less common miscellaneous aglycons such as cycloartanes, lanostanes and hopanes. Cycloartanes occupy a special position among low molecular bioregulators because they are produced by photosynthesizing organisms only, and one from the initial representatives of this range, cycloartenol, serves as the key link in the biosynthesis of different phytosterols (4). In general, the plants of *Astragalus* genera proved to be the richest source of this class of compounds. As part of our continuing studies on cycloartane-type sapogenols of *Astragalus* genus, twenty molecules were synthesized starting from cycloastragenol and its isomer astragenol, and their cytotoxicities were tested against three different cancer cell lines (HT-29: human colon cancer cell line; MDA-MB-231: human breast cancer cell line; PC-3: human prostate cancer cell line) together with a transformed cell line (HEK 293: human embryonic transformed kidney cell line). Some of the semi-synthetic derivatives such as A2 and C5 exhibited more potency compared to the starting molecules. Further studies are in progress to prepare more potent compounds versus cancer lines.

**PG79**

**Figure 1**


**PG81**

Flavonol glycosides and a saponin from *Chenopodium foliosum* Asch

Kokanova Nedialkova Z/2, B/C252cherl D/2, Nikolov S/2, Heilmann J/2, Nedialkov P/2

1Department of Pharmacognosy, Faculty of Pharmacy, Medical University of Sofia, Sofia, Bulgaria; 2Pharmaceutical Biology, Institute of Pharmacy, University of Regensburg, Regensburg, Germany

Three new flavonol glycosides and a new saponin, namely 6-methoxykaempferol-3-O-β-gentiobioside, gomphrenol-3-O-β-gentiobioside, gomphrenol-3-O-o-L-thamnopyranosyl-(1→2)-O-β-D-glucopyranosyl-(1→6)-O-β-D-glucopyranoside and 3-O-β-D-glucopyranosyl-30-normedicagenic acid-28-β-D-glucopyranosyl ester as well as the known flavonol glycosides patuletin-3-O-β-gentiobioside and spinacetin-3-O-α-gentiobioside were isolated from the aerial parts of *Chenopodium foliosum* Asch. The structures of the compounds were established by means of spectroscopic methods (1D and 2D NMR, UV, IR, and HRMS). DPPH free radical scavenging activity and cytotoxicity (MTT-test) of the new compounds were assessed as well. Acknowledgement: This study was supported by Medical Science Council at the Medical University of Sofia (Project 36/2011)
PG82
Terpenoids from the Root of Salvia hypoleuca Benth.
Gohari A1, Chamarinia M2, Saeidnia S5
1Medicinal Plants Research Center, Faculty of Pharmacy, Tehran University of Medicinal Sciences, Tehran, Iran; 2Department of Chemistry, Faculty of Science, Golestan University, Gorgan, Iran

The genus Salvia comprises nearly 900 species and is one of the largest members of the lamiaceae family. Fifty eight species of this genus are found in Iran, 17 species of them are endemic. In this study, the roots of Salvia hypoleuca Benth., were collected at flowering stage from Tehran province (Iran) and dried at room temperature, in shade. Dried plant materials were cut into small pieces and extracted with ethyl acetate by percolation method. Three sterols, sitosterol, sitosterol and stigmasterol, two diterpenoids, manool and 7α-acetoxy royleanone and five triterpenoids, ursolic acid, oleanolic acid, 3-epi- maslinic acid and coleonolic acid, were isolated and purified by column chromatography (silicagels normal and reverse phases, Sephadex LH20). The structures of these compounds were identified by spectroscopic methods including 1H-NMR, 13C-NMR, DEPT, HSQC, HMBC and H-H correlation analysis. These compounds were reported for the first time from Salvia hypoleuca of which coleonolic acid has not been previously reported from the genus Salvia. Keywords: Salvia hypoleuca, flavonoid, chromatography, spectroscopy Acknowledgement: This research was supported by the Medicinal Plants Research Center, Faculty of Pharmacy, Tehran University of Medical Sciences.

PG83
Phytochemical study of Lagochilus cabulicus Benth.
Gohari A1, Barati E2, Saeidnia S1, Shakeri A2, Motaghedi E3
1Medicinal Plants Research Center, Faculty of Pharmacy, Tehran University of Medical Sciences, PO Box 14155-6313, Tehran, Iran; 2Department of Chemistry, Faculty of cience, Golestan University, Gorgan, Iran; 3Department of Mechanical Engineering, Tabriz University, Tabriz, Iran

The genus, Lagochilus, belongs to Lamiaceae family and consists of 44 species all over the world, 33 of which grow in central Asia. Five species of this genus have been found in Flora Iranica and 4 species exclusively grow in Iran. Chemical studies on some Lagochilus species have studied [3 – 12]. One of these species, Lagochilus cabulicus Benth., was collected during flowering stage, dried at ambient temperature and shade, then powdered and cut into small pieces. Plant material was successively extracted with ethyl acetate and methanol solvents using percolation method. Main compounds were separated and isolated by column and thin layer chromatography. The isolated compounds were identified by spectroscopic methods, including 1H-NMR and 13C-NMR. In conclusion, four flavonoids, Tricetin 3′-methyl ether (1), Quercetin 3-O-L-rhamnopyranosyl (1′)-D-glucopyranoside (2), Quercetin 3-O-L-rhamnopyranosyl 1′(1β)-D-glucopyranoside (3), two steroids, β-Sitosterol acetate (5), Stigmasteryl acetate (6) and one triterpenoid, Luisop (7), have been identified, which were not previously reported from this plant species.

PG84
Unusual flavones in Cytisus Desf.
Pereira OR1, Domingues MR2, Silva AM2, Cardoso SM2, Pereira OR2
1CERNAS- Escola Superior Agrária, Instituto Politécnico de Coimbra, Bencanta, 3040 – 316 Coimbra, Portugal; 2Department of Química & QOPNA, Universidade de Aveiro, 3810 – 193 Aveiro, Portugal; 3CIMO-EScola Superior Agrária, Instituto Politécnico de Bragança, Campus de Sta. Apolónia, 5301 – 855 Bragança, Portugal; 4CIMO-EScola Superior Agrária, Instituto Politécnico de Bragança, Campus de Sta. Apolónia, 5301 – 855 Bragança, Portugal; 5CIMO-EScola Superior Agrária, Instituto Politécnico de Coimbra, Bencanta, 3040 – 316 Coimbra, Portugal; 6CIMO-EScola Superior de Saúde, Instituto Politécnico de Bragança, Av. D. Afonso V, 5300 – 121 Bragança, Portugal; 7Centro de Espectrometria de Massa, Departamento de Química, Universidade de Aveiro, 3810 – 193 Aveiro, Portugal

Cytisus Desf. (Fabaceae) is a diversified genus closing approximately sixty species, which are particularly found around the Mediterranean Sea. Many plants of this genus exhibit bioactive properties such as diuretic, hypotonic, antispasmodic, anti-diabetic and antioxidant [1] and, in particular the latter, has been closely associated to the high content in flavonoids [2]. The present work aims to contribute to the knowledge of Cytisus chemical composition, through the identification of new flavonoids in that genus. The compounds in focus were detected in ethanol extract of Cytisus multiflorus (Aiton) Sweet flowers by means of HPLC-DAD, ESI-MS and MS collisional analyses. These included the two isomers 2′-O-pentosyl-6-C-hexosyl-luteolin and 2′-O-pentosyl-8-C-hexosyl-luteolin (MW 580 Da), the two isomers 2′-O-pentosyl-6-C-hexosyl-apigenin and 2′-O-pentosyl-8-C-hexosyl-apigenin (MW 564 Da), the 6′-O-(3-hydroxy-3-methylglutaryl) -2′-O-pentosyl-6-C-hexosyl-luteolin (MW 724 Da) and the 6′-O-(3-hydroxy-3-methylglutaryl) -2′-O-pentosyl-6-C-hexosyl-apigenin (MW 708 Da). Attending that half of these compounds were herein described for first time in Fabaceae, overall, the present work is a valuable contribution for the phenolic elucidation of Cytisus genus as well as of Fabaceae family.
The genus Stachys (lamiaceae), consists about 200–300 species widespread throughout the world (1). In Iran, 34 species of this genus are present, including Stachys lavandulifolia Vahl (2). This species widely distributed in different regions of Iran and is known as the names of “Tuklidjeh” and “Chaaye Koohi”. In Iranian folk medicine, decoction of aerial parts of S. lavandulifolia is used in painful and inflammatory gastrointestinal disorders (3), anxiolytic and sedative effects of this species also are known in traditional medicine when use as tea (4), in previous phytochemical studies two phenylethanoid glycosides; Acteoside and Lavandulifolioside have been reported from this plant (5). In continuation of our phytochemical studies on medicinal plants from Iran we now report three known phenylethanoid glycosides; Acteoside, Lavandulifolioside have been reported from this plant (5). In continuation of our phytochemical studies in medicinal plants from Iran we now report five known phenylethanoid glycosides; Acteoside, Lavandulifolioside, leucosceptoside A and two new compounds; 4, 3’,4’ trimethoxy...
were performed for guiding of the bioactivity. V. chaenadry L. was found to be the most bioactive species, followed by V. serpillyfolia. V. fuhski Frey & Sint. was found to be the least active species. Thin layer chromatographies of their water extracts showed V. chaenadry to contain a large proportion of phenylethanoid glycosides, the remaining species showed the presence of a large proportion of flavonoid glycosides. Chromatography of V. serpillyfolia water extract gave five pure compounds. Their structures were determined as iridoid glucosides verproside, cat- aromatic, veronosides and flavonoid glycosides 4-O-methylapigenin-7-O-rhamnopyranosyl-acetylglucopyranoside, 3′-O-methyltulitin-7-O-rhamnopyranosyl-acetylglucopyranoside using different 1D and 2D NMR techniques. Isolation and structure determination studies on bioactive compounds of genus Veronica are still continuing. Acknowledgement: This study was supported by The Scientific and Technological Research Council of Turkey (TUBITAK) Project No: 108T518. References:

High-speed countercurrent chromatography of *Harpagophyllum procumbens* constituents and their identification by TLC-MS

Mcnwangi N1, Vermaat K1, Viljoen A1, Marston A1, 1Department of Pharmaceutical Sciences, Tshwane University of Technology, Pretoria, South Africa; 2Department of Chemistry, University of the Free State, Bloemfontein, South Africa

Harpagophyllum procumbens DC. (Pedalaceae), known as Devil’s claw, is native to the arid regions of Southern Africa including the Kalahari desert. The dried secondary root tubers have been used to reduce pain and inflammation especially in rheumatism and arthritis [1]. Iridoid glycosides are considered to be the main pharmacologically active constituents with other constituents such as phenylethanoid glycosides and flavonoids contributing to the effect [2]. Rapid isolation and identification of the constituents was necessary in order to acquire sufficient quantities of the reference compounds for use in further biological studies as well as to develop quantitative calibration models. To achieve these goals, a methanol extract of the secondary root tubers was rapidly filtered over silica gel to remove sugars and other polar compounds. The resulting fraction, which consisted mainly of iridoid and phenylpropa- noid glycosides, was subjected to high-speed countercurrent chromatography (HSCCC). This allowed a one-step separation of the major constituents. The minor constituents were obtained either by a second HSCCC operation or by a final column chromatographic step. In order to distinguish close-running compounds in the absence of reference standards, TLC-MS [3] was performed on the extract and the isolated constituents. This method could be used, for example, to distinguish the close-eluting pair 8-8-p-coumarosylylharpagide and 8-8-p-feruloylharpagide [2]. Acknowledgement: The authors thank the National Research Foundation of South Africa for financing this study. References: 1. Qij et al. (2006) Phytochemistry 67: 1372 – 1377. 2. Karioti A et al. (2011) Pharm Biomed Anal 55: 479 – 486. 3. Reich E, Widmer V (2009) Planta Med 75: 711 – 718.

Flavonoid constituents from *Morettia philaeana* and their antimicrobial activity

Marzouk MM, Hussien SR, Kawashyty SA, Ibrahim LF, 1Pharmacy and Plant Systematics Department, National Research Centre, Dokki, Giza, Egypt

A successive petroleum ether, diethyl ether, ethyl acetate and methanol extracts of *Morettia philaeana* (Delile) DC. (Cruciferae) flower- ing aerial parts were tested for their antimicrobial activity. The ethyl acetate and methanol extracts were found to be most effective against most of the tested organisms. The chemical investigation of these extracts afforded nine flavonoids using chromatographic techniques. These are kaempferol, kaempferol 3-O-p-glucoside, kaempferol 3,7 di-O-p-glucoside, kaempferol 3-O-sophoroside-7-O-p-glucoside, quercetin, quercetin 3-O-p-glucoside, quercetin 3-O-p-gentobioside, orientin and isoorientin. Their structures were established through chemical and spectral analysis. All flavonoids were evaluated to show a broad antimicrobial spectrum of activity on microorganisms including seven bacterial and two fungal species. Among them, the isolated aglycones had stronger bioactivity than their glycosides. Acknowledgement: This work was financially supported by the Phytochemistry and Plant Systematic Department, National Research Centre, Giza, Egypt.

New hexadecan-2-ol and 3-hydroxyhexadecane-2-ol with hepatoprotective Activity and Cytotoxicity Activity from *Grindelia camporum* Greene (Asteraceae)

El Moghazy AM1, Darwish EM2, El Khayat ES2, Mohamed MO2, Wink M2, El Readi MZ2, 1Pharmacognosy Dept., Faculty of Pharmacy, Assiut University, Assiut, Egypt; 2Pharmacognosy Dept., Faculty of Pharmacy, Al-Azhar University, Assiut, Egypt;

New hexadecan-2-ol and 3-hydroxyhexadecane-2-ol (10), together with 14 known compounds (1 – 7, 9, 11 – 16) have been isolated from *Grindelia camporum* Greene var. camporum. The new compounds were characterized through spectroscopic studies including 1D (1H and 13C NMR) and 2D (COSY, HSQC and HMBC) NMR and mass spectroscopy. The known compounds (Compounds 3 and 4 are hitherto unreported from the family Asteraceae) were identified by comparison of their spectral data with those reported in the literature, chemical evidence or authentic samples [1 – 3]. The total methanolic extract and the total aqueous extract exhibited cytotoxic and hepatoprotective activities respectively. Also antimicrobial, toxicological (LD50, anti-inflammatory, antipyretic and analgesic activities of the different fractions were studied [4 – 6]. References: 1. Pande A et al. (1995) Phytochemistry 39(3): 709 – 711. 2. Ageta H et al. (1995) Chem Pharm Bull 43(2): 198 – 203. 3. Chiu P et al. (1985) Phytochemistry 24(2): 263 – 266. 4. Ashour ML et al. (2009) Journal of Pharmacy and Pharmacology 61: 1079 – 1087. 5. Biignano G et al. (1994) Pharm Biol 32: 400 – 405. 6. Achliya GS et al. (2003) Ind J Pharmacol 35: 308 – 311.

Antiplasmodial and antityrososomal triterpenoids from *Salvia hydrangea* with rare carbon skeletons

Moridi Farimani M1, Bahadori B1, Taheri S2, Nejad Ebrahimi S3, Hamburger M3, 1Medicinal Plants and Drugs Research Institute, Shahid Beheshti University, G. C., Envin, Tehran, Iran; 2Laboratory of Organic Synthesis, Natural Products Department of Chemistry, Sharif University of Technology, Tehran, Iran; 3Division of Pharmaceutical Biology, Department of Pharmaceutical Sciences, University of Basel, Klingelbergstrasse 50, CH-4056 Basel

*Salvia hydrangea* DC. ex Bentham, endemic to Iran, has been widely used in traditional Iranian medicine. Fractionation of the hexane extract of the aerial parts of this plant led to the isolation of hydrangodine-A (1) and hydrangodine-B (2), two new triterpenoids with rare carbon skeletons. Their structures were established on the basis of an extensive spectroscopic analysis, including 1D and 2D NMR, and by comparison of their NMR data with those of the related compounds. The IC50 of the compound 1 and 2 were determined against two parasites and rat myo- blast (L6) cells. Hydrangodione-A (1) and hydrangodione-B (2) exhibited in vitro antimalarial activity against P. falciparum K1 strains with IC50 value 1.43 and 0.18 μM with great selectivity index (SI) 86.2 and 69.6. Also these compounds were tested against T. brucei rhodesiense STIB 900, they exhibited significant inhibition of growth with IC50 values of 4.33 and 15.92 μM. Triterpenoids with these carbon skeletons are rare in the nature and have been previously reported only from two other species: *Salvia bucharica* Popov [1] and *Perovskia abrotanoides* Kar. [2]. It is interesting to note that all three species belong to the flora of Iran and the genus *Perovskia* is a closely related to the genus *Salvia*. In a suggested proposal for the biosynthesis of hydrangodine A and B they are presumed to be synthesized from the addition of a phenylpropanoid unit (myrecene for hydrangodine A and trans-β-ocimene for hydrangodine B) to a diterpenic unit (an icetexone precursor) and this coupling may proceed via Diels-Alder type reaction.
Eight xanthones as inhibitors of monoamine oxidase have been isolated from dichloromethane extract of aerial part and roots of Polyspora supina Schreb. The isolated xanthones were characterized by spectrocscopic methods such as 1D, 2D NMR, and MS data analyses as 6,8-Dihydroxy-2,3,4-trimethoxyxanthone (1), 2,4,6,8-Tetrahydroxy-3,7-dimethoxymethoxanethone (2), 2,3,7-Trimethoxyxanthone (3), 3,7-Dihydroxy-1,2-dimethoxyxanthone (4), 1,3,6-Trihydroxy-2,7-dimethoxyxanthone (5), 1,3,5-Trihydroxy-2,6,7-trimethoxyxanthone (6). The activity of the isolated xanthones (1 – 8) from dichloromethane extract was assayed according to the method of Holt et al. Compound 5 showed the best activity with IC50 value of 0.24 mM, also 1 and 4 showed good activity with IC50 values of 2.12 mM and 3.64 mM, respectively. The compounds 2, 6, and 7 showed mild activity with IC50 values of 12.21, 43.80, and 23.96 mM. However, compound 3 and 8 were not active. References: 1. Holt A et al. (1997) Analytical Biochemistry 244: 384 – 392.

**New N-alkylamides from Anacysus pyrethrum**

Boonen J, Sharma V, Dixit V, de Spiegeler B

*Drug Quality and Registration (DruQuaR) group, Department of Pharmaceutical Research and Development, Ghent University, Ghent, Belgium; Department of Pharmaceutical Sciences, Dr. H.S. Goar University, Sagar, India*

The roots of Anacysus pyrethrum DC (Asteraceae) are frequently used in traditional medicine e.g. as aphrodisiac [1]. Depending on the extraction method and solvent, different yields of N-alkylamide constituents can be found, possibly resulting in alterations in biological effects and toxicity. Therefore, analytical profiling of the bio-active N-alkylamides in these plant preparations is an inevitable quality parameter, with liquid chromatography-electrospray mass spectrometry (HPLC/ESI-MS) as recommended technique for comprehensive analysis of alkylamides in plant extracts [2 – 4]. An N-alkylamide profiling from an ethanolic Anacysus pyrethrum root extract was performed using a gradient reversed phase HPLC/ESI-MS method on an embedded polar column. MS1 and MS2 fragmentation data were used for identification purposes, while UV was used for quantification. Thirteen N-alkylamides (five N-isobutyalamides, four N-methyl isobutyalamides, four pyrrolidines and one 2-phe-nylethylamide) were detected. Five of them are novel compounds, which have never been identified in nature. Acknowledgement: Institute for the Promotion of Innovation through Science and Technology in Flanders (IWT-Vlaanderen) (no. 091257) and the All India Council for Technical Education, New Delhi, India. References: 1. Sharma V, Thakur M, Chauhan N, Dixit V (2010) Planta Med 76: 1214 – 1214. 2. Sharma V, Boonen J, Chauhan N, Thakur M, de Spiegeler B, Dixit V (2011) Phytochemistry, in press. 3. Kartal M, Kan, Gulpinar AR (2007) Planta Med 73: 253. 4. Boonen J et al. (2010) Pharmaceut Biomed 53: 243 – 249.

**Hydrandione C, a novel triterpenoid with an unprecedented skeleton from Salvia hydrangea DC, ex Benth**

Morigi Farimani M, Bahadori B, Taberi S

*Department of Phytochemistry, Medicinal Plants and Drugs Research Institute, Shahid Beheshti University, G. C., Tehran, Iran; Department of Chemistry, Sharif University of Technology, Tehran, Iran*

The genus Salvia is a rich source of terpenoids with structural diversity. Apart from sesquiterpenoids as unusual constituents of Salvia species [12], it is a source of di- and triterpenoids with unprecedented carbon skeletons [3]. Aiming at identifying structurally interesting and bioactive metabolites from the Salvia species, we examined the extract of Salvia hydrangea DC, ex Benth. In our search for new bioactive natural products, a novel triterpenoid (hydrandione C, 1) was isolated from the hexane extract of this plant. The skeletal type displayed by hydrandione C was noticeable for its unusual carbon ring skeleton with a unique five-membered ring D substituted by an acetyl group. This is the first report of a natural triterpenoid with a five-membered ring D. The structure of 1 was established by comprehensive 1D NMR, 2D NMR, and HRMS spectroscopic analysis and subsequently confirmed by a single-crystal X-ray diffraction study.

**Figure 1:** Hydrandione C

to the *Hedysosnum brasiliense*–derived substances kept growing but were sensitive to isoniazid, an antibacterial agent.

Acknowledgement: The authors are grateful to CNPq and CAPES for financial support, and Norberto P. Lopes and José C. Tomaz (FCFRP-USP) for the antimicrobial assay.

**Figure 1:** Isolated compounds of *Hedysosnum brasiliense*

**Figure 2:** Key NOE correlations observed for compounds 4 and 5

Chemical diversity investigation of *Stemona* species using LC-MS technologies

Tang C \(^1\), Ke C \(^1\), Zhou S \(^1\), Peng S \(^1\), Ge F \(^1\), Lin G \(^2\), Ye Y \(^1\)

\(^1\)Natural Products Chemistry Department, & State Key Laboratory of Drug Research, & SIMM/CUHK Joint Laboratory for Promoting Globalization of Traditional Chinese Medicines, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, 555 Zu-Chong-Zhi Road, Zhangjiang High-Tech Park, Shanghai 201203, P. R. China; \(^2\)School of Biomedical Sciences, Faculty of Medicine, & SIMM/CUHK Joint Laboratory for Promoting Globalization of Traditional Chinese Medicines, The Chinese University of Hong Kong, Hong Kong SAR, P. R. China

*Stemona* species (Stemonaceae) are plant resources of traditional Chinese medicine ‘baibu’, which had long been used as antitussive and insecticidal agents. *Stemona* alkaloids, featuring a pyrrolo[1,2-c]azepine or pyrrol[1,2-c]azepine nucleus, are believed to be responsible for their medicinal usages. Our previous work has led to the isolation of 90-plus alkaloids from different *Stemona* species. In our effort to identify alkaloidal constituents from the extract of a Vietnamese species, an LC-MS method was established for a rapid and sensitive screening of the specific compounds. On the basis of more than 90 alkaloids, we established a MS database of all these reference compounds by UPLC/ESI-LTQ. By picking the specific peak in the LC chromatogram, extracting its MS, MS\(^2\) and MS\(^3\) spectra, and then comparing with those of the standard samples, we can do a rapid identification of main structures in the extract. Such technology was successfully applied for the chemical diversity investigation of three *Stemona* species – *S. sessilifolia* Franch. & Sav., *S. japonica* Franch. & Sav., and *S. tuberosa* Lour. The results revealed that the alkaloids varied greatly with species and habitats, but not with collecting seasons. *S. tuberosa* is the commonly-used species while having the most complicated metabolites which structural types were influenced extremely by ecological environment. *S. sessilifolia* growing in Tsuchou, Anhui province with the highest amount of the most active stemosporinone, was determined to be the best species for medicinal usage. All these data, combined with the pharmacological experiments, supplied the scientific evidence for guiding the usage of TCM baibu.

**Keywords:** the scientific evidence for guiding the usage of TCM baibu.


**Figure 1:** Isolated compounds of *Hedysosnum brasiliense*
The fungal isolate Penicillium brevicompactum which isolated from the associated marine alga Perrocladia sp. in autumn season was able to produce 11 clear and active compounds, separated by the best solvent system dichloromethane: methanol (95:5 v/v). Compounds 4 and 9 were considered as antibacterial compounds, active against Gram positive (B. subtilis) and Gram negative (E. coli) bacteria. Malt extract broth medium with initial pH 4 when incubated at 28°C in an incubator shaker at 200 rpm for 12 days were the most favorable conditions for compound 4 production (19.87 mg/l). The suitable conditions for compound 9 production (121.15 mg/l) were potato carrot broth medium, initial pH 4, incubation temperature 26°C at 180 rpm after incubation period for 10 days. Structural elucidation of the pure compounds suggested that compound 4 may be [Di(2-ethyl hexyl) phthalate], and compound 9 may be fungisterol or one of its isomers. Pure compounds were evaluated for cytotoxicity towards 6 different types of tumor cell lines performed in Cancer Biology Department, National Cancer Institute, Cairo, Egypt. The results revealed that, the maximum concentration of compound 4 (100 μg/ml) kills about 30% of lung cells. The maximum concentration of compound 9 (100 μg/ml) kills approximately 40% of the viable infected liver cells and also kills approximately 50% of the viable infected lung cells at concentration equal to 91.6 μg/ml. It can be concluded that compound 9 can be recommended as an anticancer compound.

Multiple resistance and environmental pollution to chemical pesticides with increasing world population led to the development and the improvement of new trends to pesticides from natural product. The application of natural product as well as the biological control specific agents especially nucleopolyphero virus (NPV) are considered very important tool to avoid the contradiction between pest control and clean environment. Furthermore, the biological control specific agents have many advantages such as their low mammalian toxicity and no adverse effect on plant growth and seed viability. The brown alga Sargassum species (Family: Sargassaceae) were widely distributed in worldwide. They have been used as food, as well as in industry and medicine for various purpose. Pharmacopeial constants, phytochemical screening, determination of minerals and trace elements of Sargassum asperifolium, Sargassum dentiformi, Sargassum linifolium from the Red Sea, Hurghada, Egypt, were investigated. These studies revealed that Sargassum species have high ash content and gave positive for sublimmable matter, volatile constituents, carbohydrate content, starch and/or triterpenes. In addition, they have high Ca, K, Mg & Fe contents. The protein content as well as amino acid composition of three algae were performed using Kjeldal method and amino acid analyzer, respectively. The alcoholic extracts (70%) of Sargassum species under investigation were subjected to screen their insecticidal activities in vitro and in vivo on Spodoptera littoralis and Spodoptera fungipende and their effects on the replication of Spodoptera littoralis nucleopolyphero virus and Spodoptera fungipende nucleopolyphero virus. The evaluation proved that the tested algae have various insecticidal and antiviral activities.
Iriomoteolide-12a, a 12-membered macrolide from dinoflagellate Amphidinium species, was obtained as a new compound, iriomoteolide-12a. Iriomoteolide-12a was obtained as a new 12-membered macrolide with six one-carbon branches, two ketone carbonyls, and a hydroxyl group. The skeleton is related to that of amphidinolide Q [1,2]. Detailed analyses of 2D NMR data and MS spectra. Iriomoteolide-12a is a new 12-membered macrolide with three carbonyl carbons, eight methine carbons, five methylene carbons, and a total of 25 carbon atoms due to five quaternary carbons, one of which is a halogen atom. The dichloromethane/methanol extract which exhibited the most significant activity was therefore subjected to column gradient chromatography on silica gel and led to one hundred thirty seven fractions. Active compounds showed weak value of CMI. 1 H NMR and 13C NMR provided structural information about active compounds.

Marine dinoflagellates of the genus Amphidinium are well-known as a producer of unique cytotoxic metabolites. We have isolated a new 12-membered macrolide, iriomoteolide-12a, from the benthic dinoflagellate Amphidinium species collected off Iriomote Island, Japan. In this symposium, we will discuss the isolation and structural elucidation of this new macrolide. The dinoflagellate Amphidinium sp. (strain KCA009053) was cultivated in 50 L of 1% Provasoli’s enriched seawater (PES) medium, 16 h light and 8 h dark. The algal cells obtained from 50 L of the medium were harvested with MeOH/toluene (3:1). The toluene-soluble materials of the extract were subjected to a SiO2 column, C18 column and one of the macrolide fractions containing compounds was separated by C18 HPLC to afford a new compound, iriomoteolide-12a. Iriomoteolide-12a was obtained as a colorless amorphous solid, and the molecular formula of C214HznC231 A et al. (2001) Tetrahedron 47: 2273 – 2276

Constituents of the Red Alga Laurencia obtusa

The genus Laurencia Lamouroux (Rhodomelaceae) includes about 140 species distributed worldwide. The red algal genus Laurencia (Rhodomelaceae) is known as a rich source of the halogenated sesquiterpenoids, diterpenes and acetylenes [2,3]. Although a number of studies have been done on L. obtusa, investigations are still going on this species due to high biodiversity of its constituents. In our continuing research on secondary metabolites of the alga L. obtusa Lamouroux, which has different colors in different regions of Turkey and at different times, we have obtained several halogenated sesquiterpenoids [4,5]. In this study, a sample of L. obtusa, collected from North eastern part of Aegean Sea (Bademli- Ayvalik) in Turkey and an extract was obtained by exhaustively in chloroform-methanol (1:1) and the solvent mixture was fractionated. Fractionation of the extract on a Si-gel column carried out by the elution starting petroleum ether, and gradients used were first dichloromethane, and then acetone, finally methanol with increasing amounts. The fraction, obtained by elution with the petroleum ether-dichloromethane mixture (6:4) afforded two halogenated compounds, their 1H NMR spectra indicated that they are probably sesquiterpene skeleton. Another compound was obtained during elution with the dichloromethane-acetone solvent mixture (8:2). Structure elucidation studies are still going on using by intensive NMR and mass spectral analyses. There are a number of compounds which have not purified yet. After purification and structure elucidation studies, the pure compounds will be investigated for their potential bioactivity including cytotoxic and anti-cholinesterase activity tests. Refer- ences: [1] Rodriguez MCG et al. (1992) Bot Mar 35: 227 – 237 [2] Faulkner D J (1999) Nat Prod Rep16: 153 – 198 [3] Scheuer PJ (1989) Med Res Rev 9: 535 – 545 [4] Topçu et al. (2003) Nat Prod Res: 1505 – 1508 [5] Öztnçu A et al. (2001) Tetrahedron 47: 2273 – 2276

Antimicrobial active compounds of green alga Ulva rigida collected from Ghar El Melh lagoon (North of Tunisia)

Ismail Ben Ali A1, Karlé L1, El Boum M1, Boudabbous A2
1National Institute of Marine Sciences and Technologies, Salammbô, Tunisia; 2Faculty of Mathematical, Physical and Natural Sciences of Tunis, Tunis, Tunisia

The green alga Ulva rigida is wellspread within Tunisian coast mainly in the northern region of Ghar El Melh lagoon with important blooms particularly in warm seasons [1]. The aim of this study is to evaluate its antimicrobial potential against pathogens bacteria and fungi. Thus, polar and non polar organic crude extracts of dried Ulva rigida collected from Ghar El Melh lagoon (37° 10.8’ N, 10° 16.6’ E), were tested against eighteen pathogenic species of bacteria and the yeast Candida albicans. Marine epiphytic bacteria (72 strains) were isolated from green alga Ulva rigida collected in two different biotopes (Cap Zebib, rocky shore: 30 strains and Ghar El Melh lagoon: 34 strains) and alga surrounding water (eight strains). All isolates were identified based on their 16S rDNA sequences and tested for antimicrobial effect against several human and fish pathogens (18 Gram+ and Gram- bacteria and the fungus Candida albicans) using in vitro drop method. Results obtained revealed high activities of Ulva rigida epiphytic bacteria; amongst alga isolates 36% were active with variable antimicrobial spectrum. Within active isolates, 69.5% were from the alga collected in the lagoon. High level activities of the isolates were observed for six isolates identified to Bacteroidetes bacterium, Pseudoalteromonas sp., Octadecabacter sp., Stappia marina, Stappia sp. and Ruegeria sp. Nevertheless, all free living bacteria from surrounding water were inactive. Else, we noted variable sensitivity spectrum of indicators used, Staphylococcus aureus, Micrococcus sp., Streptococcus sp. and Salmonella typhimurium were mostly inhibited by the isolates tested, while, Vibrio species (V. anguillarum, V. tapetis and V. alginolyticus) were resistant. Candida albicans was inhibited only by the two Stappia species isolated from Ulva rigida of Cap Zebib locality. Further investigations continue on the inhibition effect of Ulva rigida organic crude extracts against epiphytic bacteria isolated, in order to assess degree of affinity of epibionts to their proper host. Keywords: Ulva rigida, Epiphytic bacteria, Antimicrobial activities Acknowledgement: The authors thank Ms. Veronique Conforius-Guns, Department of Marine Microbiology, Netherlands Institute of Ecology, NIOO-KNAW, Yerseke, The Netherlands, for her assistance and help with PCR and DNA sequencing.
The red seaweed _Delesseria sanguinea_ dominantly populates a large artificial reef at Nienhagen in the Baltic Sea. It contains substantial amounts of sulfated polysaccharides (D.s.-SP), which consist of a homogenous fraction of branched sulfated xyloglucans (gal-xyl -5.4) and exhibit a pharmacological profile indicating anti-inflammatory and anti-skin aging potencies [1 - 3]. Compared with heparin, D.s.-SP revealed stronger inhibitory effects on the enzymes elastase, hyaluronidase, heparanase, collagenase as well as on complement activation, cell adhesion to P-selectin and cytokine release from LPS-activated monocytes, but have only moderate anticoagulant activity. Their hyaluronidase and complement inhibitory activities proved even superior than those of the anti-inflammatory β-1,3-glucan sulfated PS 3. Crucial for an economic use is the availability of adequate amounts of D.s.-SP with reproducible high quality. For evaluation and optimization, 30 D.s.-SP batches were harvested and extracted since 2005 resulting in almost 200 D.s.-SP batches. By a standardized procedure (extraction (EX) with water for 8 h at 85 °C), the D.s.-SP can be isolated in reproducible high quality. However, as found by a second 8 h-EX, the first 8 h-EX is incomplete. Subsequently modified EX-procedures led to following yields: 8.8%(1 8 h-EX), 13.3 %(2 4 h-EX), 15.0%(2 2 h-EX) and 17.9%(4 2 h-EX). Consequently, a 2 h-EX (15.0%) seems to be a rational compromise. Moreover, the D.s.-SP obtained by shorter EX contained less glucose, which partly represents co-extracted starch: 14.4%(1 8 h-EX), 10.92%(2 4 h-EX), 9.0%(2 h-EX) and 11.74%(4 2 h-EX). The glucose content was further reduced by precipitating the extracted D.s.-SP with 70% instead of 90% ethanol. In conclusion, after stepwise optimization of the isolation procedure, the D.s.-SP from Nienhagen are ready for an industrial application, the D.s.-SP from Nienhagen are ready for an industrial application. Consequently, three cDNA clones, plant oxidases. Then, the 3’ and 5’-end regions of cDNA were obtained by rapid amplifications of cDNA ends (RACE) with degenerate primers designed from conserved sequences in cannabinoid synthases and related plant oxidases. Then, the 3’ and 5’-end regions of cDNA were obtained by rapid amplifications of cDNA ends. Consequently, three cDNA clones, that encode polypeptides named RdOx 1 -3, were cloned. RdOx 1-3 consisted of 553, 533 and 534 amino acids containing a EAD binding motif. In addition, these polypeptides had > 50% identities with cannabinoid synthases. The heterologous expression system for RDox was established using _Pichia pastoris_ as a host. The recombinant RdOx1 and 2 could produce DCA from grifolic acid, whereas RdOx3 showed no DCA-producing activity, suggesting that RdOx 1 and 2 are active DCA synthase in _R. dauricum_. DCA synthase would be applied for biotechnological production of DCA because the substrate grifolic acid has been isolated from a mushroom _Albatrellus dispassus_ in a large amount [3].

**Figure 1:** The reaction catalyzed by daurichromenic acid synthase

**Table 1: Molecular Biology**

**PI1**

**Molecular characterization of daurichromenic acid synthase from Rhododendron dauricum**

_Taura F_, _Hashimoto T_, _Asakawa Y_

*Kyushu University, Kyushu, Japan; *Tokushima Bunri University, Tokushima, Japan

_Daucus carota_ is one of the most economically important and the most popular vegetables cultivated worldwide among the members

**PI2**

**Development of new genomic and genic SSR primer pairs for carrot**

_Ince AG_, _Karaca M_

_Akdeniz University, Faculty of Agriculture, 07059 Antalya, Turkey

Carrot ( _Daucus carota L_ ) is one of the most economically important and the most popular vegetables cultivated worldwide among the members.
of family Apiaceae. Despite its importance for human nutrition, health, and development of new drugs, genic resources in carrot relatively underdeveloped and the use of molecular markers in carrot has limited to a few results of several researches [1]. Among the molecular markers microsatellites or simple sequence repeat (SSR) has much superiority in genetic studies since they are co-dominant, highly polymorphic, and reliable PCR procedure [2,3,4]. But, the number of microsatellite primer pairs flanking the microsatellites in ESTs and genomic DNA library limited in carrot. In order to utilize microsatellites in carrot genetic studies, new microsatellite primer pairs are required. To date at the NCBI, 3845 nucleotide sequences and 93 expressed sequence tag (EST) sequences are available for all Daucus species (March 2011). We developed 14 microsatellite primer pairs using ESTs and genomic DNA library data bases in the NCBI databases. Microsatellites were determined using Exact-Tandem Repeat Analysis program and primer pairs flanking the microsatellites were designed using Primer3 software [5,6]. Microsatellite primer pairs developed in the present study (Table 1) will enhance genetic studies in carrot. Besides, transferability of these microsatellite primer pairs from carrot to other members of the Apiaceae family is important for future genetic studies in the Apiaceae family. Acknowledgement: This research is supported by the Scientific Research Projects Coordination Unit of Akdeniz University. References: 1. Cavagnara PF et al. (2009) Mol Genet Genomics 281: 273 – 288. 2. Karaca M, Ince AG (2008) J Genet 87: 83 – 86. 3. Ince AG et al. (2010) Mol Breeding 25: 645 – 658. 4. Ince AG et al. (2010) Mol Breeding 25: 491 – 499. 5. Ince AG et al. (2008) Plant Cell Tissue Organ Cult. 94: 281 – 290. 6. Ince AG et al. (2010) Mol Breeding 25: 645 – 658. 4. Ince AG et al. (2010) Mol Breeding 25: 491 – 499. 5. Ince AG et al. (2008) Plant Cell Tissue Organ Cult. 94: 281 – 290. 6. Ince AG et al. (2010) Mol Breeding 25: 491 – 499.

Transferability of EST-Microsatellite Markers to some Labiatae Genera

Ince AG1, Karaca M1, Ay S2

1Akdeniz University, Faculty of Agriculture, 07059 Antalya, Turkey
2West Akdeniz Agricultural Research Institute, 07110 Antalya, Turkey

In recent years, molecular markers have been used in identification and differentiation of chemical compositions in some aromatic species [1,2]. Among the SSRs or microsatellites are the marker of choice, however, the development of microsatellite markers is often a laborious and costly process since the construction of a DNA library and screening of the library with probes corresponds to the repetitive sequences required. Fortunately after the discovery of microsatellites in 1985, EST microsatellites became an important source for development of new microsatellite markers [4]. Many studies indicated that unlike genomic microsatellites, genic ones could amplify genomic regions of related genera [5,6]. Based on these findings this study developed one hundred microsatellite primer pairs using a total of 13641 ESTs from Origanum, Salvia, Stemonitis, and Thymus species. A total of 20 primer pairs obtained from Origanum ESTs were used to investigate transferability of EST based microsatellites to Salvia, Sideritis, Melissa, Teucrium, Rosmarinus, Thymus, Pholomis and Capsicum. All 20 primer pairs amplified genomic DNA of Origanum. Three of the 20 microsatellite primer pairs were failed to amplify genomic DNAs of the species tested. Analyses indicated that the level of transferability of Origanum EST microsatellite markers were considerable higher in species belonging to Labiatae genera [5,6]. Based on these findings this study developed one hundred microsatellite primer pairs using ESTs and genomic DNA library data bases in the NCBI databases. Microsatellites were determined using Exact-Tandem Repeat Analysis program and primer pairs flanking the microsatellites were designed using Primer3 software [5,6]. Microsatellite primer pairs developed in the present study (Table 1) will enhance genetic studies in carrot. Besides, transferability of these microsatellite primer pairs from carrot to other members of the Apiaceae family is important for future genetic studies in the Apiaceae family. Acknowledgement: This research is supported by the Scientific Research Projects Coordination Unit of Akdeniz University. References: 1. Cavagnara PF et al. (2009) Mol Genet Genomics 281: 273 – 288. 2. Karaca M, Ince AG (2008) J Genet 87: 83 – 86. 3. Ince AG et al. (2010) Mol Breeding 25: 645 – 658. 4. Ince AG et al. (2010) Mol Breeding 25: 491 – 499. 5. Ince AG et al. (2008) Plant Cell Tissue Organ Cult. 94: 281 – 290. 6. Ince AG et al. (2010) Mol Breeding 25: 491 – 499.

The effect of silicon on membrane integrity and antioxidative pigments on Echium amoenum that exposed to cadmium stress

Amin F1, Enteshari S2, Delavary K3

1Tibology Department, Payame Noor University, 19395 – 4697-Tehran, Iran; 2Islamic Azad University, Ashtian Branch, Department of Biology, Arak, Iran

Some researchers reported that silicon (Si) increase tolerance in some higher plants against biotic and abiotic stress. The beneficial effects of Si are mainly associated with its high deposition in plant tissue and enhancing their strength and rigidity. We investigated the role of Si against cadmium stress in (Echium amoenum Fisch. & C.A.Mey.) in greenhouse condition. When the seventh leaf appeared, plants were pretreated with five levels of Si: 0.0, 0.5, 0.7 and 1.5 mM Si (as sodium silicate, Na2(SiO3)) and then the plants were treated with two levels of Cd (30 and 90 mM). The effects of Silicon and Cd were investigated on some physiological and biochemical parameters such as: lipid peroxidation (malondialdehyde (MDA) and other aldehydes), anticyanin and flavonoid content. Our results showed that Si significantly increased MDA, other aldehydes, anticyanin and flavonoid content in Echium and silicon offset the negative effect and increased tolerance of Echium against Cd stress. From these results we concluded that Si increase membrane integrity and antioxidative ability in this plant against Cd stress.

Effect of the extracts of Piper cumanense and Piper eriopodon in the behavior of genes involved in oxidation process of the skin

Amao Y1, Acvedo AC2

1National University of Colombia, Pharmacy Department, Bogotá, Colombia
2National University of Colombia, Pharmacy Department, Bogotá, Colombia

Nowadays the role of the genes is a tool of study to achieve an accurate form to avoid in this particular case the skin degeneration. The propose of this study was the method design to evaluate the behavior of certain genes as MMP1, MMP7, MMP9, MMP11, MMP12, COL1A2, COL3A1, H2O2 content significantly decreased but growth parameter and free radicals scavenging compounds increased. We concluded that MeJA increased antioxidative capacity in this plant against Cd as heavy metal stress.

Enhancing effect of Methyl jasmonate on antioxidative capacity of Bumilium persicum under Cadmium stress

Enteshari S1, Delavary K2

1Biotechnology Department, Payame Noor University, 19395 – 4697 Tehran, Iran; 2Islamic Azad University, Ashtian Branch, Department of Biology, Arak, Iran

Methyljasmonate (MeJA) is a compound that used as plant growth regulation and is currently being used in cancer research. Bumilium persicum

B.Fedtsch. is one of important medicinal plants that cultivated widely and has numerous usage in medicine. The present study emphasizes the important of MeJA (0.01 and 0.1 μM) against oxidative stress in this plant that exposed to cadmium (0.30 and 60 Mm CdCl2) stress. Our results show that in plants that only treated with Cd growth parameters, flavonoids, ascorbic acid and total phenol content reduced significantly but protein, MDA and H2O2 content and superoxide dismutase (SOD) activity enhanced significantly. On the other hand, in plants that were pretreated with MeJA and then treated with Cd, MDA and H2O2 content significantly decreased but growth parameter and free radical scavenging compounds increased. We concluded that MeJA increased antioxidative capacity in this plant against Cd as heavy metal stress.
Planta Med 2011; 77: 1229–1472 Georg Thieme Verlag KG Stuttgart · New York · ISSN 0032-0943

Abstracts | 59th International Congress of the GA | 4th–9th September 2011, Antalya, Turkey

Identification of methyl jasmonate-inducible cytchrome P450 s and diterpene cyclase involved in cyclic diterpene biosynthesis in Scoparia dulcis
Yamamura Y, Mizuguchi Y, Emori Y, Inoue S, Kurosaki F
University of Toyama, Graduate School of Medicine & Pharmaceutical Science for Research, Laboratory of Plant Resource Sciences, 2630 Sugitani, Toyama, Toyama 930 – 0194, Japan

Diterpenes including the phytoalexins and phytohormone gibberellins are one of biologically important pharmaceutical sources from natural origin. Scoparia dulcis L. (Scrophulariaceae), a tropical medicinal plant, produce tetracyclic diterpenes such as scopadulcic acid B (SDB) and scopadulcic acid C (SDC). SDB exhibits various pharmacological activities including inhibitory effects on replication of herpes simplex virus type 1 and antitumor. Furthermore, SDB formation in S. dulcis leaf tissue is rapidly and transiently stimulated by addition of methyl jasmonate (MJ) as an elicitor. In order to gain insight into the molecular mechanisms underlying diterpene biosynthesis, we focused on cytochrome P450 (P450) enzymes often appear to form key regulatory steps in plant secondary metabolism. As a first step, we performed differential display analysis of P450 genes induced during elicitation of SDB biosynthesis after MJ addition to S. dulcis leaf tissues. As a result, nine genes were found to be up-regulated and were highly homologous to the corresponding region of P450 CDNA. We further examined the gene expression in the course of induction of SDB synthesis by MJ or Yeast extract. In addition, we also isolated a gene encoding ent-kaurene synthase (KS) which catalyzes the cyclization of copalyl diphosphate to ent-kaurene from S. dulcis and analyzed its function. The KS gene has been duplicated in the S. dulcis genome and is highly expressed in mature leaves. Here, we discuss the physiological roles of these isolated P450s and KS in diterpene biosynthesis in S. dulcis.

Effects of black cohosh (Cimicifuga racemosa) extract on apoptosis and proliferation rates in hMSCs, mCF7, mDA-MB-231 and LNCaP cells
Krugers D, Schuster M, Schoeller A, Pfister H, Gorg A
Orthopedic Center for Musculoskeletal Research; University of Würzburg; Germany

Recently, herbal therapeutics such as Cimicifuga racemosa (L.) Nutt. (CR) have increased as an alternative to hormone replacement therapy. New findings, which additionally demonstrate osteoprotective effects of CR in ovariectomized rats, would make CR an optional herbal drug for osteoporosis prevention and treatment. Purified fractions of CR containing saponin (S) and aqueous (A) soluble contents were examined in vitro to determine their effects on cell viability and proliferation on a series of tumor cells and osteogenic precursors, respectively. Human mesenchymal stem cells (hMSCs), estrogen receptor-positive (mCF7) and estrogen receptor-negative (MDA-MB-231) human breast adenocarcinoma cell lines as well as androgen-sensitive human prostate adenocarcinoma cells (LNCaP) (n = 3) were stimulated with the whole CR extract, S and A fractions in a range of 0 to 1000 ng/ml. After 72h incubation, apoptosis (Caspase-Glo 3/7-Assay, Promega) and proliferation (CellTiterGlo-Luminescent Cell Viability Assay, Promega) rates were determined. The apoptosis rate is decreased down to 33% compared to untreated cells in all investigated cells and cell lines. No noticeable effect regarding the proliferation rate of hMSCs, mCF7 and MDA-MB-231 cells was detected except for a border line stimulating effect on LNCaP cells. The overall toxicity of such extracts appeared to be very low, cell death occurred outside concentrations of 1 mg/ml. The obtained data show inhibitory effects of CR extracts on apoptosis but no cytotoxicity as measured by proliferation assays in hMSCs, mCF7 and MDA-MB-231 cells. Hence, CR does not possess toxic effects at concentrations of up to 1000 ng/ml extract upon the cells mentioned.

Genomic characterization of γ-terpene synthase from Thymus caespitius
Lim A5, Lukas B6, Novak f, Figueiredo AC5, Pedro LG4, Barroso JC, Trindade H1
1Universidade de Lisboa, Faculdade de Ciências de Lisboa, Departamento de Biologia Vegetal, Instituto de Biotechnologia e Bioprocessos, Centro Biotechnologia Vegetal, C2, Campo Grande, 1749 – 016 Lisboa, Portugal;
2Institute for Applied Botany and Pharmacognosy, University of Veterinary Medicine, Veterinärplatz 1, 1210 Wien, Austria

Thymus caespitius Bro. , commonly known as ‘tormento’ or ‘erva-úrula’, is a Lamiaceae aromatic species endemic of the NW Iberian Peninsula, and of the Azores and Madeira archipelagos characterized for showing high essential oil chemical variability [1, 2]. Using eight chemically distinct Thymus caespitius accessions, collected at Pico and São Jorge islands (Azores) and in the Mainland Portugal, the genomic characterization of exon and intron numbers, sizes and placement, of a putative gene encoding a monoterpene synthase, γ-terpene synthase (TCtPS2), was performed. TCtPS2 is responsible for the first step of the ‘cymyl’-pathway, giving rise to phenolic terpene isomers thymol and carvacrol and related compounds, main components in two of the chemotypes from T. caespitius essential oils. The putative gene was organized in seven exons and six introns. With almost no variability on the plants analysed, TCtPS2 putatively encoded for a protein sequence of 598 amino acids from an open reading frame of 1794 bp, comprising a total of 2291 bp nucleotide sequence content. The deduced amino acid sequence of the putative gene showed a 98% pairwise identity, sharing 93% similarity with closely related Origanum species. A BLAST search on GenBank revealed a high identity (65 – 58%) with other known terpene synthases from different members of other Lamiaceae species. Hereewith reported for the first time for the genus Thymus, this nucleotide identification approach improved the understanding of the genome organization of these genes. Acknowledgement: This study was partially funded by the Fundação para a Ciência e Tecnologia (FCT) under research contracts PTDC/AGR-AMM/70136/2006 and PTDC/AGR-GPI/103344/2008. References: 1. Figueiredo AC. et al. (2010) Natural Product Communications 5: 1465 – 1476. 2. Figueiredo AC et al. (2008) Cur Pharm Design 14: 3120 – 3140.

PCR-based Assays for the Authentication of Black Cohosh Products
Williams S, Howard C, Brenner PD, Fowler MR, Scott NW, Slater A
Biomolecular Technology Group, De Montfort University, Leicester, U.K. LE1 9BH

Black Cohosh (Actaea racemosa L.) is one of the highest selling medicinal plants, ranking as the eighth best seller in the US in 2005. However, this popularity has been damaged by links to cases of hepatotoxicity and other significant health implications. The investigation of these reports has not been able to confirm that Black Cohosh plant material is responsible. This has led to the suspicion that some cases of adverse reactions may result from substitution or adulteration with Asian species of Actaea, rather than to A. racemosa (1). This context demonstrates the requirement for correct identification of A. racemosa in Black Cohosh products. We report the development of the PlantID assay for Actaea species; a DNA-based assay capable of discriminating A. racemosa from potential adulterant species, particularly those associated with hepatotoxic. A group of cohoosh species were chosen on the basis of their widespread growth, commercial availability and/or knowledge of use as an adulterant of Black Cohosh preparations. DNA sequences for each species were aligned to identify hotspots of sequence variation. Species-specific primers were then designed to these regions and optimised for qPCR and multiplex PCR. The product from each reaction was designed to differ in size to enable their resolution by capillary electrophoresis; fluorescent labels attached to each forward primer allow detection of each fragment. The profile of peaks generated is indicative of each species present in the sample. References: 1 Jordan S A, Cunningham DG & Marles RJ,(2010) Toxicology and Applied Pharmacology 243: 198 – 216.
PI11 Insights from P-Glycoprotein in-silico modelling
Ferreira RJ, Ferreira MU, dos Santos DJ
Research Institute for Medicines and Pharmaceutical Sciences (Med.UL), Faculdade de Farmácia, Universidade de Lisboa, Av. das Forcas Armadas, IP00 - 083, Lisbon, Portugal

P-glycoprotein (P-gp) is the most representative member of the ABC transmembrane transporter superfamily, often implicated in the multi-drug resistance phenomenon (MDR) [11]. Several models have been proposed for the efflux mechanism, namely the hydrophobic pore, the flip-flop model and, more recently, the hydrophobic vacuum-cleaner model. Using the bacterial transporters Sav1866, BtuCD or MFSbA, several homology models have been constructed. However, the majority proved to be inaccurate due to errors introduced during the homology process, originating misleading results. The recently published crystallographic structure of the murine P-glycoprotein [2] constitutes a more suitable working model. However, a linker known to regulate substrate’s specificity and to be involved in the conformational changes that accompanies ATP hydrolysis was not mapped [3]. Starting with the murine P-gp crystallographic structure, we built a system comprising a correctly protonated P-gp structure inserted in a lipid bilayer inside a molecular dynamics simulation box with respective counter-ions and waters to solvate all the system. Variations on this system were studied that allowed examining the influence of the linker and lipid type on the P-gp structure stability. The lipid environment and bilayer rigidity was also tested by studying systems with and without cholesterol. Different field parameterizations were used for quality assessment. The molecular dynamics systems were simulated for tens of nanoseconds using the GROMACS simulation package and the new insights gathered from the simulations namely dynamic and static properties both from P-gp and lipids will be presented and discussed. Acknowledgement: This study was supported by FCT, Portugal (project PIDC/QUI/QUI/099815/2008) References: 1. Juliano R et al. (1976) Biochimica et Biophysica Acta 455: 152 – 162 2. Aller S. et al. (2009) Science 323: 1718 – 1722 3. Sato T et al. (2009) FEBS 276: 3504 – 3516

PI12 DNA-based molecular screening and identification of Veronica sp
Icim MC
NIRDBS/’Stejarul’ Research Centre for Biological Sciences, Alexandru cel Bun St., 6, Piatra Neamt, 610004, Romania

Southeastern Europe represents an important center of genetic diversity for many groups of Veronica. It was estimated that about 80 species of Veronica, representing 10 subgenera, are found in Europe [1]; out of these, about 40 have been reported in literature as being present on the Romanian territory [2]. Data about the chemical composition have been found for ten Veronica species from the Romanian flora; these species have a complex and variable biochemical composition, with many secondary metabolites used in pharmacognosy [3]. We aimed to apply molecular techniques to different Veronica species present in Romania, in order to obtain reliable means of authentication of the raw plant material and medicinal products which contains V. officinalis and other species of the genus. Nuclear ribosomal internal transcribed spacer region (ITS) and plastid DNA (cpDNA) intron sequences have been used for PCR amplification. The rpoB-trnC spacer region, one of the most variable plant markers of the plastid genome [4] and the pshA-trnR spacer, a highly variable cpDNA region [5] were amplified from different veronica species. The length of both DNA fragments taken into evaluation for their putative usefulness as markers for plant authentication were highly variable among the Veronica species tested; the length variability in coherent with the molecular data reported from phylogenetic studies [6]. These two spacers could be successfully used as potential DNA barcode marker and as an alternative way to rapidly authenticate the plant species. Acknowledgement: This study was supported by UEFISCDI/ project 32151/2008. References: 1. Albcach DC et al. (2004) Taxon 53: 429 – 452. 2. Icim MC et al. (2010) Bulletin USAVM Agriculture 67(2): 482. 3. Crisan G et al. (2008) Rev Med Chir Soc Med Nat 113(2): 81 – 85. 4. Shaw J et al. (2005) Am J Bot 92: 142 – 166. 5. Kress WJ et al. (2005) PNAS 102(23): 8369 – 837. 6. Albach DC, Meudt HM (2010) Phy! Evol 54: 457 – 471.

PI13 Evaluation of the Effect of Licorice Extract on Proliferation and Differentiation of Human Mesenchymal Stem Cells into Osteoblast cells
Azizi Sani A1, Piri K1, Semaani M2, Mohammadi M*1
1Department of Biotechnology, Faculty of Agriculture, Bu-Ali Sina University, Hamedan, Iran; 2Hematology Department, Faculty of Medical Science, Tarbiat Modares University, Tehran, Iran; *Hamedan Science and Technology Park, Hamedan, Iran

Estrogen deficiency caused osteoporosis during the first decade after Menopause. Estrogen replacement therapy is effective in osteoporosis caused by menopause, but it has some effects, such as carcinogenesis and uterine bleeding. Recent studies have focused on replacement natural compound that contain phytoestrogen, phytoestrogen is natural compound derived from plant, which exhibit estrogen-like activities. Licorice is one of the medicinal plant that have phytoestrogen and its extract indicate activity as Estradiol in some parameters. To evaluate the effect of licorice extract on the proliferation and osteogenesis of human mesenchymal stem cells we used by MTT method. In real-time PCR. Our results show that licorice extract were increased the proliferation and differentiation of hMSC in a dose dependent manner (significant at 10,25,50,100 µg/ml). Real-time pcr analysis shown that licorice extract treatment induced an increase in the expression of BMP-2, Runx-2, Alp, osteocalcin and sp-1 in day 6 and 12, hence ICI 182780, an specific estrogen receptor antagonist inhibit the effect of licorice extract on differentiation, we found that licorice extract stimulates osteoblastogenesis via estrogenic activity and can be used as alternative natural medicine for bone disease such as osteoporosis.

PI14 Methyl jasmonate-induced biosynthesis of taxol and expression of certain related genes by Hazelnut (Corylus avellana L.) cells
Rezaei A1, Ghanati F2, Behnamesh M*2
1Department of Plant Biology, Faculty of Biological Science, Tarbiat Modares University, Tehran, Iran; 2Department of Genetics, Faculty of Biological Science, Tarbiat Modares University, Tehran, Iran

Taxol (Paclitaxel), a diterpene alkaloid against cancer, was originally isolated from Taxus sp. and recently was shown to be produced by hazelnut as well. To develop an optimal bioprocess for paclitaxel supply, taxane biosynthetic pathway regulation must be better understood. In the present study, the effects of methyl jasmonate (MJ) on taxol production and phenyl alanine ammonia-lyase (PAL), deoxycyclulose phosphate reductoisomerase (DXR) and 3-hydroxy-3-methylglutaryl CoA reductase (HMGR) genes expression were investigated in suspension-cultured hazelnut cells. The cultures were treated with MJ (0.25, 50 and 100µM) 8 days after subculture. According to the results, cell growth and viability decreased but lipid peroxidation rate, phenolics and taxol production increased by these treatments. In those cells treated with 100µM of MJ, extracellular and intracellular taxol were respectively 168µg/L and 20.6 µg/g DW (dry weight) for, 3 and 2.3 times higher than those of the control cells. The expression of the PAL and DXR genes were respectively maximum after 48 h and 72 h of the MJ treatment, but the expression of HMGR gene was suppressed by MJ suggesting that the terpenoid part of taxol is more derived from non-mevalonate route and is originated from plastidic terpenoid pathway rather than cytosolic route of terpenoids production. References: Bestoso F et al. (2006) BMC Biotechnol 6: 45. Hoffmann A et al. (1998) Spectroscopy 13: 22 – 32. Ottaggio L et al. (2008) J Nat Prod 71: 58 – 60. Rezaei A, Ghanati F, Behnamesh M (2010) 6th International Workshop on Biological Effects of Electromagnetic Fields pp 70 – 71. Wu J, Lin L (2003) Appl Microbiol Biotechnol 62: 151 – 55.

PI15 Quantitation of Underivatized Omega-3 and Omega-6 Fatty Acids in Foods by HPLC and Charged Aerosol Detection
Acworth J*, Plante M, Crafts C, Bailey B
EXA – a Dionex Company, Applications Department, Chelmsford, USA

The omega fatty acids are a group of compounds that include essential n-3 and n-6, and nonessential n-9 analytes. The omega-3 fatty acids,
which also include eicosapentaenoic acid [EPA] and docosahexaenoic acid [DHA], are required for normal growth and health. Although both omega-3 and -6 fatty acids can give rise to eicosanoid-signaling molecules (prostaglandins, prostanoyclins, thromboxanes and leukotrienes), the omega-6 eicosanoids are generally pro-inflammatory and may play a role in disease. It appears that the amounts and balance of omega fatty acids in a person’s diet affect their eicosanoid-controlled functions. A proper balance of omega fatty acids in the diet is important. Traditionally, omega-3 fatty acids are measured using gas chromatography (GC). For foods, analytes are extracted from the samples prior to hydrolysis to release the fatty acids from their triglycerides, and then converted to their volatile methyl-esters prior to analysis by GC. Regardless, this approach is tedious, time-consuming, and the high temperatures can affect polyunsaturated fatty acid stability. Charged aerosol detection (CAD), a universal mass-based approach, is sensitive, has a wide dynamic range, and has a major advantage in that all nonvolatile analytes give similar response independent of chemical structure. No derivatization is required, and unlike UV detection, the analyte does not need to contain a chromophore. Presented here is a simple and direct HPLC-CAD method that can be used to measure omega-3, -6, and -9 fatty acids in traditional and commercially produced meat, fish, and oils, as well as over-the-counter supplements.

Potential preventive and therapeutic effects of date palm (Phoenix dactylifera L.) pollen grain on cadmium – induced testicular injury in rats

El Neweshy MS, El Muddawy ZK
Faculty of Veterinary Medicine, Alexandria University, Alexandria, Egypt

This study was investigated the possible preventive and therapeutic effects of date palm (Phoenix dactylifera L.) pollen grain (DPP) on cadmium (Cd)-induced testicular damage using quantitative, biochemical and histopathological approaches. A total of 25 adult male rats were randomly divided into five groups: control; DPP treated group received a 56 days DPP suspension; Cd treated group received only CdCl₂; pretreated group received a 15 days of DPP suspension followed by CdCl₂ injection. CdCl₂ (1.2 mg/kg bwt) was intraperitoneally injected as a single dose and DPP (120 mg/kg bwt) was given via gavage suspended in distilled water. Cd treated group showed significantly decrease reproductive organs index weight, sperm count and motility, reduced glutathione, serum testosterone and Johnsen’s score. Meanwhile, sperm abnormalities, lipid peroxidation were significantly elevated. Necrotic changes with poor spermatogenesis to complete spermatogenic arrest were the key histopathological finding. Although the mechanism is not clear, improved sperm quality and antioxidant status, elevated testosterone levels, restored testicular spermatogenesis were noticed in DPP post-treated group as therapeutic intervention. While, DPP pretreatment as preventive intervention failed to attenuate the adverse effects of cadmium.

Anti-metalloproteinase-9 Activities of Selected Indonesian Zingiberaceae Rhizome Extracts in Lipopolysaccharide-induced Human Vascular Endothelial Cells In Vitro

Yanti Y, Steven N, Whihara A, Fajaranto S
Faculty of Biotechnology, Atma Jaya Catholic University, Jakarta, Indonesia

Atherosclerosis arises from chronic inflammation triggered by bacterial infection that activates degradation process by matrix metalloproteinases (MMPs). Zingiberaceae, a group of tropical food crops grown in Indonesia and other Southeast Asia regions, has been traditionally used for food coloring, seasoning, culinary, and medicinal purposes. However, its efficacy as natural vascular protection has not been explored. Our previous studies demonstrated that Kaempferia pandurata Roxb. possessed MMP-2 and MMP-9 inhibitory effects in human gingival and oral epithelial cells induced by Porphyromonas gingivalis, suggesting its potential therapeutic for natural periodontal therapy. Here, we examined the effects of 10 Indonesian Zingiberaceae rhizome extracts on inhibition of MMP-9 expression in human vascular endothelial cells treated with lipopolysaccharide (LPS) in vitro by conducting gelatin zymogram, Western blotting, and RT-PCR assays. LPS (2 μg/ml) significantly elevated the expression of MMP-9 secretion, protein, and mRNA in vascular endothelial cells. Selected Zingiberaceae extracts (1 and 5 μg/ml), i.e. Curcuma xanthorrhiza, C. aeruginosa, C. mangga, C. longa, Kaempferia galanga, Alpinia galanga, and Zingiberaceae officinalis, effectively attenuated the expression of MMP-9 secretion, protein, and mRNA in LPS-induced vascular endothelial cells. Furthermore, MMP-9 expression were specifically blocked by MAPK inhibitors, i.e. PD 98059 (ERK1/2 inhibitor), SB203580 (p38 inhibitor), SP600125 (JNK inhibitor), and PI3K inhibitor (LY294002), indicating that MAPK and PI3K signaling pathways are involved in regulation of MMP-9 gene expression in LPS-induced vascular endothelial cells. These results suggest that selected Indonesian Zingiberaceae rhizomes with potent MMP-9 inhibitory activity may have potentials on prevention and protection of vascular diseases particularly atherosclerosis.

5-Hydroxymethylfurufural (HMF) is derived from dehydration of sugars and has been identified in processed foods [1]. The biological function of HMF have revealed as anti-sickling agent and tyrosinase inhibitor [2,3]. This study was performed to find out the amount of HMF and free sugars from the aged garlic when it is treated by temperature at 60 and 75°C and different incubation period from 7 to 35 days. HMF and free sugars from the hot-water extracts of aged garlics were analyzed with GC/MS, LC/MS, and HPLC. The amount of HMF was high at 75°C and 35 days incubation. Among free sugars, the only fructose except glucose and sucrose was formed and converted to HMF at high temperature and long incubation period. However, fructose formed in low temperature making aged garlic was rarely converted to HMF. This result indicates that formation of HMF can be dependent on the temperature and incubation period for making aged garlic. References: 1. Chen S et al. (2009) Food Chem 114: 582 – 588. 2. Abdulkalim O et al. (2005) British J Haematol 128: 552 – 561. 3. Sharma V et al. (2004) Phytotherapy Res 18: 841 – 844.

Sesamin and sesamolin contents in various commercial sesame oils

Cheng Y¹, Shao Y², Yan W¹
¹Department of Chemistry, Zhejiang University, Hangzhou, 310027, China; ²Skyherb Ingredients, Anji, 313300, China

Sesame (Sesamum indicum L.) seed and oil have been categorized as one of the representative health food and widely used for good flavor and taste in China, Japan, and other East Asian countries for a long times. Sesame seed and oil contain abundant lignins [1] such as sesamin, sesamolin, and others. Sesamin and sesamolin are major lignians in sesame oil, and its biological effects have been extensively studied. These components are believed to play an important role in the oxidative stability of sesame oil [2]. It is important to understand the variation in the contents of these physiologically active constituents in sesame oil. This knowledge of their levels and forms in sesame seed, sesame oil, and functional foods derived from sesame is beneficial to control the quality of the sesame seed and sesame oil, and to develop the sesame oil manufacturing technique. The aim of this study was to establish the methods of sample pretreatment and simultaneous determining the sesamin and sesamolin in the sample of sesame oils by RP-HPLC. The methods developed in this work were used to determine the sesame and sesamolin in ten different brands of sesame oils collected in the Chinese markets. The mean contents of total lignians, sesamin and sesamolin were 8.16, 5.14, and 3.02 mg/g respectively. The results shown that can be used to control the quality of sesame oils, and to estimate the dietary intake of sesame lignans. And also it will be beneficial to improve the processing technique in industry. Acknowledgement: This research work was supported by the Research Council of Zhejiang University and Skyherb Ingredients. References: [1] Daisuke N et al. (2006) J Pharm Exp Ther 318: 328 – 335 [2] Nakai M et al (2006) Biosci Biotech Biochem 70: 1273 – 1276.

Formation of 5-HMF in making aged garlic (Allium sativum L.) under different condition

Cho K, Cha J, Lim J, Kim J
National Academy of Agricultural Science, Suwon, Korea
Secoisolariciresinol diglucoside (SDG) (Fig.1) is an essential component (11.9 to 25.9 g/kg [1]) of lignans in flaxseeds. Recently, SDG has drawn more and more attention because of its health benefits. A number of animal studies have shown that SDG may help fight many diseases in the modern society, including breast cancer [2], cardiovascular malfunction [3], diabetes [4] and prostatic hyperplasia [5]. With a novel perspective, we focused our study on the effect of food processing methods, including steamed, boiled, fried and deep fried, and processing times on the stability of SDG in flaxseed powder. In this paper, the samples of four processing times were prepared for each processing method. The concentrations of SDG in different samples were determined by RP-HPLC [6]. The chromatographic analysis was performed on a Diamonsil C18 column (150*4.6 mm, 5 µm). Acetonitrile and 1% aqueous acetic acid was selected as the mobile phase. The detection wavelength was 280 nm. A comprehensive study on the effect of different processing methods was made in Fig. 2. For steaming process, little effect on SDG content was observed. While for the boiled dishes, i.e. the medicated soups, although little amount of SDG was released from flaxseed powder remnant, small amount of SDG was released from flaxseed into the filtrate (the soup). As for fried and deep fried dishes, the loss of SDG was inevitable, but shorter processing time lead to less decrease in SDG, flaxseed could also be employed if cooked properly.

Figure 1: Chemical structure of SDG

Figure 2: Comprehensive comparison of SDG content in different processing methods. a. Steaming; b. Boiling; c. Frying; d. Deep Frying.


Quality control of red clover based nutritional supplements by FTIR and chemometric analysis
Kasper J, Metzger MF
Institute of Pharmacy, Freie Universitaet Berlin, Koenigin-Luise-Str. 2+4, D-14105 Berlin, Germany

Botanical preparations of red clover (Trifolium pratense L.) have gained interest as an alternative treatment for menopausal problems such as hot flushes. This shows the need for simple and rapid analysis methods. In the present study, FTIR-ATR spectroscopy has been applied for the characterization and identification of the active compounds of red clover belonging to the class of isoflavones, e.g. formononetin and biochanin A. Information about functional groups and chemical composition could be obtained, making FTIR a powerful tool for a fast and non-destructive quality control. Moreover, using chemometrics calibration models based on the partial least squares (PLS) regression were employed. The results of the multisivariate calibration demonstrated the potential of this method to quantify the main isoflavones in red clover.

Protective effects of saffron and trans-crocetin on glutamate mediated excitotoxicity in rat neuroblastoma cells
Berger F, Hensel A, Nieher K
1University of Leipzig, Institute of Pharmacy; D-04013 Leipzig, Germany; 2University Münster, Institute for Pharmaceutical Biology and Phytochemistry; D-48149 Münster, Germany

Neuronal dysfunctions or even cell death are often accompanied by an exceeding release of glutamate. Excessive glutamate level induces unregulated stimulation of NMDA receptors. In previous studies we found an antagonistic effect of hydro-ethanolic saffron extract (CSE) and trans-crocetin, a carotenoid from saffron, on NMDA receptors (Berger et al., Neuroscience, 180; 238 – 247, 2011). In this study we evaluated the protective effects of CSE and trans-crocetin on glutamate mediated excitotoxicity on rat B104 neuroblastoma cells using cell-based cytotoxicity tests and a fluorescence stain with 4,6-diamidino-2-phenylindole (DAPI-staining). Glutamate applied for 24 h decreased cell viability from 27 ± 4% to 63 ± 5% and decreased LDH activity from 207 ± 5% to 125 ± 18%. The number of annexin-V and 7-AAD positive cells was also increased (0.1 – 20 mM) cell viability (MTT-test) and increased LDH activity (LDH-test). The extract was also subjected to the evaluation of α-amylase inhibitory activity. The IC50 value was found to be 552 ± 6.09 µg/ml. This study provides evidence on the potential health benefits of lectins from Vigna radiata, thus confirming the traditional claim. References: 1. Heller VG (1927) JBC 435 – 442.

Protocol for lectins from Vigna radiata - A potential health supplement
Singh SJ, Tatke PA, Naharwar VP
1C.U.Shah College of Pharmacy, S.N.D.T University, Mumbai, India; 2Amsar Pvt Ltd, Indore

There has been growing interest in use of nutraceuticals of plant origin, because of their impact on the status of human health and disease prevention. Plant lectins, a unique group of proteins and glycoproteins with potent biological activity, occur in commonly consumed foods such as legumes. The lectins can be used as dietary supplements due to their potential benefits of enzyme inhibition. The present research work discusses lectins from Vigna radiata (L) R.Wilczek as an effective and economical source of natural antioxidants and α-amylase inhibitors which can prove to be a potential dietary and health supplement. Lectins from Vigna radiata were extracted by macerating the seed meal in 50 mM phosphate buffer saline (pH 5.2) containing D(-)-galactose at 40°C. The lectin rich extract was prepared by salt precipitation. The precipitate was dialysed against distilled water for 48 hours. The lectins were purified by Size exclusion Chromatography by using Sephadex G75. The presence of lectins was confirmed by hemagglutination assay. The fractions showing agglutination were pooled together and lyophilized. The yield of relatively purified lectins was found to be 0.044%w/w. Lectin rich extract was evaluated for anti-oxidant property by TBARS assay. The IC50 value of the lectin rich extract was found to be 598.32 ± 3.2 µg/ml. The extract was also subjected to the evaluation of α-amylase inhibitory activity. The IC50 value was found to be 552 ± 6.09 µg/ml. This study provides evidence on the potential health benefits of lectins from Vigna radiata, thus confirming the traditional claim. References: 1. Heller VG (1927) JBC 435 – 442.
Red clover (Trifolium pratense, Fabaceae) is important source of isoflavones, among which the most present are: daidzein, genistein, for- mononetin and biochanin A [1]. These substances are considered to be beneficial for reduction of menopausal symptoms, prevention of osteoporosis, cancer and cardiovascular diseases. Since red clover extracts are used for production of dietary supplements, it is important to evaluate profile of these active compounds in plant parts. The aim of this study was to determine content of daidzein, genistein, formononetin and biochanin A in different plant parts of red clover, and to investigate which isoflavone is present in highest concentration. Stems, leaves and flowers of five red clover cultivars were grounded and mixed with water 30 minutes on 37°C. After that, 3 M HCl and 96% ethanol were added and mixture was heated to boiling. Extracts were than purified by solid phase extraction on HLB cartridges. Isoflavones were identified and quantified in samples by high-performance liquid chromatography (HPLC), using corresponding standard compounds [2]. Total isoflavone content was on average the highest in leaves (2.73 mg/g), while the lowest average content had biochanin A in stems of red clover cultivars (0.47 mg/g). Isoflavone content in flowers was on average the highest in leaves (2.73 mg/g), and the lowest (HPLC), using corresponding standard compounds [2]. Total isoflavone content was on average the highest in leaves (2.73 mg/g), and the lowest in stems of red clover cultivars (0.47 mg/g). Isoflavone content in flowers varied between 0.53 – 1.05 mg/g. In leaves formononetin was dominant (1.62 mg/g), while the lowest average content had biochanin A in stems (0.04 mg/g). The highest individual concentrations of all investigated isoflavones were found in leaves of different cultivars. On average, in all analyzed samples formononetin was the most present isoflavone.

References:

Fatty acid profile from samples of hemp seeds of dioecious and monoecious hemp varieties approved in Romania

Cannabis sativa L. hemp seeds can be a complete and balanced source of fatty acids, with an optimal omega 6/omega 3 ratio of 3:1 [1], but with limited use in Romania because of the stigma of drug. Whole hemp seeds have oil content of about 25 – 35% [2], but in Russia there is a cultivated variety called “olifera” which contains 40% oil [3]. About 30 – 35% of the weight of hempseed is an edible oil that contains about 80% as essential fatty acids (EFAs), linoleic acid, omega-6 (LA, 55%), alpha-linolenic acid, omega-3 (ALA, 22%), in addition to gamma-linolenic acid, omega-6 (GLA, 1 – 4%) and stearidonic acid, omega-3 (SDA, 0 – 2%). This study aims to investigate the impact of the extraction technology of oil content and fatty acid profile from samples of hemp seeds of dioecious and monoecious hemp varieties obtained in research stations from different areas of the country and approved in Romania. Hemp oil has been extracted from the seeds by cold pressing using a press and by Soxhlet method with a Velp block of mineralization. Investigation of fatty acid profile and oil content was performed by gas chromatography GC-MS whith Shimadzu GC MS QP 2010. Hemp seeds not only contain essential fatty acids, but come with substantial contribution of 20 – 25% protein, essential amino acids, which make hemp seed an ideal food for vegetarians, successfully replacing the lack of animal protein, with a high nutritional value for human consumption in salads, bread and even chocolate. References: [1] Callaway JC. (2004) Euphytica 140: 63 – 72 [2] Sandru ID, Parascoviu R, Găucă C (1996) Cultura cânepei, Helicon, Timișoara [3] Defere JL and Pate DW (1996) Journal of the International Hemp Association 3(1): 4 – 7

The contents of heavy metal (Pb, Cd and Zn) in plant Taraxacum officinale Weber

The species of Taraxacum officinale Weber (Asteraceae) is a very popular medicinal and edible herb. Since time immemorial have been used in traditional phytotherapy in Bosnia and Herzegovina (B-H) [1]. The young shoots and inflorescences are used as health food [2]. Dandelion is widespread. Most often inhabit different anthropogenic habitats that are loaded with different pollutants, including heavy metals [3]. This is a serious limitation for safe and sustainable use of this plant in medicine and dietetics. Investigation the content of heavy metals in roots and aerial part of the dandelion included 30 localities of B-H which are under different anthropogenic influence. Samples have been prepared using standard methods. Measurement of concentrations of heavy metals was carried out by atomic absorption spectrophotometry. The content of heavy metals cadmium, zinc and lead in Taraxacum officinale varied depending on the vegetative part of plants, season, location, then the type of soil, the intensity of anthropogenic influences, soil pH, the interaction of the tested elements, climate conditions and other environmental factors. The concentration of cadmium ranged from 0, 02 – 0,8 mg/kg, the concentration of zinc was 30 mg/kg to 100 mg/kg. The concentration of lead varied from 0,1 – 10 mg/kg. There are significant differences in the concentration of metals between the sites under different anthropogenic influence. Samples have been prepared using standard methods. Measurement of concentrations of heavy metals was carried out by atomic absorption spectrophotometry. The content of heavy metals cadmium, zinc and lead in Taraxacum officinale varied depending on the vegetative part of plants, season, location, then the type of soil, the intensity of anthropogenic influences, soil pH, the interaction of the tested elements, climate conditions and other environmental factors. The concentration of cadmium ranged from 0, 02 – 0,8 mg/kg, the concentration of zinc was 30 mg/kg to 100 mg/kg. The concentration of lead varied from 0,1 – 10 mg/kg. There are significant differences in the concentration of metals between the sites under different anthropogenic influence. Samples have been prepared using standard methods. Measurement of concentrations of heavy metals was carried out by atomic absorption spectrophotometry. The content of heavy metals cadmium, zinc and lead in Taraxacum officinale varied depending on the vegetative part of plants, season, location, then the type of soil, the intensity of anthropogenic influences, soil pH, the interaction of the tested elements, climate conditions and other environmental factors. The concentration of cadmium ranged from 0, 02 – 0,8 mg/kg, the concentration of zinc was 30 mg/kg to 100 mg/kg. The concentration of lead varied from 0,1 – 10 mg/kg. There are significant differences in the concentration of metals between the sites under different anthropogenic influence. Samples have been prepared using standard methods. Measurement of concentrations of heavy metals was carried out by atomic absorption spectrophotometry. The content of heavy metals cadmium, zinc and lead in Taraxacum officinale varied depending on the vegetative part of plants, season, location, then the type of soil, the intensity of anthropogenic influences, soil pH, the interaction of the tested elements, climate conditions and other environmental factors. The concentration of cadmium ranged from 0, 02 – 0,8 mg/kg, the concentration of zinc was 30 mg/kg to 100 mg/kg. The concentration of lead varied from 0,1 – 10 mg/kg. There are significant differences in the concentration of metals between the sites under different anthropogenic influence. Samples have been prepared using standard methods. Measurement of concentrations of heavy metals was carried out by atomic absorption spectrophotometry. The content of heavy metals cadmium, zinc and lead in Taraxacum officinale varied depending on the vegetative part of plants, season, location, then the type of soil, the intensity of anthropogenic influences, soil pH, the interaction of the tested elements, climate conditions and other environmental factors. The concentration of cadmium ranged from 0, 02 – 0,8 mg/kg, the concentration of zinc was 30 mg/kg to 100 mg/kg. The concentration of lead varied from 0,1 – 10 mg/kg. There are significant differences in the concentration of metals between the sites under different anthropogenic influence. Samples have been prepared using standard methods. Measurement of concentrations of heavy metals was carried out by atomic absorption spectrophotometry. The content of heavy metals cadmium, zinc and lead in Taraxacum officinale varied depending on the vegetative part of plants, season, location, then the type of soil, the intensity of anthropogenic influences, soil pH, the interaction of the tested elements, climate conditions and other environmental factors. The concentration of cadmium ranged from 0, 02 – 0,8 mg/kg, the concentration of zinc was 30 mg/kg to 100 mg/kg. The concentration of lead varied from 0,1 – 10 mg/kg. There are significant differences in the concentration of metals between the sites under different anthropogenic influence. Samples have been prepared using standard methods. Measurement of concentrations of heavy metals was carried out by atomic absorption spectrophotometry. The content of heavy metals cadmium, zinc and lead in Taraxacum officinale varied depending on the vegetative part of plants, season, location, then the type of soil, the intensity of anthropogenic influences, soil pH, the interaction of the tested elements, climate conditions and other environmental factors. The concentration of cadmium ranged from 0, 02 – 0,8 mg/kg, the concentration of zinc was 30 mg/kg to 100 mg/kg. The concentration of lead varied from 0,1 – 10 mg/kg. There are significant differences in the concentration of metals between the sites under different anthropogenic influence.

References:

Alpha lipoic acid (ALA) has become a common ingredient in many commercially available supplements. The aim of the present work is to compare the dissolution profiles of the certain samples of ALA soft gelatin capsules and tablets 300 mg and 600 mg) commercially available from the local market. Fixed volumes of the dissolution medium were withdrawn at 15, 30, 45 and 60 minutes. Dissolution tests were performed on the USP Apparatus 2 (Dissolution tester ERWEKA DT 800; rotating speed 75 rpm at 37 ± 0.5°C, 900 mL, distilled water). Also, the purpose of this work was to adapt and use the HPLC method proposed by Salem for the determination of the amount of the active ingredient released [1,2]. HPLC was performed with a mobile phase composed of 0.05 M phosphate buffer pH 3.5:acetonitrile = 650:350/vv, and peaks were detected at 330 nm. Degassed and diluted samples were analysed on Zorbax Eclipse XDB-C 18 column (250 x 4.6 mm, 5 μm), at 25°C and 1.5 mLMin⁻¹ flow rate. The dissolved amounts of ALA in soft capsules and tablets at the end of testing were in the range of 12.9 ± 2.8% - 18.6 ± 1.9% and 85.2 ± 7.6% - 90.6 ± 4.9%, respectively. The results of dissolution studies are summarized in Figure 1. which show the percentage of ALA dissolved as a function of time. The results obtained in this study indicated problems in drug release from the investigated ALA soft gelatin capsules. According to the results obtained, we can presume differences in therapeutic response of the investigated ALA soft gelatin capsules and tablets.
The high fat load in the knee joint is a source of high cytokine production which exerts inhibitory effects on development of cartilage tissue in the knee joint. Both, E2 and Ecd seemed to prevent these lipotoxic effects. Acknowledgement: This work was in part funded by VerdeVital GmbH

References:

PJ14
Beta-ecdysone prevents the metabolic syndrome in ovariectomized rats: effects on metabolic parameters
Wuttke W, Seidlova Wuttke D
University Medical Center Göttingen, Robert-Koch-Str. 40, D-37075 Göttingen, Germany

Ovariectomized (ovx) rats develop a metabolic syndrome which includes hypercholesterolemia, hypertriglyceridemia and an impaired oral glucose tolerance test. These impaired metabolic parameters are due to increased cytokine secretion by the increased number of adipocytes. Estradiol (E2) and beta-ecdysone (Ecd) are known to reduce visceral and subcutaneous fat loads and E2 normalizes the deranged metabolic parameters. Whether this can also be achieved by a treatment with Ecd was not studied hitherto. Ovx rats were orally treated with E2 (0.108 mg/animal/day) or Ecd (18.56 mg/animal/day) and following necropsy 4 weeks later serum cholesterol, LDL, HDL and triglycerides were determined. In addition oral glucose tolerance test (OGTT) was performed. Serum cholesterol, LDL and HDL were reduced by E2 whereas triglycerides were increased. Ecd decreased cholesterol, LDL and also triglycerides but increased HDL. Clearance of glucose following an OGTT lasted longer in the ovx controls than in the E2 and Ecd treated animals. It is concluded that Ecd shares the positive effects of E2 on cholesterol and glucose clearance but prevents the adverse acting stimulation of triglycerides by E2. Hence, Ecd may be a novel non-estrogenic alternative for prevention and treatment of the metabolic syndrome. Acknowledgement: This work was in part funded by VerdeVital GmbH

PJ15
Beta-ecdysone (Ecd) prevents the metabolic syndrome in ovariectomized (ovx) rats: joint cartilage tissue
Seidlova Wuttke D, Wuttke W
University Medical Center Göttingen, Robert-Koch-Str. 40, D-37075 Göttingen, Germany

We have recently shown that Ecd prevents osteoporosis and decreases visceral fat mass in ovx rats. This animal model is known to develop a metabolic syndrome and this disease was shown to be associated with increased visceral and bone marrow fat tissue. The adipocytes in bone secrete large amounts of cytokotines which have cytotoxic effects on osteoblast thereby augmenting estrogen deficiency induced osteoporosis. The reduction of bone mass by Ecd is suggestive that fat load in the knee joint is a source of high cytokine production which exerts inhibitory effects on development of cartilage tissue in the knee joint. Both, E2 and Ecd seemed to prevent these lipotoxic effects. Acknowledgement: This work was in part funded by VerdeVital GmbH

Elemental Compositions of Soybean Cultivars Cultivated in Turkey
Kan A. Selçuk University, Vocational School of Technical Sciences, Program for Food Technologies, 42070 Konya, Turkey

Soybean (Glycine max [L.] Merr.) is an annual plant species and is locally known as “Soya Faslıyeli”. Soybean is consumed as a food and vegetable plant and its recorded folkloric usages for the medicinal purposes. In the present study macro (N, P, K, Ca, Mg, Na,) and trace elements (Fe, Mn, Zn, Cu) of soybean cultivars cultivated under the controlled conditions in Turkey have been studied. Macro and trace elements were determined by using various techniques. N was determined by the dry combustion method using elemental analyses, P was measured by a colorimetric method, whereas K and Na by flame photometry. Finally Ca, Mg, Fe, Cu, Zn and Mn was detected and quantified by atomic absorption spectroscopy (AAS). All experiments were performed qualitatively and quantitatively with comparison to a certified reference plant material statistically. The results of elemental analyses showed that N ranged 5.41 – 5.82%, P ranged 5250 – 6100 ppm and K ranged 15915 – 19645 ppm. To the best of our knowledge, this is the first report on micro and macro elements of cultivated Turkish soybean cultivars. As a conclusion, the elemental composition and the nutritional value soybean cultivars are worthwhile to investigate with comparison to other Glycine sp. used medicinally.
Histopathological and immunohistochemical study of the effect of Punica granatum extract on Azoxymethane induced colon cancer in Rats

Hetta MH 2, Ali ME 1, Yassin NZ 3, El Guindi OD 4

1Pathology Department, National Research Center, 12622 Dokki, Cairo, Egypt; 2Pharmacology Department, National Research Center, 12622 Dokki, Cairo, Egypt; 3Chemistry of Tannins Department, National Research Center, 12622 Dokki, Cairo, Egypt

Chemoprevention has become an important area in cancer research due to the failure of current therapeutic modalities. Polyphenol-rich dietary foodstuffs have attracted attention due to their chemopreventive and chemotherapeutic properties. The modulating effects of aqueous methanol of Punica granatum L. peels at doses (200 and 400 mg/kg) on colon carcinogenesis initiated with azoxymethane (AOM), were investigated in male rats by weekly s.c. injections of 15 mg/kg body wt for 12 weeks. Histopathological studies on AOM-treated rats revealed dysplasia of the colonic histoarchitecture, which showed signs of improvement following P. granatum administration, was found to significantly and dose dependently decrease the total number of aberrant crypt foci (ACF) per rat. Cell proliferation in the colon, as shown by proliferating cells nuclear antigen (PCNA), was also reduced in those treatments. AOM-treated rats exhibited alterations in cancer tumour markers gamma glutamyl transpeptidase (gamma-GT), carcinoembryonic antigen (CEA), pathophysiological markers (alkaline phosphatase (ALP) and lactate dehydrogenase (LDH)) and oral administration of P. granatum restored the levels of these marker enzymes. Also, pro-inflammatory proteins (inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2)) and pro-inflammatory cytokines tumour necrosis factor (TNF)-alpha and interleukin (IL-6) in AOM group exhibited elevated expressions of all these inflammatory proteins. P. granatum administration reduced COX-2, iNOS, TNF-alpha and IL-6 as confirmed by immunohistochemical analysis during AOM-induced colon carcinogenesis. Our results suggest that P. granatum could exert a significant chemopreventive effect on AOM induced colon carcinogenesis is probably due to combined effect of polyphenolic compounds.

Investigation on Compositions of Seed Oil and Yield of Silimyrin of Seeds from Silbuly marianum (L) Gaertn. Cultivated in Konya Ecological Conditions

Celal S. Gunay 1, Kartpinar F.

1Sel/C231uk University, Agricultural Faculty, Department of Field Crops, 42070 Kampus-Konya, TURKEY; 2Ankara University, Faculty of Pharmacy, Department of Pharmacognosy, 06100 Tandogan-Ankara, TURKEY

Milk thistle (Silybum marianum (L) Gaertn.), is an herbal supplement used to treat liver and biliary disorders. The active constituent of milk thistle is silymarin, a mixture of flavonolignans. It is also contains important fatty oil acid anisaturated. In this study, researches have been conducted in Medicinal – Aromatic Plants laboratory and Medicinal and Aromatic Plants Experimental Farm of Field Crops Department, Agriculture Faculty, Selcuk University. The aim was to determine the effect on yield and quality some characters of organic fertilizers applied at the different doses on milk thistle (S. marianum) cultivated under Konya (Turkey) ecological conditions. It were applied at three different doses sheep manure as organic fertilizer. In this study; plant height, plant seed yield, yield of crude oil, composition and yield of oil and flavonoid (silymarin) were examined. According to results of this research; plant height, seed yield, yield of crude and composition and yield of oil and flavonoid (silymarin) varied between 75 – 218 cm, 720 – 1480 kg/ha, 20 – 28% and 1% – 3%, respectively. In this research; high silymarine yield, crude oil yield, fatty acid composition and drug of milk thistle (S. marianum) cultivated in Konya ecological conditions were obtained from 15000 kg/ha from applied organic fertilizer.

Antioxidant properties of wild Solanum nigrum ripe fruit

Aly IS, Shallon MA

Cairo University, Faculty of Agriculture, Biochemistry Department, Giza, Egypt

This work was to examine hepatoprotective properties of Solanum nigrum, commonly known as Black Nightshade, a medicinal herb grown in Egypt used traditionally in oriental medicines and believed to have various biological properties. A crude ripe fruit ethanol extract was made, lyophilized to give 2.86 g/100 g. Two sets of experiments were done, in vitro experimental and in vivo biological assays. The results revealed that crude ethanol extracts of Black Nightshade ripe fruit had strong antioxidative activity, for example in a DPPH assay at 500 ppm of the extract, a 68% reduction of DPPH radicals was detected, with total antioxidiant capacity at 8.45 ± 0.031 as ascorbic acid equivalents. In vivo, the extract; 100, 200 or 300 mg/Kg, showed valuable activity as a hepatoprotective agent on oral one dose CCl4-treated experimental rats shown by an increase in total serum soluble protein, albumin, and a remarkable reduction in the serum activity of AST, ALT and ALP as well as bilirubin and uric acid. For example, the serum total albumin level was reduced from 6.29 ± 0.12 g/dl in the healthy normal control animals to 3.32 ± 0.17 g/dl (53% at normal control) for CCl4 intoxicated control rats, but recovered to 5.62 ± 0.39 g/dl by 300 mg extract per kg body weight for rats on daily oral post-treatment for 5 days. Collectively, Black Nightshade ripe fruit ethanol extracts was shown to be an effective hepatoprotective agent in vivo due to high content of antioxidiant and bioprotective plant secondary metabolites. Key words: Solanum nigrum L, Black Nightshades, Hepato-protective, Antioxidant, CCl4 Acknowledgement: The authors thanks Dr. Enam Abdel-Mobbed, Biochemistry department, Faculty of Agriculture, Cairo University for his great support. Thanks to Kamela Alegre, American PhD student at Universitat Autonoma de Barcelona, for Language revision. References: Lin H et al. (2008) Chemico-Biological Interactions 171: 283 – 293 Loganayaki N, Sidduraju P and Manian S (2010) Food Science and Biotechnology 19(1): 121 – 127

Hypocholesterolemic Effects of Glyphaea brevis (Spreng.) Monarch. in Normal And Streptozotocin-Induced Diabetic Rats

Dakan W, Ntemie FR, Oben J

Department of Biochemistry, Faculty of Science, University of Yaounde 1, Yaounde, Cameroon

Hypercholesterolemia, sometimes linked to diabetes, is a public health concern since it paves the way to severe complications such as hypertension and stroke. The search for innovative, natural and safe treatments to reverse the condition remains imperative. This study was aimed at evaluating the hypocholesterolemic potential of Glyphaea brevis (Spreng.) Monarch. aqueous extract (AE) in both normal and streptozotocin-induced diabetic rats. AE was administered to rats by gastric intubation at 500 mg/kg for 30 days in a controlled study. At the end of experiment, administration of AE significantly reduced the levels of fasting blood total cholesterol (normal: -38.54%, p < 0.01; diabetic: -22.08%, p < 0.01); LDL-cholesterol (normal: -72.85%, p < 0.01; diabetic: -38.15%, p < 0.01) while no significant change was observed in triglycerides. Moreover, atherogenicity indices total cholesterol/HDL-cholesterol (TC/HDL-c) and LDL-cholesterol/HDL-cholesterol (LDL-c/HDL-c) were significantly reduced at the end of study (TC/HDL-c normal: -43.88%, p < 0.01; diabetic: -52.43%, p < 0.01; LDL-c/HDL-c normal: -76.14%, p < 0.01; diabetic: -44.11%, p < 0.01). These results suggest the hypocholesterolemic effect of G. brevis. Such effect would be accountable of the presence of flavonoids (revealed by phytochemical screening) that may inhibit enzymes such as hydroxymethyl-CoA (HMGoA) reductase that are involved in cholesterol biosynthesis. The outcome of our study could find applications in the development of alternative means of treatment or prevention of hypercholesterolemia and its associated complications.

The pericarp of Pismum sativum L (Fabaceae) as a biologically active waste product

Taha KF, Hetta MH2, Ali ME2, Yassin N2, El Guindi OD4

1Phytochemistry Department, NODCAR, Cairo, Egypt.; 2Pharmacognosy Department, Faculty of Pharmacy, Beni Suef University, Beni Suef, Egypt; 3Pharmacology Department, National Research Centre, Cairo, Egypt; 4Pharmacognosy Department, Faculty of Pharmacy, Al Azhar University, Cairo, Egypt

Food industries generate large amounts of wastes and byproducts which contain biologically active compounds. The recycling of these wastes could be of economic benefits. Pericarp of Pismum sativum L. pods is separated from the seeds which are processed as frozen foods. Most of the phytochemical studies on Pismum sativum dealt mainly with the seeds. With the aim of utilization of waste products as biologically ac-
Auricularia auricula-judae (Bull.) J.Schr/C246t. and Mushrooms have been used for many years in oriental culture as tea and photodiode array detector (HPLC-DAD). Twelve of the 14 phenolic compounds identified in these edible mushroom species have been carried out by high-performance liquid chromatography coupled to photodiode array detector (HPLC-DAD). Twelve of the 14 phenolic compounds were identified and quantified by comparing their chromatographic characteristics and absorption spectra with that of the standard compounds. The analysis showed that p-hydroxybenzoic acid, catechin, gallic acid and caffeic acid were the major phenolic components in the extracts. Protective effect of these mushroom on H2O2 induced oxidative cell damage was determined by using MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, a yellow tetrazole) assay. All the extracts exhibited protective effect against H2O2 induced oxidative cell damage but the highest activity was observed for A. auricula-judae aqueous extract (89.5 ± 1.8% cell viability at 0.1 mg/ml) (Fig. 1.). A. auricula-judae extracts (at concentration of 0.025 – 0.100 mg/ml) were not toxic to baby hamster kidney fibroblast cell line (BHK 21) (Fig. 2.). The results of this study indicated that the extracts exhibited interesting protective effect against H2O2-induced baby hamster fibroblast cell line and they may be used as natural sources in pharmaceutical industry for the prevention of conditions that occur due to oxidative damage.

Figure 1: Protective effect of the mushrooms extracts in BHK 21 cells pretreated with the extracts at concentrations (0.100 mg/ml) for 24 h and exposed to 1 mM H2O2-induced oxidative stress. Each value represents the mean ± SD of five wells. AAE; A. auricula-judae methanol extract; AME; A. auricula-judae aqueous extract; PAE; P. eryngii aqueous extract; PME; P. eryngii methanol extract

Figure 2: Cytotoxic effect of the mushrooms extracts in BHK 21 cells pretreated with the extracts at concentrations (0.025, 0.050 and 0.100 mg/ml) for 24 h. Each value represents the mean ± SD of five wells.

Artemisinin, a sesquiterpene lactone endoperoxide isolated from the herb *Artemisia annua* L. (*Asteraceae*), is a highly potent antimalarial compound, which is efficient against multidrug-resistant strains of *Plasmodium falciparum*. The promotion of artemisinin-based combination therapies (ACTs) by the WHO during the past years lead to a strong pressure on the world market of artemisinin. The artemisinin world market is volatile and therefore efforts to improve performance of this culture are often limited. The use of varieties with high artemisinin content is a key factor for the development of such cultures. This should secure the supply of artemisinin, lower its cost of production and improve the competitiveness of this new culture with other commercial crops. After the variety Artemis, Mediplant launches a new variety called Apollon with about 20% yield increase. Performances of this new hybrid, with artemisinin content nearing 1.5%, are being presented.

**PK2**

Apollon, a new *Artemisia annua* variety with high artemisinin content

Simonneau X, Quenzel M, Carten C

Mediplant, 1964 Conthey, Switzerland; 2Agroscope ACW, 1964 Conthey, Switzerland

Improvement of high-fat-diet-induced metabolic syndrome by ethanol extract of *Polygonatum falcatum* (ID 1215B) in mice

Kweon H, Ko J, Yoon J, Jang H, Yoon S, Kang J

Research laboratories, Idong Pharmaceutical Co., Ltd., 23 – 9, Seoga-Dong, Hwasung-Si, Gyeyong-Di, Korea

**PK3**

Use of paclitaxel on balloon catheters against restenosis

Yoon MC, Speck U, Kolodziej H

1Institute of Experimental Radiology, Charité, Campus Mitte, Humboldt-Universität zu Berlin, Charitéplatz 1, 10117 Berlin, Germany; 2Freie Universität Berlin, Institute of Pharmacy, König-Luise-Str. 2+4, 14195 Berlin, Germany

Besides the established use as chemotherapeutic agent against breast or ovarian cancer, paclitaxel coated on medical devices such as stents and balloon catheters has recently been applied in local prophylaxis and therapy of arterial stenosis/restenosis [1]. Paclitaxel is particularly suitable to inhibit injury-induced excessive intravascular scar formation following balloon angioplasty because of its strong and persistent anti-proliferative properties [2]. The aim of this work was to optimize the balloon coating for clinical application. The challenge is to guarantee firm adherence of coated paclitaxel on its way through a hemostatic valve and atherosclerotic arteries to the target, and to allow optimal release at the lesion site. Paclitaxel was coated on the surface of angioplasty balloon catheters by a semiautomatic Hamilton microsyringe. HPLC showed a coating of ca. 3.0 µg/mm² of paclitaxel. When introducing the catheter into the artery, the loss of paclitaxel was shown to be in the range of 15%. Upon the inflation of a balloon catheter in the stenotic segment of the artery, the surface gets in contact with the vessel wall for a few seconds up to one minute only [3]. After removal of the device from the artery ca. 10% of the drug were retrieved on the balloon, indicating the release of ca. 90%. The proportion absorbed by the vessel was ca. 10% as assessed in a porcine model. The pharmacological effect measured by angiography using the diameter stenosis as a parameter supported the benefits of the processed coating for clinical practice. References: 1. Rowinsky EK and Donehower RC (1995) N Engl J Med 332: 1604 – 1614. 2. De Labriolle A et al. (2000) Catheter Cardiovasc Interv 73: 643 – 652. 3. Waksman R and Pakala R (2005) Circ Cardiovasc Interv 2: 352 – 358.

**PK4**

Preclinical evaluation of red grapes seeds extract from *Vitis vinifera*, Burgund Mare, Recas, Romania as skin photochemoprotective agent

Bojla P1, Sarcu F1, Filip A1, Gal A1, Taulescu M1, Cuc C1, Nagy A2, Tabaran F2, Borza C2, Cotoi C1

1University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Faculty of Veterinary Medicine, Pathology Department, Calea Manastur, no 3 – 5, 400372 Cluj-Napoca, Romania; 2University of Oradea, Universitatii no. 1, Dermatology Department, Oradea, Romania; 3University of Medicine and Pharmacy Iuliu Hatieganu, Physiology Department, Cluj-Napoca, Romania

Several studies have shown that polyphenols from grape seeds possess anti-inflammatory, antioxidant effects and inhibit the oxidative stress – mediated action of MAPK and Nrf2/ARE pathways [1]. For the development of newer and more effective photochemopreventive agents we assessed the effect of grapes seed extract from Vitis vinifera L, Burgund Mare variety, Romania, with proved anti-oxidant and anti-inflammatory effects in vitro and on SKH-1 hairless mice [2]. Two groups of volunteers (n = 10) were exposed to one minimal erythema doses (1 MED) of UVB. For each subject we used 3 skin study areas, of the same size, from the posterior thorax: 1. ctrl – irradiated with 1 MED; 2. HA + I – pre-treated with vehicle (40 µl/cm² hydroalcoholic solution) HA and irradiated after 30 min with 1 MED; 3. BI + I – pretreated with BM extract 4 mg polyphenols/40 µl/cm² in HA, irradiated at 30 min with MED. Skin biopsies were sampled 1 hour respectively at 24 hours after UVB irradiation. Samples were examined histo-pathologically and by immunohistochemistry for DNA damage and apoptosis (using Anti Cyclobutane Pyrimidine Dimers – CPD antibody and Caspase 3 antibody). The BM extract reduced sunburn cells number, acute inflammation and formation of UVB radiation-induced DNA damage as demonstrated by reduced amounts of CPD, ultimately leading to reduced apoptosis. Our results suggest that BM extract might be a potential chemopreventive candidate in reducing skin cancer risk. Acknowledgements: Project PN II-42104/2008 References: 1. Chakraborti S, Chakraborti T (1998) Cellular Signalling 10: 675 – 683. 2. Muresan A (2010) Acta Physiol Hung 97(2): 240 – 246.

**PK5**

Improvement of high-fat-diet-induced metabolic syndrome by ethanol extract of *Polygonatum falcatum* (ID 1215B) in mice

Kweon H, Ko J, Yoon J, Jang H, Yoon S, Kang J

Research laboratories, Idong Pharmaceutical Co., Ltd., 23 – 9, Seoga-Dong, Hwasung-Si, Gyeyong-Di, Korea

**PK6**

Researches regarding skin anti-photoaging effect of *Linum usitatissimum* L. oil by using an in vivo skin imagistic dermatologic evaluation

Pop C1, Dragomirescu A2, Bojla P1

1Banat’s University of Agricultural Science Timisoara; 2University of Medicine and Pharmacie Victor Babes, Timisoara, Romania

Due to its content of unsaturated fatty acids and lignans (acting like phytostrogens) *Linum usitatissimum* L. oil has been proved several pharmaceutical properties in dermatologic and cosmetic field. The current stage of knowledge concerning the dermatological uses of this oil is: - photoprotection effect, an well-known propriety, already used by a lot of production pharmaceutical laboratories; - skin moisturizing and non-codemogenic effect of linum seeds lignans. The main lignan in flaxseed is secoisolariciresinol diglucoside (SDG) which plays a major role in the sebum rate decrease, by being a 5 alpha-reductase inhibitor. - unsaturated fatty acids are essentials for maintain the epidermal physiology. LA is the most abundant fatty acid in the epimeris. Importantly, it is the precursor to ceramides, a major component of the extracellular lipid matrix that forms the stratum corneum permeability barrier (SCPB). [2,3] Additionally, omega 3 fatty acids are involved in prevention of skin cancers. Our study is advocated the skin anti-photoaging effect of *Linum usitatissimum* oil. This effect was performed in vivo, on healthy volunteers, by registering the wrinkles involution with ProDerm Skin Analyzer. We admitted in study 22 females volunteers, ages between 33 an 56 years. The imagistic evaluations were registered after 14 days, 21 days and respectively 28 days of daily application. The oil content of the
Bilia A

Inclusion studies of falcarinol in β-cyclodextrin

Karioti A1, Leonti M2, Bergonzi M2, Bilia A1

1) Department of Pharmaceutical Sciences, University of Florence, Via Ugo Schiff 6, 50019, Sesto Fiorentino (FI), Firenze, Italy; 2) Dipartimento Farmaco Chimico Tecnologico, Facoltà di Farmacia, Università di Cagliari, Via Ospedale 72, 09124 Cagliari (CA), Italy

Falcarnel is a natural C17-polyacetylenic pesticide (phytotoxin) present in Apiaceae vegetables such as carrot (Daucus carota L.). Recently, the substance has attracted a lot of attention due to its interesting biological activities such as cytotoxic, antibacterial, and anticoagulant [1]. However, falcarnel suffers from photo- and thermal degradation, due to the presence of unstable triple bonds in its structure, which limits its possible applications. In the present work the thermal and photo-stability of falcarnel alone and in complex with β-cyclodextrin was studied. Falcarnel was isolated from the endemic Sardinian plant Sesieli praecox (Gamisans (Apiceae)) [2]. Falcarnel/β-cyclodextrin complexes were prepared and the inclusion complex was initially characterised by NMR (ROESY) spectroscopy. Accelerated thermostabilty testing proved to be an extremely aggressive method for this type of constituent resulting in the complete degradation of both, the compound and its inclusion complex. On the other hand, photo-stability studies were carried out successfully as the β-cyclodextrin complex provided protection to the substance which kept its macroscopically properties and protected the substance from degradation. In comparison the photostability assay generated a loss of 15% in uncomplexed falcarnel. Therefore, inclusion in β-cyclodextrin was proved to be a good method for the photoprotection of falcarnel.

Figure 1: Falcarnel/β-cyclodextrin inclusion complex


Curcumin is a natural polyphenolic constituent of Curcuma longa L. It has been generally associated with a large number of biological activities, including anti-oxidant, anti-inflammatory [1] and anti-cancer [2] properties. Although curcumin is a safe molecule even at high doses, its therapeutic use is limited by its low hydro-solubility in acid or physiological pH [3] and, consequently, by its poor bioavailability. Another drawback for clinical application of curcumin is its rapid hydrolysis under alkaline conditions and its photochemical degradation. The overall aim of this project is to increase solubility and stability of curcumin by microinclusion in cyclodextrins. Solubility studies of curcumin in presence of different concentrations of natural (α, β, γ) and semi-synthetic cyclodextrins (HEC, DMJ, TMJ, RAMEB, HPβ, HPγ) were carried at different temperatures (25 – 37°C). Thermodynamic parameters related to complex formation (∆G, ∆H and ∆S) were also evaluated. Stoichiometry of curcumin inclusion and apparent equilibrium constants (K1, K2) were evaluated by Job’s plot method using UV detection. Inclusion of curcumin into selected cyclodextrins was obtained by co-fusion, co-lyophilization, co-evaporation and physical mixture. Complex characterization was achieved by DSC, UV, NMR and HPLC/DAD analysis. Between the cyclodextrins tested the most efficient in order to maximise curcumin solubilisation was DMJ and the most effective complexation technique was the co-lyophilization. This latter was then employed, after curcumin complexation, for the realization of a topical formulation, useful as local anti-inflammatory medication, and pharmacokinetic was evaluated by in vitro test using Franz cells apparatus. References: 1. Dong-Oh M (2008) Bioch Bioph Res Comm 375: 275 – 279 2. Preetha A et al. (2008) Cancer Lett 267: 133 – 164 3. Tonnesen HH et al. (2002) Int J Pharm 244: 127 – 135.

Effects of different irrigation intervals on yield and yield components of black cumin (Nigella sativa)

Taeheh Noori M1, Seyyed Rahmani S2, Ghassemi Colezani K3

1) Agricultural Department, Azad University Maragheh branch, Maragheh, Iran; 2) Agricultural Insurance Fund, West Azerbaijan, Iran; 3) Department of Agronomy and Breeding, Faculty of Agriculture, University of Tabriz, Iran

Black cumin (Nigella sativa L.) is a medicinal plant with economic influences, especially in medicine production. A randomized complete block (RCB) experiment with three replications was conducted in 2010, to evaluate yield and yield components of black cumin (Nigella sativa) under no irrigation and three different irrigation intervals (7, 14 and 21 days) at research station of Islamic Azad university of Maragheh. Plants were sown in plots 20 cm plant to plant distance and 50 cm apart rows. Three irrigation intervals had significant effects on all studied characters. Results showed that increasing irrigation intervals to 14 days, increased number of capsules per plant, number of seeds per capsules and grain yield per plant, but produced smaller seeds. The lowest numbers of capsules and grain yield per plant were obtained in no irrigation treatment. Lowest Number of seeds per capsules and the largest grains produced in 21 days intervals. Increasing yield per plant in 14 irrigation interval was mainly attributed to the highest number of capsules per plant and number of seeds per capsules. References: 1. Ghamarinia H, Khosravy H, and Sepehri S (2010) J Medicinal Plants Research 4(16): 1612 – 1616. 2. Mohhebi M and Maleki H (2010) Advances in Environmental Biol 4(1): 10 – 13. 3. Nourouzpour Gh and Moghadam P (2007) Agronomy and Horticulture 19: 43 – 47.
Pharmacoeconomic evaluation of peppermint tea-bag products using graph theory
Elezovic A1, Elezovic A1, Uzunovic A1, Pilipovic S2, Hadidic S1
1Agency for Medicinal Products and Medical Devices, Titova 9, 71000, Sarajevo, Bosnia and Herzegovina; 2Faculty of Pharmacy, University of Sarajevo, Cekalusa 90, 71000 Sarajevo, Bosnia and Herzegovina

Peppermint (Mentha x piperita L.) has very long tradition of medicinal use due to its essential oil content. It is often also used as tea and food flavoring. The content of essential oil of peppermint leaf is crucial for its medicinal, but also flavoring effects. There are plethora of tea products on the market for the consumers to choose from, usually based on the product's price and the external package appearance. The consumers commonly don't have the insight into the tea-bag's content pharmacognostic and chemical quality. We have used pharmacoeconomic framework to make the surveillance of ten peppermint tea-bag products present on Bosnian and Herzegovinian market. Consumers were asked to give grades 1 – 10 for the products external packaging. The unit price was determined for each product. Pharmacists were asked to rate organoleptic appearance of herbal content of tea-bags. Modified methods of GC and GC-MS analysis described by Kowalski and Wawrzynkowski where used for the essential oil determination. The graph theory was chosen to sum up all results of different parameters for each product and give a quantitative estimate of the pharmacoeconomic acceptability. Relationship between tested parameters is presented in Figure 1. The obtained results reflect balance between external value on one side (> 1) and pharmacognostic quality (> 1), while value of 1 represents perfect balance of tested opposites. Of ten tested products, 8 predominated external value (0,19 to 0,70), while in 2 products predominated pharmacognostic quality (1,67 and 1,77). The graph theory was useful in assessment of herbal products.

Figure 1: Relationship between tested quality parameters


Phytochemical and hypoglycemic effect investigation of methanolic flower extract from Piper claussenianum
Marques A1, Cavalcante C2, Sudo S2, Pereira S2, Sudo R2, Zapata S2, Kaplan M1

Species of the genus Piper, the most important genus of Piperaceae Family, are widely used in traditional medicine for the treat many conditions. Chemical investigations of Piper species revealed many bioactive metabolites as amides, lignans, alkaloids, terpenes, flavonoids among others. In order to investigate native Piper species, phytochemical analysis of Piper claussenianum flowers was tested for hypoglycemic effect in rats with type 1 diabetes. Seven days after the induction, rats with glucose levels above 200 mg/dL began to be treated with vehicle or extract. Treatment lasted for 14 days and rats had glucose levels measured on days 0, 5 and 14. Glucose levels of both groups were also measured after 7 days of treatment interruption. Glucose levels of vehicle and extract groups were: day 0 (346.14 ± 41.67 and 275.29 ± 27.13, p > 0.05, n = 7), day 5 (290.14 ± 32.65 and 122.71 ± 7.19, p > 0.05, n = 7), day 14 (370.75 ± 77.89 and 137.50 ± 17.70, p < 0.05, n = 4). Seven days after treatment interruption, glucose levels were 304.14 ± 71.16 (vehicle, n = 7) and 255.50 ± 114.86 (extract, n = 4). Thus, the results suggest the remarkable presence of 2,6-Dihydroxy-4-Methoxychalcone on methanolic flower extract of Piper claussenianum has a noteworthy role to reduce blood glucose levels in rats with type 1 diabetes. Acknowledgement: The authors thank to CNPq.

Stability and staining property of gel from roselle calyx extract and butterfly pea flower extract
Kaeawmanee K, Pripream A, Preeprame S
Faculty of Pharmaceutical Sciences, Khon Kaen University, Khon Kaen, Thailand

Roselle calyx and Butterfly pea flower are among plants which have been used for coloring of food and beverage. The combination of the water extraction of the two plants present tanning color and was found interesting to study the property of an herbal staining gel. The herbs were extracted with boiling water for 2 hours, filtered and lyophilized until dried. The gel base was prepared by using combination of sodium carboxy methyl cellulose gel base and hydroxyethylcellulose 4000 gel base in 1:1 ratio. The herbal extractions were varied amount to add to the gel base. The color number in an expanded color chart were used to evaluate the usable color. The staining gel was tested for cracking, precipitation, changing of color, changing of pH before and after heating cooling cycle. The gel was tested for staining property by applied 0.5 g of gel on the pig skin and left for 1 hr then determine the color by using a color scale. The Mexameter MX 18 was used for determined of color uniformity of skin. The permanent of staining was tested by stirring the pig skin after staining for 24 hrs with 500 mililitres of water for 20 minutes and compare the color change. The gel with 1.35% of roselle extracted and 0.15% of butterfly pea extract was selected to be a staining gel. The color pH and physical property of gel are not changing after freeze and thaw for 3 cycles. The gel present consistency and permanent of staining. Acknowledgement: The faculty of pharmaceutical science Khon Kaen university for supportive of grant. References: 1. Kazuma K et al (2005) Phytochemistry 64: 1131 – 1139. 2.Therkildsen P et al (1998) Skin Res Technol 4: 174 – 179.

Stem gum of Moringa oleifera as pharmaceutical excipient
Hurakadle PJ, Patil DN
Department of Pharmaceutical Biotechnology, KLE University College of Pharmacy, Nehrunagar, Belgam-590 010, Karnataka, India

The herbal gum exudates from the stem of Moringa oleifera Lam. is sparingly soluble in water but swells in contact with water, giving a highly viscous solution. It consists of arabinose, galactose, and glucorononic acid. In the present study the formulation of paracetamol tablets by

The authors thank to CNPq.
using *Moringa oleifera* gum as a binder was performed. The four different tablet formulations loaded drug as paracetamol were prepared by wet granulation method. The binder concentrations used in the formulation were 2, 4, 6, and 8% w/w of *Moringa oleifera* gum. Tablets were subjected for evaluation of hardness, friability, drug content uniformity. The preliminary evaluation for granules was done by measuring the granule size, angle of repose and percentage fines. The percent friability was in the range and tablet showed 98% to 99% of labeled amount of paracetamol indicating uniformity in drug content with 6 to 20 min disintegration time and more than 90% dissolution at 80 to 90 min. Tablets at 6% w/w binder concentration showed more optimum results as tablet binder. Paracetamol tablets were prepared using newly developed herbal gums as binder for the controlled release. The results indicated that tablets were successfully formed and displayed good binding properties that can come as the new source of binder. The herbal gums obtained from *Moringa oleifera* stem gum could be utilized in the development of new pharmaceutical formulations.

**PK15**

Development and evaluation of conventional and PEGylated curcumin liposomes, absorption and tissue distribution studies in mice

Mazzacuva F, Iacchi B, Bergonzì M, Artigucci S, Pallamì S, Novelli F, Bilia A

1Department of Pharmaceutical Science, University of Florence, via U. Schiff 6, 50019 Sesto Fiorentino, Florence (Italy); 2Department of Preclinical and Clinical Pharmacology, University of Florence, Viale Pieraccini 6, 50139, Firenze, Italy

Curcumin is the main biological active polyphenolic compound present in the rhizomes of turmeric (*Curcuma longa* Linn.), has a wide biological and pharmacological profile. It has been reported to possess anti-oxidative, anti-inflammatory and anti-carcinogenic properties [1–3]. Many clinical study reports have revealed that curcumin has many beneficial properties in the treatment of various diseases in man such as pancreatic cancer and inflammatory bowel disease [4,5]. Despite these promising effects a poor oral absorption due to its extremely low aqueous solubility and rapid metabolism results in very low oral systemic bioavailability, thus limiting its clinical use. In order to overcome bioavailability drawbacks, this work proposes inclusion of curcumin into liposomal carriers. Liposomes were prepared by thin layer evaporation technique using phospholipid 90G, cholesterol and PEG–GSPE. Temperature of rehydration of thin film and curcumin amount were optimized in order to maximise efficiency of entrapment of drug inside the vesicles. Vesicles were characterized by dynamic light scattering and HPLC/DAD. The pharmacokinetic profile was tested in mice after i.p administration using stealth and conventional vesicles and an alcoholic solution of the drug. After 2 h, blood samples (liver, spleen, intestine, muscle, lung and heart) were collected and curcumin concentrations were determined using HPLC method. The results indicated that PEGylated liposomes have higher bioavailability compared to conventional liposomes. The aim of this study was investigating anticarcinogenic and antioxidative effects of *Cyclotrichium origanifolium* (Labill.) Manden. & Scheng. The anticarcinogenic activity of the methanolic and the water extract from *C. origanifolium* (at 10–1000 μg/ml concentration) in CCL-221 (colorectal cancer cell line) and Caco-2 (colon cancer cell line) was determined using trypan blue exclusion test. On the other hand, antioxidant activity of the extracts was evaluated by using DPPH radical scavenging, metal chelating, plasma lipid peroxidation and β-carotene bleaching assays. In addition, the measurement of total antioxidant compounds in the extracts were carried out. The most effective anticancer activity has been shown at concentrations 500 and 1000 μg/ml of the extracts against CCL-221. The water extract showed anticarcinogenic properties in CCL-221 with maximum inhibition of 81.3 ± 2.2% over the control at 1000 μg/ml. Also, no cytotoxic effect of the extracts was observed against normal cell line (human fibroblast cell line). *C. origanifolium* water extract (36.17 ± 0.35%) showed the highest inhibitory effect against plasma lipid peroxidation than the methanolic extract (20.02 ± 2.17%). *C. origanifolium* water and methanol extract showed effective scavenging activity against DPPH radicals (IC50 0.040 ± 0.003 mg/ml and IC50 0.051 ± 0.003 mg/ml, respectively). These results showed that *C. origanifolium* may be used in pharmaceutical applications due to its remarkable antioxidant and anticancer activity against CCL-221. Further studies such as fractionation and purification of the extract must be carried out in order to identify the active compounds.

**PK16**

Supercritical CO2 Extraction And Optimization of Total Phenols From Strawberry tree (Arbutus unedo L.)

Akay S, Alpak I, Yesil Celiktas O

Ege University, Faculty of Engineering, Bioengineering Department, 35100 Bornova, Izmir, Turkey

*Arbutus unedo* L. is an evergreen shrub of *Ericaceae* family growing on rocky slopes or in pine forests at the Mediterranean countries. Fruits of *A. unedo* were reported to possess astrigent, diuretic and antiseptic properties and to have high flavonoid content, mainly proanthocyanidins, anthocyanins as glycosides of cyanidin and delphinidin with cyanid-3-galactoside. Moreover, elagic acid, vitexin and kaempferol were also quantified. The aim of this study was to optimize total phenol composition and radical scavenging activities of *A. unedo* fruits by supercritical fluid extraction, using response surface methodology and to compare the total phenol contents with that of traditional water and ethanol extractions. The independent variables were temperature (30–80 °C), pressure (50–300 bar) and co-solvent ratio (0–205). The antioxidant capacities were determined by Folin-Ciocalteu and DPPH radical scavenging methods, whereas β-carotene bleaching method was used to evaluate the oxidative stability of the extracts in the linoleate emulsion model. The results demonstrated that temperature and co-solvent were more effective on yields of the compounds, whereas the effect of pressure was not noticeable. Optimum extraction conditions were elicited at 60 bar, 48 °C and 19.7% yielding 25.72 mg GAE total phenols/g extract and 99.9% radical scavenging capacity which were significantly higher than water (24.89 mg/g; 83.8%) and ethanol extracts (15.12 mg/g; 95.8%), whereas oxidation rate ratio (0.892) was close to that of water extract (0.661) demonstrating challenges as a green separation process for industrial applications.

**PK17**

Anticarcinogenic effect of *Cyclotrichium origanifolium* on human colorectal cancer cell line and associating with its antioxidative properties

Rostami S, Oke Altuntas F, Aslim B, Duman H

1Department of Biology, Faculty of Science, Gazi University, Ankara 06500, Turkey; 2Molecular Biology Research Center, Gazi University, Ankara 06500, Turkey

Urtica dioica L. (stinging nettle), a member of the Urticaceae family is a plant which has been used as a remedy for diabetes mellitus (1), benign prostatic hyperplasia (2), arthritis (3), allergic rhinitis (4), hypertension and cardiovascular disease (5). In recent studies it was shown that extract of *Urtica dioica* exhibited significant growth reduction in human prostatic epithelial cells (6) and inhibition on adenosine deaminase activity in prostate tissue. In this study we aimed to investigate the effect of *Urtica dioica* on proliferation of HCT-116 colon cancer cell line. Herbal preparation of *Urtica dioica* was made by ethanol extraction which was followed by evaporation. HCT-116 cells were incubated with different doses of *Urtica dioica* ranging from 3.33 mg/ml to 42.8 mg/ml for 24 hours. Cell viability was measured by MITT test. Results showed that *Urtica dioica* (33.3 mg/ml; 42.8 mg/ml) inhibited HCT-116 colon cancer cell proliferation significantly (p < 0.001). Further studies are needed to reveal the effectiveness of *Urtica dioica* as an alternative therapy for colon cancer. References: 1) Rasal VP, Shetty BB, Srinathambi A, Yeshmanna S, Ashok P (2006) Int J Pharmcol 4(2): 22. 2) Krzeski Tet al.
In recent years, the design of multifunctional polymeric materials in the submicrometer size has been considerably improved due to their wide applications in the fields of biomedicine. Particularly, hollow polymeric nanospheres and micelles have attracted a great deal of attention due to their wide range of applications. These structures have potential utility in encapsulation and controlled release of various biomolecules such as drugs, peptides and genes. A variety of multi stimuli-responsive nanoparticles have been synthesized that are capable of conformational and chemical changes on receiving an external signal. These changes are accompanied by variations in the physical properties of the polymer. The signal is derived from changes in the materials’ environment, such as a change in temperature or in pH. On the one hand, we have synthesized, characterized and studied organic micro- and nano-spheres for magnetic and non magnetic properties. Specifically, pH and thermal responsive hollow microspheres were prepared using the distillation precipitation polymerization method with magnetic nanoparticles encapsulated either in the shell or in the core. These novel hybrid microstructures were characterized with transmission electron microscopy, scanning electron microscopy, dynamic light scattering, vibrating sample magnetometry, X-ray diffraction and FT-IR spectra. On the other hand, polymeric micelles seem to be one of the best carriers for delivering hydrophobic drugs. They are formed by the self-assembly of amphiphilic block copolymer in aqueous solutions and have a spherical shape and a size in nano-range. Anticancer drugs that are incorporated into micelles were shown to improve their stability and efficiency. Acknowledgement: This work was supported by scientific program “IDEAS”, ERC Advanced Grand Nanotherapy. Project Reference: 232959. References: [1] Kataoka K. et al. (2003) Angew Chem Int Ed. 42: 4640 – 4643. [2] Minko S et al. (2010) Nature Materials 9:101 – 113. [3] Piskin E et al. (2006) Angew. Chem. Int. Ed. 45: 7671 – 7674. [4] Grabner W (1997) Phytomedicine 4: 105 – 108. [5] Daher CF, Baroody KG, Baroody GM (2006) Planta Med 56: 44 – 47. [6] Konrad L, Müller HH, Lenz C, Laubinger H, Aumüller G, Lichius J (2000) Planta Med 66(1): 44 – 7.

PK19


This study investigated the burn healing efficiency of Black seeds (Nigella sativa L.) oil on the second degree burn wound models in rats. Many of pharmacological activities of Nigella seeds such as anti-inflammatory and antioxidant were due to unsaturated fatty acids and essential oil [1,2,3]. In this study the hexanic extract of seeds was topically applied to evaluate the healing activity of seeds oil. Animals were randomly divided into three groups of six for each group. Burn wounds were created on dorsal part of shaved rats by a soldering iron with a flat contact surface (diameter 1.5 cm) on top (100 °C for 10 seconds). Silver sulfadiazine (SSD) was used as an antibiotic standard drug. Wound healing was evaluated by the rate of contraction and histological characteristics in treated and untreated groups. On day 12, the extract-treated animals showed 81.20% decreasing in the wound district and were significantly (P<0.05) more than control group 63.31%. Histological study showed fully grown regenerated epidermis on day 12 in treated animals. The results of this study suggest that burn wound healing potential of seeds may be due to anti-inflammatory, antioxidant and antimicrobial activities of main compounds oil.

PL2

Genetic variation study among Lepidium sativum resources in Egypt Ottai ME, Mostafa EA, Ibrahim MM. Genetic & Cytology Department National Research Center, Dokki, Giza, Egypt

Three local resources (Rajab, Haraz and Khider) of Lepidium sativum L. were used to study the genetic variation in Egypt. The study included quantitative characters, fatty acid and DNA fingerprint. Five quantitative characters were studied among each three successive seasons. Separated and combined statistical analysis presented significant variation among resources and among interaction between resources and seasons in the plant characters. GLC analysis of fatty acid methyl esters were carried out for each resource. Arachidonic acid was the most abundant acid followed by linoleic in Khider resource, while behenic acid was the most abundant acid followed by arachidonic acid in both Haraz and Rajab resources. By using six primers, DNA fingerprint showed differences in the number of bands among resources. The variations among Lepidium sativum sativum resources were confirmed on quantitative characters, fatty acids and DNA fingerprint level.

PL3

Anti-Dengue virus activity of Polygonum spectabile (Polygonaceae) Rodrigues RA1, Gomes Ruiz AC1, Brandão GC3, Evangelista KS1, Oliveira Junior HA1, Kroon EG2, De Oliveira AB2

1Viriontech do Brasil Indústria de Insumos e Serviços em Biotecnologia Ltda, Belo Horizonte, Brazil; 2Departamento de Microbiologia, Instituto de Ciencias Biologicas, Universidade Federal de Minas Gerais, Belo Horizonte, Brazil; 3Departamento de Produtos Farmacêuticos, Faculdade de Farmácia, Universidade Federal de Minas Gerais, Belo Horizonte, Brazil

Dengue is considered a worldwide public health problem and is responsible for thousands of deaths annually. However, the main strategies for prevention and control of dengue are focused on the virus vectors, since there is no specific therapeutic agent or vaccine for dengue viruses. In the quest of new anti-dengue drugs we have screened plant extracts which disclosed the activity of the aerial parts of Polygonum spectabile Mart., a plant traditionally used in Brazil for treatment of several infections diseases. However, the compounds isolated have shown no activity against DENV-2 (1) what has motivated a further investigation of this extract. We report here the evaluation of fractions obtained from the crude ethanolic extract by partition between immiscible solvents and of the fractions from the ethyl acetate chromatography over a Sephadex LH20 column. The in vitro cytotoxicity (LLCMK2 cells) and antiviral activity were evaluated by the MTT colorimetric method. Three, out of the seven fractions from the Sephadex LH20 column of the ethyl acetate fraction, have inhibited the viral multiplication cycle with EC 50 values between 41.52 ± 3.7 µg/ml and 5.58 ± 2.9 µg/ml. The determined CC 50 values were between 197.78 ± 19.7 µg/ml and 257.34 ± 24.1 µg/ml. Good SI values (CC 50/EC 50) have been calculated: 5.88, 11.52 and 46.11. These results show that chemical constituents of these fractions might be promising as anti-dengue drugs. Phytochemical and molecular studies are on progress aiming to determine the active compounds and mechanisms of action. Keywords: Dengue virus, antiviral activity, Polygonum spectabile

Inter-population variation in phenolic content of *Teucrium chamaedrys* L. from the localities in the Balkan Peninsula

Stankovic MS, Vassilev K, Stankovic MN, Milošević T, Topuzov M, Markovic A, Solyić S

1Department of Biology and Ecology, Faculty of Science, University of Krševnjevac, Radoja Donamovica 12, 34000 Krševnjevac, Serbia; 2Institute of Botany, Bulgarian Academy of Sciences, Acad. G. Boschev St., Bl. 23, Sofia 1113, Bulgaria; 3Special Nature Reserve – Zasavica, Svetog Save 19, 22000 Sremka Mitrovica, Serbia; 4Department of Pharmacognosy, School of Pharmacy, University of Athens, Panepistimiotepolis, Zografou, 157 71, Athens, Greece; 5Department of Chemistry, Faculty of Science, University of Krševnjevac, Radoja Donamovica 12, 34000 Krševnjevac, Serbia

Total phenolic content and flavonoid concentrations in methanolic extracts obtained from *Teucrium chamaedrys* L. in five natural populations of the Balkan Peninsula and a garden population were investigated and compared. The above-ground parts of plants were collected during the flowering phase and the methanolic extracts were prepared. The total phenolic content of the extracts was determined using Folin–Ciocalteu reagent and expressed as gallic acid equivalent. The obtained values varied between 142.04 mg GA/g and 265.91 mg GA/g. The concentration of flavonoids was determined using AlCl₃ and expressed as rutin equivalent. The obtained values ranged between 55.66 mg Ru/g and 90.48 mg Ru/g. The highest phenolic content was found in the plants collected from the mountain areas (Bulgaria, Serbia, Bosnia and Herzegovina) and somewhat lower content was found in plants from Mediterranean localities (Montenegro, Croatia). The lowest level was found in the extract obtained from the cultivated plant (Greece). The highest concentration of flavonoids was found in the plants from Mediterranean localities (Croaţia, Montenegro), while the levels were lower in the other samples and ranged between 50 and 70 mg Ru/ml. On the basis of comparative analysis, the plants collected at higher altitude localities were found to be richer in total phenolics, while higher concentration of flavonoids was found in *T. chamaedrys* from Mediterranean localities. A cultivar of *T. chamaedrys* had lower concentration of phenolics in comparison with natural populations. The results obtained in the analysis point out that the concentration of phenolics depend on the ecological properties of the plant habitats. Acknowledgement: Ministry of Science and Education, Republic of Serbia (NII/01010)

**PL5**

Phytochemical and pharmacological studies of *Ficus auriculata* Lour. (Family Moraceae) cultivated in Egypt

Al Fishawy A, Zayed R, Afji S

1Department of Pharmacognosy, Faculty of Pharmacy, Cairo University, Cairo, Egypt; 2Department of Pharmacognosy, Faculty of Pharmacy, Sini University, 55441 North Sinai, Egypt

This study scientifically examined the phytochemistry, antibacterial and anti-inflammatory potencies of two extracts of *Ficus auriculata* Lour. Eight known compounds, including: betulinic acid, lupeol, stigmastanol, bergapten, scopoletin, β-sitosterol-3-β-D-glucopyranoside, myrictin and quercetin-3-β-D-glucopyranoside were isolated from the petroleum ether, chloroform and ethyl acetate fractions of alcoholic extracts of the leaves and fruits of *Ficus auriculata*. The structures of these compounds were elucidated on the basis of various spectroscopic methods. This is the first report on compounds separation from *Ficus auriculata* (Moraceae). Concerning the biological studies, the results revealed that both extracts were effective against gram + ve bacteria (Staphylococcus aureus) and gram – ve bacteria (Escherichia coli) by agar well diffusion method. However, ethanolic extract of leaves exhibited greater antibacterial activity than the ethanolic extract of fruits. Meanwhile, the ethanolic extract of leaves at dose of 500 mg/kg exhibited significant anti-inflammatory effect using carrageenin-induced rat hind paw edema model. Keywords: *Ficus auriculata*, Moraceae, antibacterial activity, anti-inflammatory

**PL6**

Moringa oleifera-treated dry season-turbid Well-water in Enugu Metropolis, Nigeria: A comparative evaluation

Nnamdi OP, Otua CI, Attama AA, Inyia Agha St, Ibezim CE

1Drug Delivery Research Unit, Environmental Research Unit, Department of Pharmaceutics, Faculty of Pharmaceutical Sciences, University of Nigeria, Nsukka 410001, Enugu State, Nigeria; 2Department of Pharmacognosy and Environmental Medicine, University of Nigeria, Nsukka 410001, Enugu State, Nigeria

Water and sanitation services provide a cost-effective solution for alleviating the impact of water-borne diseases. Polluted water is gateway to infectious pathogens leading to a both acute and chronic-diseases worldwide. With the ultimate objective of contributing to the improvement of the quality control of drinking water, we report here, the main application of *Moringa oleifera* Lam. seed extract in the treatment of 25 natural underground well-water samples randomly collected from the three most populous cities in Enugu Metropolis, in southeastern Nigeria. The assessed parameters were salinity, pH, conductivity, total dissolved solids (TDS), total solids (TS), total suspended solids (TSS), turbidity and microbial load before and post-treatment with both alum (as a standard agent) and *M. oleifera* aqueous and ethanolic extracts at equal concentrations of 60 mg/L. The result of the finding showed the ability of *M. oleifera* seed extract to remove organic matter (natural humic substances and micropolllutants) thereby avoiding water degradation (mainly bad odours and taste, formation of disinfection by-products such as trihalomethanes) and in addition to having a potent antimicrobial activity which alum naturally lacked. The ethanolic extract of *M. oleifera* had broader spectrum of antibacterial activity than aqueous extract. The alum-treated water samples showed increased salinity and pH in addition to other by-products. From the foregoing, the use of *M. oleifera* aqueous and ethanolic seed extracts as alternative biocompatible flocculants in water treatment in Enugu Metropolis could be recommended. Acknowledgement: This work is a product of research for a Fellowship award of Nigerian Institute of Science Laboratory Technology (NISLT).

**PL7**

The effect of *Salvia virgata* on GSH-Px Activities of HepG2 cells.

Yerer Aycan MB, Seker Karatoprak C, Aylan C, Inanir M, Kosar M

1Department of Pharmacology, Faculty of Pharmacy, University of Erciyes, Kayseri, Turkey; 2Department of Pharmacognosy, Faculty of Pharmacy, University of Erciyes, Kayseri, Turkey; 3Department of Biochemistry, Faculty of Pharmacy, University of Erciyes, Kayseri, Turkey

Turkey is an important country for *Salvia* species. The flora of Turkey includes 88 species of the genus *Salvia*. *Salvia virgata* Jacq. which has been shown to be extremely rich with the phenolic compounds that allows this species to be a important member of antioxidant plants. This study was performed to investigate the effect of different *Salvia* extracts on GSH-Px activities of HepG2 hepatocarcinoma cells. The 70% methanol and water extracts were prepared from the aerial parts of *S. virgata* collected from Bursa, Turkey. Gallic acid and rosmarinic acid were used as positive controls. The cells at a number of 2 x 10⁵ cells per well were incubated for 24 h with the extracts and the positive controls under 5% CO₂ at 37 °C. The GSH-Px activities of the cells were then analysed spectrophotometrically via a multifunctional microplate reader. Phenolics rich extract of aq. methanol has enhanced the GSH-Px activity more than water extract where their effect was just in between the rosmarinic acid and gallic acid positive controls. These results reveal that both extracts mostly the phenolics rich extract of aq. methanol has enhanced the antioxidant activity in the hepatocarcinoma cell line and these results confirm that it can further effect the glutathione reserves of these cells. This preliminary results needs to be further investigated over the GSSG, GSH and total glutathione and selenium levels. Keywords: *Salvia Virgata*, HepG2, GSH-Px, antioxidant References: 1. Kosar M, Goger F, Baser KHC (2008) J Agric Food Chem 56(7):2369 – 74 2. Tosun M, Ercisli S, Sengul M, Ozer H, Polat T, Ozturk E (2009) Bials Res 42(2):175 – 81 3. Tepe B (2008) Bioreasur Technol 99(6):1584 – 8
Acrocarpus fraxinifolius Wight & Arn leaves were assayed using the disc diffusion method for their antibacterial activity against Escherichia coli, Staphylococcus aureus, and methicillin-resistant Staphylococcus aureus. The results indicate that these medicinal herbs can be used for the treatment of infections caused by these bacteria. The present study aimed to detect the antibacterial activity of Acrocarpus fraxinifolius leaves and to evaluate the nutritional and pharmacological activities of the medicinal plants. The results showed that the leaves of the plant had significant antibacterial activity against the tested bacteria, and the nutritional and pharmacological activities were also observed. The future research will focus on the isolation of active compounds and the development of new antimicrobial agents.
A large with four different doses of the fungal pathogen Seimatosporium hypericum and the soil bacterium Pseudomonas putida. Secondary metabolites were analysed by HPLC-DAD. An analysis of covariance was used to measure the overall effect of different inoculation doses of microorganisms on concentrations of metabolites. According to the results, inoculation of H. perforatum with both microorganisms had a significant effect on positive changes of hypericins, hyperforin, rutin, hyperoside, isoorientin and total phenolics. In H. triquetrifolium the amounts of hypericins and phenolic compounds did not vary significantly under the higher doses of inoculums with exception of a positive accumulation of hyperforin. The increased accumulation of hyperforin can be described to be very important in plant defense mechanism triggered by some of the components excreted by the microorganisms. The increased accumulation of hyperforin can be described as a most important compound in plant defense mechanism triggered by some of the components excluded by the microorganisms. The comparison of the microorganisms' effect on the biosynthesis of secondary metabolites showed that pathogenic fungi seem to have more influence than bacteria. The two species of Hypericum showed differences in the accumulation of secondary metabolites induced by biostress. Biological stimuli of microorganisms may allow a specific modulation of the biosynthesis of some desirable metabolites in plants. Acknowledgement: The research was supported by Research Council of Lithuania; project number MIP-57/2010. References: 1. Conceição et al. (2006) Floriculture, Ornamental and Plant Biotechnology Vol. 3: 487 – 487. Global Science Books. UK.

**PL13**

The content of fagopyrin and polyphenols in common buckwheat (Fagopyrum esculentum Moench) sprouts depends on growing conditions and the phase of development

Janos D1, Kreft S2, Kreft P2

1Department of Pharmaceutical Biology, Faculty of Pharmacy, University of Ljubljana, Slovenia; 2Department of Agronomy, Biotechnical Faculty, University of Ljubljana, Ljubljana, Slovenia

Dried buckwheat herb (Fagopyrum esculentum Moench) is used in medicinal products and the fresh green plant parts, especially sprouts, are consumed as a vegetable [1,2]. The herb contains fagopyrins, which cause sensitivity to light after the ingestion of large amounts of the green parts of buckwheat [3]. The aim of this study was to investigate the impact of different growing conditions and development phase on the content of fagopyrin and phenolic compounds in buckwheat sprouts. Total flavonoid and total phenol contents, fagopyrin content and antioxidant activity were determined spectrophotometrically. Fagopyrin and flavonoids were located almost exclusively in cotyledons. It was found that the content of fagopyrin in 14-days-old buckwheat sprouts grown in a sprouter was nearly the same as reported for mature plants, but the content of polyphenols was only at approximately 20% to 30%. The safe intake of buckwheat sprouts was then estimated to be at least 40 g per day. References: 1. Hinneburg I, Neubert Reinhard HH (2005) Agric Food Chem 53: 3 – 7. 2. Kreft I et al. (2006) Food Chem 98: 508 – 513. 3. Chick H, Ellinger P (1941) Physiol Plant 100: 212 – 230.

**PL14**

Comparative phytochemical study on Veronica officinalis L. and Veronica chamaedrys L. (Plantaginaceae sensu APG 2003, formerly Scrophulariaceae) are urgently needed [1], especially Veronica chamaedrys L. (germander speedwell), widely spread and without therapeutic action. We have studied the differential phytochemical characters, for the two species regarding the iridoids and polyphenolic compounds content. In these species we have determined the aucubin and catalpol content by using a HPLC analysis with mass spectrometry detection. The content of aucubin is 107.4 µg/g for V. officinalis and 328.6 µg/g for V. chamaedrys. The content of catalpol is 232.2 µg/g for V. officinalis and 144.4 µg/g for V. chamaedrys [2]. The polyphenolic compounds were determined in the two species before and after acid hydrolysis. The identification of these compounds was achieved through a HPLC analysis with mass spectrometric detection, by comparison with 18 polyphenol standards. The quantitative analysis of the polyphenols, based on UV detection, was performed using an external standard method [3]. The most significant difference between the two species is in the qualitative and quantitive content of polyphenolic compounds and it can be a criteria to phytochemical differentiation of V. officinalis and V. chamaedrys. Acknowledgement: This work was supported by the grant PN II 3251/2008 financed by MECI Romania. References: [1] APG (Angiosperm Phylogeny Group). An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG II (2003) Bot J Linn Soc. 141: 393 – 436 [2] Crisan Get al. (2010) Farmacia 58(2): 237 – 242 [3] Crisan G, Vlase L, Balica G, Crisan O (2009), Rev Med Chir Soc Med Nat Iasi 113(2); Suplement nr. 4, 81 – 85.

**PL15**

Pharmacognostic studies and establishment of quality parameters of Albizia altissima (Hook.f) Hutch et Dandy

Agboola OI, Chidiobi C, Omobuwajo OR

Department of Pharmcognosy and Herbal Medicine, Faculty of Pharmacy, Niger Delta University Wilberforce Island Bayelsa State Nigeria

Albizia altissima (Hook.f) Hutch et Dandy is a deciduous tree that grows up to 15 m in height and 25 cm in diameter and is found in various parts of Africa from Sierra Leone to West Cameroon, Sudan and up to Angola. It is used as a fish poison and in traditional medicine for the treatment of mental illness, snake bites, stomachache and toothache. The use in ethnomedicine for the treatment of mental disorders has been scientifically investigated and the results validated the ethno medicinal use Pharmacognostic studies of the leaves were carried out following the World Health Organization guidelines on the establishment of quality standards for medicinal plants. Other physiochemical parameters were also determined. Pharmacognostic investigations include macro and microscopic studies on fresh and powdered leaves, physicochemical constants like total ash, extractable (water and alcohol) material and chromatographic fingerprint analysis. The results showed the presence of paracystic stomata in the leaves exclusively on the lower surface, free prisms of calcium oxalate and clusters of calcium oxalate crystals embedded in the epidermal cells. The amount of water extractable matter was 130 mg/g, that of alcohol extractable matter 150 mg/g and the ash value was 30 mg/g. The results of this study are helpful for the preparation of a monograph for the Albizia altissima. Acknowledgement: The authors acknowledge Ajibesin K K and Raji R References: World Health Organization (1998): Quality control methods for medicinal plants, Geneva.

**PL16**

Development of a novel botanical drug (DA-9701), as a new prokinetic agent


Dong-a pharm, Seoul, Korea

Functional dyspepsia (FD) is a highly prevalent chronic gastrointestinal disorder that causes a considerable burden to both the patient and society. In the past ten years, several herbal extracts were reported from natural sources in our laboratory. This research was also carried out as a continuous work on bioactive extracts and for the development of prokinetic drugs from natural sources. Based on our prokinetic prescreening data, Cordialis tuber (Cordyceps sinensis Nakai) and Pharbitis seed (Pharbitis nil Choisy) were selected for this research. A prokinetic agent, DA-9701 has newly formulated with Cordialis tuber and Pharbitis seed. We evaluated the gastroprokinetic effects of DA-9701 to develop a therapeutic for FD. Cordialis tuber have been used as traditional Chinese medicine (TCM) in the treatment of gastric and duodenal ulcer. Pharbitis seeds are the seeds of Pharbitis nil Choisy of the Convolvulaceae family, has been used as a folk medicine for analgesic effects on the abdomen in the TCM. Oral administration with DA-9701 significantly accelerated gastric emptying and gastrointestinal transit. Furthermore, DA-9701 increased the gastric accommodation in Beagle dogs. These results indicate that DA-9701 has potential as a safe and effective prokinetic agent capable of lessening gastrointestinal symptoms and increasing quality of life in FD patients with abnormalities in GI motor function. At the present time, product development is in progress for complement of phase
PL17

A novel botanical drug (DA-9801) for the treatment of diabetic neuropathy
Dong-a pharm, Seoul, Korea

Diabetic neuropathy is one of the most common causes of chronic neuropathic pain. In our search for bioactive constituents from plant sources, we found a diabetic neuropathy agent, DA-9801. Ethanol extract of two herbal mixture (the rhizome of Dioscorea japonica Thunberg and Dioscorea nipponica Makino. DA-9801 induces increases in endogenous Nerve growth factor (NGF) levels, and thereby has a protective effect against diabetic neuropathy. NGF plays an important role in the survival and maintenance of neurons in the nervous system and in nerve injury repair. The rhizome of D. japonica has been used in traditional medicine and as food in East Asia to strengthen stomach functions and to dilute sputum in TCM. The rhizome of D. nipponica has been used in traditional medicine in East Asia for treatment of rheumatoid arthritis and diabetes. After phytochemical investigation we found 2 new furanotaxones besides 13 known compounds in DA-9801. We evaluated the anti-diabetic neuropathic effect of DA-9801 in a streptozotocin (STZ)-induced animal model. After treatment with DA-9801, NGF levels increased significantly in STZ-induced diabetic rats. Results from a nociceptive test (thermal & mechanical hyperalgesia) showed an increased latency time in groups treated with DA-9801 when compared with control and reference drug groups. The results suggest that DA-9801 may improve the damage produced by diabetic neuropathy via increasing the level of NGF in target tissue, shows improvement on nerve conduction velocity (NCV) and recovery from nerve degeneration. Therefore DA-9801 may have a potential therapeutic effect in patients with diabetic neuropathy. Acknowledgement: This study was supported by grant from the Korea Healthcare Technology R&D Project, Ministry for Health, Welfare & Family Affairs, Republic of Korea (2011-A11082).

PL18

A polyphenol enriched Theobroma cacao L. extract for cosmetic application
Ferrari CR, Jorge A, Lago JC, Arrotexta RF, Medina S, Martinelli M, Ambrosio V, d, Rocha V, Junior SD, Gesztesi JL. Natura Cosmetics, Cajamar, Brazil

It was found that long-term ingestion of cocoa flavonols was able to prevent a variety of dermal disorders associated to UV exposure, like decrease of skin thickness and skin density (1), possibly by means of production of glycosaminoglycans and collagen (2). The antioxidant activity seems to be the underlying mechanism (3). These findings led to the development of a polyphenol enriched cocoa bean (Theobroma cacao L.) extract for further use as a cosmetic ingredient. The proposed method of extraction of polyphenols was able to avoid the formation of insoluble tannins that normally occurs in the production of chocolate (4).

The extract was evaluated by three different and complementary DPPH, Liperoxidation and Plasminoidal DNA Protection Assay. In the DDPH assay, the extract showed an IC50 of 7 x 10-3 mg/mL, the same protection of 62% was achieved by the control BHT at 0.01%. In the liperoxidation assay the lowest concentration tested, 25,ug/mL showed a reduction of oxidation of 33.8% of the liposomes. The maximum level of protection was greater concentration tested under UVA radiation. The extract showed an IC 50 of 7 x 10-3 mg/mL, the same protection of 62%. In the Plasminoidal DNA Protection Assay, the damnification of the supercoiled (SC) DNA is done by UVA (4.7 J/cm2) and riboflavin (phototoxic In the Plasmidial DNA Protection Assay, the damnification of the supercoiled (SC) DNA is done by UVA (4.7 J/cm2) and riboflavin (phototoxic In the Plasmidial DNA Protection Assay, the damnification of the supercoiled (SC) DNA is done by UVA (4.7 J/cm2) and riboflavin (phototoxic In the Plasmidial DNA Protection Assay, the damnification of the supercoiled (SC) DNA is done by UVA (4.7 J/cm2) and riboflavin (phototoxic

...
**PL21**

**Sanguisorba hybridra: pharmocagnostic and antimicrobial activity evaluation**

Moreira I	extsuperscript{1}, Madureira AM	extsuperscript{1}, Duarte A	extsuperscript{1}, Feijó MD	extsuperscript{2}, Correia A	extsuperscript{1}, Teixeira CA	extsuperscript{1}

	extsuperscript{1}Universidade de Lisboa, Faculdade de Farmácia de Lisboa, 
	extsuperscript{2}Med Universidade de Lisboa, Avenida Prof. Gama Pinto, 1649 – 003, Lisboa, Portugal; 

**Abstracts | 59th International Congress of the GA | 4th–9th September 2011, Antalya, Turkey**

Sanguisorba L. is a Rosaceae distributed throughout the northern hemisphere. Some species are known to show hypoglycemic and hemostatic properties (1), antimicrobial (2) and antiviral activities (3). Sanguisorba hybridra (L.) Nordhög is endemic in Portugal (4) and was selected for pharmacognostic studies including a preliminary phytochemical survey and an evaluation of its potential against human pathogens. Samples were collected in SW Portugal (38° 8’ N – 8° 33’ W) during 2009 – 10 and identified at LISU. Under microscopy techniques non glandular and glandular multicellular trichomes were seen on both leaf surfaces. With histochemical tests the terpenoids and phenols were the most relevant compounds detected. Powdered plant material was extracted with n-hexane, dichloromethane, ethyl acetate, methanol and water. Their phytochemical survey, through TLC on silica gel plates and the proper reactions, was performed and the previous tests were confirmed. All extracts were tested against reference and multiresistant bacterial strains:

- **Gram-positive** (Enterococcus faecalis, Staphylococcus aureus, Staphylococcus epidermidis and Mycobacterium smegmatis); 
- **Gram-negative** (Pseudomonas aeruginosa, Salmonella typhimurium, and Klebsiella pneumoniae) and the yeast C. albicans.

The minimum inhibitory concentrations (MIC) were determined by the serial broth microdilution method. Appropriate antibiotics were used as controls. The methanol and water extracts showed better antimicrobial activity than n-hexane, dichloromethane, ethyl acetate extracts. Gram-positive bacteria were the most sensitive and the MIC values of 3.50 – 1.75 μg/mL were obtained using those polar extracts against Staphylococcus aureus, including strains resistant to meticillin (MRSA- Methicillin Resistance Staphylococcus aureus). 


---

**PL23**

**In vitro antiviral activity and cytotoxicity of the extracts of Salvia wiedemannii Boiss.**

Ustun O	extsuperscript{1}, Ozcelik B	extsuperscript{2}

	extsuperscript{1}Department of Pharmacognosy, Faculty of Pharmacy, Gazi University, Ankara, Turkey; 
	extsuperscript{2}Department of Microbiology, Faculty of Pharmacy, Gazi University, Ankara, Turkey

**Abstracts | 59th International Congress of the GA | 4th–9th September 2011, Antalya, Turkey**

Herpes simplex type 1 (HSV-1) and Parainfluenza-3 (PI-3) viruses are important pathogens for humans. Although antiviral drugs are available, resistance to these antiviral medications have been increasing (1,2). Salvia L. (Lamiaceae) is widely distributed in Turkey by 94 taxa belonging to 89 species, with a 50% ratio of endemism (4). Some parts of Salvia species have been used in Turkish folk medicine for the treatment of various disorders and symptoms, including catarrh, cold, wounds, stomatitis, flatulence, constipation, rheumatic pain, wards, sunstroke, and hemorrhage. In addition, there are also some reports on the antiviral effects of Salvia species. In this study, we evaluated the antiviral efficacy of S. wiedemannii Boiss. extracts on HSV-1 and PI-3 viruses by using Vero cell lines. Antiviral efficacy of these extracts, obtained from aerial parts of S. wiedemannii, was compared to that of acyclovir and oseltamivir. The H2O, CHCl3, and EtOH extracts of S. wiedemannii (16 – 0.0625 μg mL\(^{-1}\)) showed a significant antiviral activity on HSV-1 with the IC50 of 16 μg mL\(^{-1}\). Only the BuOH extract of S. wiedemannii demonstrated important antiviral activity on PI-3 with a range of 64 – 16 μg mL\(^{-1}\) of inhibitory concentration for CPE, which was close to the ant PI-3 activity of oseltamivir. This study has showed that S. wiedemannii extracts have important antiviral activities and can be used as a source for drug development. References: 1. Rebecca CB et al. (2004) Antiviral Res 61: 73 – 81. 2. Hall CB (2001) N Engl J Med 344: 1917 – 28. 3. Davis PH. (1982) Flora of Turkey and the Aegean Islands. Edinburgh.

---

**PL22**

**New results on saponins and saponin-rich plant-extracts enabling synergistic cytotoxicity with type-I-RIPs/lectins**

Böttger S, Melzig MF

Institute of Pharmacy, Free University Berlin, König-Luise-Str. 2+4, 14195 Berlin, Germany

**Abstracts | 59th International Congress of the GA | 4th–9th September 2011, Antalya, Turkey**

The synergistic cytotoxicity between saponins and lectins, especially the naturally very low cytotoxic activity showing type-I-RIPs (ribosome-inactivating protein type I)/lectins is known for years by now (1). It has become a promising strategy in anti-cancer research (2). While the pre-appliance of certain saponins can drastically amplify the cytotoxicity of the type-I-RIPs [3, 4], it may also minimize the required effective dose of these very expensive (especially when linked to human antibodies) and time-consuming to purify/to create substances [5]. In our work we searched for new saponins and saponin-rich plant-extracts capable of increasing the cytotoxicity of the naturally very low cytotoxic activity showing lectin saponin, considered as a standard type-I-RIP. The spotlight of our research was put on the plant-family of Caryophyllaceae, but saponins and saponin-rich plant-extracts from other plant-families were also tested when fulfilling certain structural conditions. All tests were performed in a cell culture model using ECV-304 cells. The cytotoxicity was measured by MTT assay and DNA quantification. References: 1. Hebestreit P, Melzig MF (2003) Planta Med 69: 921 – 925. 2. Bachran C et al. (2008) Immunother 32: 713 – 725. 3. Hebestreit P et al. (2006) Toxicicon 47: 330 – 335. 4. Weng A et al. (2008) Chem Bio Int 176: 204 – 211. 5. Bachran C et al. (2010) Brit J Pharmacol 159: 345 – 352.

---

**PL24**

**Evaluation the bioactivities of some extracts of Cistus laurifolius**

Ustun O, Ozcelik B, Baykal T

**Abstracts | 59th International Congress of the GA | 4th–9th September 2011, Antalya, Turkey**

S. wiedemannii

Evaluation the bioactivities of some extracts of Cistus laurifolius

Ustun O, Ozcelik B, Baykal T

Department of Pharmacognosy, Faculty of Pharmacy, Gazi University, Ankara, Turkey; Department of Pharmaceutical Microbiology, Faculty of Pharmacy, Gazi University, Ankara, Turkey

Some Cistus species (Cistaceae) can be found in Turkey; five of them have been identified (1). The leaves of Cistus species have been used against high fever, rheumatic pain, peptic ulcer, stomachache and urinary inflammations in Turkish folk medicine (2,3). For Cistus some biological effects are reported, including antimicrobial, antibacterial, antiviral, anti-inflammatory, anti-Helicobacter pylori, antiulcer, analgesic, anti-oxidant, antihypertonic, antiaggregant, and anticoagulant activity. In the present study, ethanol, hexane, chloroform, butanol, and water extracts of C. laurifolius L. were screened for their in vitro antibacterial, antifungal and antiviral activity. Antibacterial and antifungal activities were tested by the microdilution method against both, standard and isolated strains, of gram negative (Escherichia coli, Pseudomonas aeruginosa, Proteus mirabilis, Klebsiella pneumoniae, Acinetobacter baumannii) and gram positive (Staphylococcus aureus, Enterococcus faecalis) bacteria, as well as fungi (Candida albicans, C. parapsilosis). Antiviral activities of these extracts were tested against Herpes simplex virus Type-1 (HSV-1) and Parainfluenza-3 virus (PI-3) by using Madin-Darby Bovine Kidney and Vero cell lines. All extracts (32 – 64 μg mL\(^{-1}\)) exerted strong antimicrobial activity against standard and isolated strains of E. coli which are close to effects with the control antibiotic ampicillin (MIC: 64 μg mL\(^{-1}\)). The hexane extract (CPE of 32 – 8 μg mL\(^{-1}\)) had remarkable antiviral activity against PI-3. References: 1. Davis PH (1998) Flora of Turkey and the East Aegean Islands. Edinburgh. 2. Yesilda G et al. (1995) Ethnopharmacol 46: 133 – 152. 3. Honda G et al. (1996) J Ethnopharmacol 50: 1 – 13. 4. Smith SB et al. (1997) Flora of turkey. 5. Davis PH. (1982) Flora of Turkey and the Aegean Islands. Edinburgh. 2011; 77: 1229–1472 Georg Thieme Verlag KG Stuttgart · New York · ISSN 0032-0943

Planta Med 2011; 77: 1229–1472 Georg Thieme Verlag KG Stuttgart · New York · ISSN 0032-0943
Biologically active flavonoid glycosides from *Horwoodia dicksoniae* Turrill
Fawzy GA, Al Taweel AM, Abdelbaky NA, Marzouk MS  
1Pharmacognosy Department, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia; 2Pharmacology Department, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia; 3Pharmaceutical Chemistry Department, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia

Three flavonoid glycosides and one aglycone have been isolated from the ethanolic extract of *Horwoodia dicksoniae* Turrill for the first time, and their structures were assigned from 1H- and 13C-NMR spectra (DEPT) and from negative ESI-MS, as luteolin-7-O-D-glucopyranoside (1), apigenin-6-C-D-galactopyranoside (2), luteolin-6-C-b-D-galactopyranoside (3) and luteolin (4). The SRB cytotoxicity assay was used to investigate the antitumor activities of the ethanolic extract, compounds 1, 3 and 4. Compound 1 showed the highest cytotoxic activity against the three human cell lines; HEPG2, HCT 116 and MCF 7 (*IC_{50}* 10.7, 9.3 and 9.9 μg/mL, respectively), compared with the standard cancer drug doxorubicin. Compound 4 showed selective antitumor activity against the colon cell line (*IC_{50}* 9.5 μg/mL). The present investigation also demonstrates the protective effect of compounds 1, 3 and 4 with antioxidant potential, in glycerol-induced myoglobinuric acute renal failure in rats. Treatment with each of these compounds attenuated renal dysfunction, and restored the oxidant balance by decreasing renal MDA levels, increasing the activity of the depleted renal antioxidant enzymes, and the non enzymatic antioxidant GSH. They also, decreased the elevated serum inflammatory marker (TNF-α), and ameliorated apoptosis and kidney damage by reduction in caspase-3 activity. Compound 1 showed the highest biological activity.

---

**PL26**

Biodiversity assessment of *Veronica* sp. in Romania for their characterization, preservation and sustainable use in pharmacognosy
Ichim MC, Racatariu AC, Paramon PP, Toth ET  
1NIORDS’Stefaru’ Research Centre for Biological Sciences, Alexandru cel Bun St, 6, Ploiesti Neamt, 60004, Romania; 2Targu Mures University of Medicine and Pharmacy, Cheghe Ore Marinescu St., 38, 540139, Targu Mures, Romania

*Veronica* is the most species-rich genus of Plantaginaceae family with about 500 species. The majority of the species are herbs, but there are also perennials and they are very diverse from an ecological point of view [1]. Based on morphological and molecular analyses it was estimated that about 80 species of *Veronica* are found in Europe; about 40 species found in nine different subgenera are endemic to Europe and about 40 species have been reported in literature as being present in Romania [1, 2]. The genetic diversity and the chemical composition, useful for pharmacognosy [3] (*V. officinalis*, especially), of the *Veronica* species have encouraged different types of research to be carried out. Our aim is to assess the biodiversity of the species from *Veronica* genus on the Romanian territory in order to contribute to the protection of their biodiversity. These studies will subsequently result in offering new Veronica species as alternatives to pharmacognosy and to long-term biodiversity reconstruction and sustainable use of these sources of pharmacologically active compounds. Different Veronica sp. have been identified in different types of habitats, including some from NATURA 2000 sites, and distributed all across Romania: West (Timisoara, Arad and Hunedoara counties), South (Gorj and Valcea counties), Center (Mures, Cluj and Harghita counties) and North-East (last, Neamt and Sucuavea counties). Have been identified 21 taxons, out of which 20 species and one sub-species. The most abundant Veronica species proved to be: *V. chaemaeleyri*, *V. officinalis*, *V. beccabunga*, *V. persica*, *V. piretii*, *V. spicata* L. and *V. spicata* L. ssp. *orchidea* (Crantz) Hayek. Acknowledgement: This study was supported by UEFISCDI/project 32151/ 2008. References: 1. Albach DC et al. (2006) Mol Ecol 15: 3269 – 3286. 2. Ichim MC et al. (2010) Bulletin UASVM Agriculture 67(2): 482. 3. Crisan G et al. (2010) Farmacia 58(2): 237 – 242.

---

**PL27**

Centipeda cunninghamii, an australian traditional medicinal plant
Beattie KD, Waterman PG, Leach DN  
Center for phytochemistry and pharmacology, southern cross university, lismore, australia

*Centipeda cunninghamii* (DC.) A.Braun & Asch. is an endemic Australian Asteraceae with a long history of traditional use as a medicinal plant for treating wounds, infections and inflammation. Whilst its essential oil composition, principally chrysanthenyl and sabinyl acetates, has been known for some time, there was little scientific information regarding its phytochemistry and biological activity. Investigations on aqueous ethanol extracts confirmed its anti-inflammatory and antioxidant (ORAC) activity. Detailed investigations suggest the extract acts against a range of inflammatory markers including COX-1, COX-2, NO and TNF-α, but not through the lipoxigenase pathway. Seventeen compounds were isolated and subsequent bioassays indicated that the anti-inflammatory activity was linked to flavonoids, whilst the antioxidant activity was attributed to both flavonoids and a group of novel heptenedioic acid cinnamoyl esters. The latter compounds are ring-opened quinic acid derivatives and appear to be unique to this species. Optimisation of growing, post-harvest and extraction conditions based on quality markers have been developed for future production and product development.

---

**PL28**

Biodiversity of carrot genetic resources – variation in secondary metabolites
Baranski R, Allender C, Kaminska P, Jemioła Rzemińska M  
1Department of Genetics, Plant Breeding and Seed Science, Faculty of Horticulture, University of Agriculture in Krakow, Krakow, Poland; 2Warwick Genetic Resources Unit, The University of Warwick, Wellesbourne, Camp, Wellesbourne, Warwick, The United Kingdom; 3Department of Botany and Plant Physiology, Faculty of Horticulture, University of Agriculture in Krakow, Krakow, Poland; 4Department of Plant Physiology and Biochemistry, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Krakow, Poland

*Daucus* genetic resources comprises a few thousand accessions collected in gene banks mainly in USA, Europe and Russia. In 2009 a sub collection of 94 accessions representing edible and wild carrot species was established that should represent available biodiversity. The choice of the accession was done mainly based on their passport data supplemented with data obtained during European programme on carrot characterization during which morphological characters were assessed. The aim of the presented work was to assess variation of the chosen accessions with regard to their composition of secondary metabolites. The analytical investigation was focused on carotenoids, including alpha-, beta-carotene, lutein, lycopene and their precursor phytoene, reducing and non-reducing sugars, phenolics, including anthocyanins, flavonoids and phenylpropanoids and tocopherols. The results obtained revealed considerable variation of secondary metabolites content depending on genetic background. Edible carrots possessed higher carotenoid content, while phenolics dominated in wild relatives. Several accessions with high level of these compounds with importance for human health were identified. These materials may be prioritized in genetic and breeding programs for the development of high nutritional carrot cultivars. Acknowledgement: Research was supported by Polish Ministry of Agriculture and Rural Development (grant No. H01 RN/H-078 dec-1/10).

---

**PL29**

Quantitative Determination of Lycorine in *Galanthus xvalenthei* nothosubsp. subpicatus
Unver Somer N, Cicek Polat D, Onur MA, Kaya G  
1Department of Pharmacognosy, Faculty of Pharmacy, Ege University, Bornova-Izmir, 35100, Turkey

*Galanthus xvalenthei* (J. Allen) Beck nothosubsp. *subpicatus* (N. Zeybek) A. P. Davis (Amaryllidaceae) is a hybrid between *G. nivalis* L. and *G. plicatus* M. Bieb. subsp. *hyazinzus* (Baker) D. A. Webb. This *Galanthus* L. hybrid is endemic and it occurs naturally in north-western Turkey [12]. Lycorine, a common alkaloid found in Amaryllidaceae plants, has been shown to possess important biological activities including antiviral [3], cytotoxic [4] and antimalarial activities [5]. In the present study, a reversed-phase high-performance liquid chromatographic method has
been used for the quantitative determination of lycorine in the aerial parts and bulbs of G. xalvantenii nothothubis subplicatus [6]. A simple method for the extraction of lycorine in low-mass plant samples was employed utilizing pre-packed columns with diatomaceous earth (Ex- trel®) [7]. The chromatographic separation was carried out on an isocratic system with a mobile phase of trifluoroacetic acid-water-acet- onitrile (0.01: 95: 5) applied at a flow rate of 1mL min⁻¹ using diode array detector. The linearity of the method was studied by injecting five known concentrations of lycorine in the range of 0.5–8 µg µL⁻¹. The calibration curve for lycorine was determined as Y = 14.9668622x + 0.771199. The content of lycorine in the bulbs of G. xalvantenii notho- subsubplicatus was found to be 0.0028%. Lycorine was not detected in the aerial parts of this hybrid. Acknowledgement: This study was financially supported by Ege University Research Fund 09/ECZ/037 and partially supported by TUBITAK (TBAG-104T272) and EBILTEM (2007-BIL-007). References: 1. Davis AP et al. (2001) Kew Bull 56: 639–647. 2. Davis A (2006) The Genus Galanthus—Snowdrops in the Wild, in Bishop M., Davis A.P., Grimshaw J. (Eds.), Snowdrops, A Monograph of Cultivated Galanthus. Griffin Press Publishing Ltd. Cheltenham. 3. Szlávik L et al. (2004) Planta Med 70: 871–873. 4. Weniger B et al. (1995) Planta Med 61: 77–79. 5. Sener B et al. (2003) Phytotherapy Res 17: 1220–1223. 6. Mustafa NR et al. (2003) J Liq Chromatogr R T 26:3217–3233. 7. Berkov S et al. (2008) Phytochem Anal 19: 285–293.

Medicinal plants of the Royal Botanic Garden site at Tell Ar-Rumman in Jordan
Taftour H, Nawash OS, Al Amen A
The Royal Botanic Garden, Jordan, PO Box 99 Amman 11910 Jordan


From structural studies of natural products to the discovery of a selective antimalarial derivative: a serendipity story
Benidir M¹, Litaudon M², Rossanoaio P², Grellier P², Gueritte P²
¹Centre de Recherche de Gif, Institut de Chimie des Substances Naturelles, CNRS, Avenue de la Terrasse, 91958, Gif-sur-Yvette, France; ²Institut Malgache de Recherches Appliquées, B.P. 3833, 102 Antananarivo, Madagascar; ³Muséum National d’Histoire Naturelle, 63, rue Buffon, 75231 Paris cedex 05, France.

In our continuing effort directed to the search for new antimalarial natural compounds from plants of the tropical biodiversity, the phytochemical study of Canthium majus Drake, a Madagascan plant belonging to the family Rubiaceae, was carried out. Bioguided fractionation of the ethyl acetate leaf extract led to the isolation of eight new diarylhepta- noic glucosides together with the known jatroponol, which showed a weak antimalarial activity due to a possible stromatocytogenic[1] ef- fect. The structures of the diarylheptanoid glucosides were similar to those isolated from the rhizomes of Taccu chantrieri André by Yokosuka and co-workers[2]. After chemical modifications of the natural gluco-}

Antiprotozoal and cytotoxic activities of some mushrooms from Turkey
Usun O¹, Kaiser M², Tasdemir D³
¹Department of Pharmacognosy, Faculty of Pharmacy, Gazi University, Ankara, Turkey; ²Institute of Veterinary Parasitological and Infection Biology, Swiss Tropical and Public Health Institute, CH-4002 Basel, Switzerland; ³Centre for Pharmacognosy & Phytotherapy, The School of Pharmacy, University of London, London WC1 AX, UK

Protozoal infections still constitute a major health problem worldwide. Due to emerging resistance to common antiprotozoal agents, new drugs are urgently needed. Mushrooms are simple, non-photosynthetic organisms widespread in the world flora. Some are inedible or toxic, but many of them are used medicinally or in cooking. Some biological effects, e.g. antioxidant, immunomodulatory, antitumor, antimicrobial and antipro- tozoal of mushrooms have been shown [1–4]. In this study, we evaluated the in vitro antiparasitic and cytotoxic potential of ethanolic ex- tracts of some mushrooms growing in Turkey, namely Polyopara gigus, P. sulphureus, P. amnusos, P. rhedes, P. pinicola, P. volvatus, P. bodius, Cantharelus cibarius, Canodermia applanatum, Fomes fomentarius, Clavu- lina cinerea, Cortinarius orellanus and Trametes versicolor. The test organisms used were Trypanosoma brucei rhodesiensis, T. cruzi, Leishmania donovani and Plasmodium falciparum. Cytotoxic effects of the extracts were also assessed towards primary mammalian L6 cells. All mushroom extracts were active against T. brucei rhodesiensis with P. rhedes being the most potent (IC₅₀ 0.59 µg/mL). The most potent extracts against L. donovani and P. falciparum were those of P. gigus, P. sulphureus, P. amnusos, P. pinicola, F. fomentarius, C. cinerea and T. versicolor (IC₅₀ values 1.39–2.73 µg/mL). The extracts were also assessed cytotoxic activity against HL-60, KB and MRC 5 cell lines. To our knowledge, this is the first biological activity and anti- protozoal screening study carried out on Turkish mushrooms. The activity-guided isolation of the most active extracts is in progress. References: 1. Ribeiro B et al. (2008) Food Chem 110: 47–56. 2. Guerra DCM et al. (2007) Int Immunopharmacol 7: 1160–1169. 3. Kaneko R et al. (2004) Food Chem Toxicol 42: 909–916. 4. Samchial S et al. (2009) J Biol Sci 9: 778–783.

Climate change impact on conservation status of wild Melissa officinalis L. (Lamiaceae) populations in Armenia
Abrahamyan A¹, Telians A², Zorins A²
¹Department of Environmental Protection, Rezezke Augustskola, Rezezke, Latvia; ²Department of Computer Sciences and Mathematics, Rezekne Augustskola, Rezekne, Latvia

Climate change and temperature may lead to long-term irregularities in inter-specific interaction and may alter plant populations’ dynamics, its structure and ecosystem functioning in the region [1,3]. Studies on possible effects of climate change on medicinal plants biodiversity and conservation status are particularly significant due to their value within traditional systems of medicine and as economically useful plants. Current- ently, only limited data on conservation status under the impact of global climate change of these species is available in Armenia [2]. Anthropic threats to biodiversity (overpopulation, deforestation and urbanization) have simultaneously hindered research and increased the need for it. From 2006–2009, field studies were conducted to find
out changes the growth, phenological and habitat characteristics of Melissa officinalis L., population size and location (GPS mapping). In 2010, we have implicated these research data to carry out future assessment of the risk analyze and impact of global climate change on its population distribution and conservation status. Neural network and genetic algo-

rithms have been identified as stochastic self-learning methods to in-

vestigate hidden regularities between different data. Certain factors, such as biological characteristic of plants, habitat of the populations, anthropogenic threats and climate change have been identified as the key elements. In fact, vulnerability of plant population, particularly will increase central and northern part of the country, as they identified to be comparatively stressful environment under global climate change and anthropogenic threats, which included: poor land management, increasing population pressure, and excessive collection of plants. References: 1. Hughes L (2000) Trends Ecol Evol 15: 56 – 61 2. IUCN-WHO-WWF (1993) Guidelines on the Conservation of Medicinal Plants, IUCN, Gland, Switzerland, 50 p. 3. Bishop JG, Schemske DW (1998) Ecol 79: 534 – 546

Chemotaxonomy, or chemotaxonomy, is the attempt to classify and identify plants, according to demonstrable differences and similarities in their secondary metabolites. Thus, chemotaxonomic markers are powerful tools for the identification of a wide variety of plants [1]. Chinese forest scientists have been making significant efforts to develop fast-growing trees due to the extreme shortage of wood resources. Tri-

ploid Populus tomentosa Carr. (Salicaceae), the cloned hardware poplar species from Populus tomentosa, has been receiving the most attention [2]. However, secondary metabolites of triploid P. tomentosa have never been studied to date, though poplars have been widely used in folk medicines for the treatment of various diseases [3]. This work was car-

ried out to investigate the secondary metabolites and the chemotaxy-

matic markers from triploid P. tomentosa. Column chromatographic pur-

ification of triploid P. tomentosa extracts resulted in the isolation of twelve phenolics: grandidentatin, isograndidentatin A, isograndident-

tatin B, caffeic acid, populoside A, salirepose, luteolin, salicin, apigen-

in, populoside, p-coumaric acid, and 7-O-cafeoylsalirepin. The struc-

tures of the isolated secondary metabolites were extensively elucidated and characterized by spectroscopic method, including 1D and 2D NMR, and EI, FAB and MALDI-TOF MS. This was the first investigation of the secondary metabolites of triploid P. tomentosa wood. The isolation of isograndidentatin A, isograndidentatin B, grandidentatin, here in triploid P. tomentosa was interesting and glucosides of 1,2-dihydroxyxyclohex-

ane acylated by p-coumaric acid (or p-coumaric acid derivatives) could be considered as useful chemotaxonomic marks within the Salicaceae family, which was also well in accord with the our previous conclusion [4]. Acknowledgement: This work was financially supported by the Project for New Century Excellent Talents in University (NCET-2010). Foundation for the Development of Science and Technology in Tianjin Universities (No. 200808616), National Natural Science Foundation of China (NSFC, No. 31000279) and Natural Science Foundation of Tianjin University (No. 09JCJB14000). References: 1. Bohm BA (1987) The Bot Rev 53: 197 – 279. 2. Si CL et al. (2009) Biochem Syst Ecol 37: 221 – 224.


Effects of chemical and organic fertilizers on number of corm and stigma yield of saffron (Crocus sativus)

Effects of chemical and organic fertilizers on number of corm and stigma yield of saffron (Crocus sativus)

Saffron (Crocus sativus L.) is the world’s most expensive spice and 95% of the production is coming from Iran. [1]. The aim of this study was to better understanding the effects of different organic and chemical ferti-

izers on number of corm and stigma yield of saffron. This experiment was conducted in Organic Farm of Ferdowsi University of Mashhad, Iran, based on CRBD with three replications. The experimental treatments were four different fertilizers including chemical (50 – 250; 100 – 250 and 300 – 250 kg/ha N-P2O5), cow manure (20, 40 and 60 t/ha), sheep manure (20, 30 and 40 t/ha) and hen manure (5, 10 and 15 t/ha). The results which is reporting here, came from fifth year of the experiment. Results showed that the highest fresh flower and dry stigma yield were
obtained from chemical fertilizer (300 – 250 kg/ha N-P2O5) and then from cow manure (20 kg/ha) treatments. Fresh flower and dry stigma yield were increased by increasing the nitrogen level in chemical fertilizer and increasing sheep manure levels. The same results have been reported by Behnia et al. [1], Behzad et al. [2] showed that application of 200 kg ammonium phosphate plus 30 tons of cow manure produced the highest stigma yield. Rezvani moghadam et al. [4] reported that cow and chemical fertilizers produced more flower and stigma yield than hen manure. Sheep manure at 40 t/ha produced the highest mother corn and replacement corn per clump. Saffron is a low nutrient demand plant and requires a modest amount of nutrients [3]. References: 1- Behnia MR et al. (1999) Agron Crop Sci 182: 9 – 15. 2- Behzad S et al. (1992) Acta Hort 306: 337 – 339. 3- Housini M (1998) Iranian Scientific and Industrial Research Organization, Press -Khorasan Center. 4- Rezvani Moghadam et al. (2006) 2nd International Symposium on Saffron Biology and Technology, Iran.

In the order Asterales only two species are known to have proteolytic activity in their latices, first Taraxalis from dandelion Taraxacum officinale Webb s.l., and second Partnahren from Guayule Parthenium argentatum L. Both are characterized as serine endopeptidases [1]. Proteolytic enzymes isolated from plant latex have received special attention in the pharmaceutical industry and biotechnology due to their property of being active over wide range of temperature and pH. Nearly half of the commercially available enzymes are proteases, frequently used in food processing, tenderization of meat, brewing, cheese elaboration, bread manufacturing, leather and textile industries [1]. In this investigation the latex of 40 species of the Asteraeaceae family and 8 species of the Campanulaceae, which are not biochemical characterized before, were collected in the Botanical Garden Berlin. To determine proteolytic activity we used the fluorogenic substrate BODIPY FL-casein (Molecular Probes, Inc., USA) [2]. To investigate the type of endopeptidases, the latex samples were pre-incubated with specific inhibitors for serine proteases (fluoride hydrochloride)), aspartatic proteases (Pepstatin A) and metalloproteases (E64 (4-(2-Aminoethyl) benzenesulfonyl chloride), cysteine proteases (E64 (4-(2-Aminoethyl) benzenesulfonfonyl fluoride hydrochloride)), aspartic proteases (Pepstatin A) and metalloproteases (EDTA) and the remaining activity was determined. In both families highly active serine proteases were found. References: 1. Dom-salla A, Melzig MF (2008) Planta Med 74: 1 – 13 2. Menges DA et al. (1997) Anal Biochem 251: 144 – 147

Acidoid Properties and Phenolic Composition of Viburnum opulus from Turkey Koşar M, Orakçı EE, Şeker Karataşoglu G Faculty of Pharmacy, Department of Pharmacognosy, Erciyes University, 38039 Kayseri, TURKEY

Viburnum opulus L. (Caprifoliaceae) growing in Kayseri and surrounding areas is named as gilaburu. The fruit juice of gilaburu is consumed as a spasmodic, anti-inflammatory, anti-allergic, sedative and diuretic pure. The fruit juice of gilaburu is consumed as a means plant samples, were carried out by a simple and a rapid method approved standards 5th ed. NCCLS document M7-A5. NCCLS: Wayne, PA, USA.

Quantitative Determination of Galanthamine and Lycorine in an endemic Galanthus species: G. cilicicus Kayra G, Cicik Polat D, Onur MA, Unver Somer N Department of Pharmacy, Faculty of Pharmacy, Ege University, Bornova- Izmir 35100, Turkey

Galanthus cilicicus Baker, an endemic species of the genus Galanthus (Amaryllidaceae), is distributed in southern Turkey mainly in the province of Icel [1]. Galanthus, the most important alkaloid found in Amaryllidaceae species, is used for the treatment of Alzheimer’s disease [2]. Lycorine, another important and also a widespread alkaloid found in Amaryllidaceae plants has been proven to have several biological activities [3,4]. A reversed-phase high-performance liquid chromatographic method has been used and validated for the determination of lycorine and galanthamine in G. cilicicus. The extraction of both alkaloids in low-mass plant samples, were carried out by a simple and a rapid method using pre-packed columns with diatomaceous earth (Extralut®) [5]. The chromatographic separation was performed using an isocratic system with a mobile phase of trifluoroacetic acid-water-acetonitrile (0.01: 92.5: 7.5) and diode array detector [6]. The linearity of the method was studied by injecting five known concentrations of lycorine in the range of 1 – 10 μg mL⁻¹ and five known concentrations of galanthamine in the range of 2.5 – 20 μg mL⁻¹. The calibration curves for lycorine and galanthamine were calculated by the least squares method. All experiments were performed in triplicate. Y = 0.4848x + 0.048861 and Y = 10.1354031x + 0.5465348, respectively. Validation procedures showed that the method was specific, accurate and precise. The above-mentioned method was applied to the aerial parts and bulbs of G. cilicicus. The contents of galanthamine and lycorine in the bulbs of G. cilicicus

**PL42**

Screening of Zambian Ficus species for antibacterial and antymycobacterial activity

Bwalya AG1, Stapleton P1, Phiri P2, Montamat Sicotte D2, Hingley Wilson S3, Lalvani A1, Tasdemir D1

1Centre for Pharmacognosy and Phytotherapy, School of Pharmacy, University of London, London WC1N 1AX, UK; 2School of Mathematics and Natural Sciences, Copperbelt University, P.O Box 21692 Kitwe, Zambia; 3Tuberculosis Research Unit, Department of Respiratory Medicine, National Heart & Lung Institute, Imperial College London, London W2 1HC, UK

Members of the genus Ficus (Moraceae) are traditionally used in Zambia against many infectious diseases, including bacterial (oral, chest and diarrhoeal), mycological (Koal and fungal (ringworms) infections [12]. Based on this information, we collected different plant parts (leaves, stem and root barks) of eight Zambian Ficus species; F. ovata Vahl, F. wakefieldii Hutch, F. natalensis Hochst, F. sanisbarica Warb. subsp. macroperma, F. lutea Vahl, F. ingens (Miq.) Miq., F. sycomorus L. subsp. gnaphalocarpa (Miq.) Miq., and F. sycomorus L. subsp. sycomorus. The dried plant materials were extracted with methanol (CR-Me) and further partitioned (H37Rv strain). The CR-Me extracts of the stem bark exhibiting the highest activity (MIC 128 μg/ml). These results part to n-hexane (K-Hex), chloroform (K-CHCl3) and aqueous methanol (K-MeOH) subextracts. We recently investigated the antifungal effect of CR-Me extracts against Trichophyton species, the causative agents of ringworm infections [3]. Herein we screened the CR-Me extracts and the subextracts for antibacterial and antymycobacterial activity using agar disc diffusion and MITT assays, respectively. Test organisms were Gram-positive [Staphylococcus aureus NCTC 12695, methicillin-resistant Staphylococcus aureus MRS 11998, Enterococcus faecalis 13379], and Gram-negative [Escherichia coli NCTC 10418] bacteria, plus Mycobacterium tuberculosis (H37Rv strain). The CR-Me extracts of the stem barks were active against all Gram-positive microorganisms. Of the subextracts, K-MeOH-solubles exhibited the best activity with inhibition zones of 11 mm at 100 μg/disc concentration against all three Gram-positive bacteria. Moderate antibacterial activity was observed in some K-Hex and K-CHCl3 subextracts, with K-CHCl3-solubles of F. ovata stem bark exhibiting the highest activity (MIC 128 μg/ml). These results provide a scientific basis supporting the use of Zambian Ficus species in traditional herbal preparations in Zambia. Acknowledgement: UK Commonwealth Scholarship Commission, the fick-Codd Travel Fund of the School of Pharmacy are acknowledged for funding. References: 1. Kuette V et al. (2008) Ethnopharmacol 124: 556 – 561. 2. Fowler DG (2007) Zambian Plants: Their vernacular names and uses. Royal Botanical Gardens, Kew, UK. 3. Bwalya AG et al. (2010) Planta Med 72: 1301.

**PL44**

Antioxidant Properties and Phenolic Composition of Salvia virgata from Turkey

Seker Karatoprak G, Kusar M

Erciyes University, Faculty of Pharmacy, Department of Pharmacognosy, Kayseri, Turkey

Several biochemical reactions generate reactive oxygen species and these are crucial in damaging crucial bio-molecules [1]. Free radicals are very important in food products, because oxidative degradation of lipids is one of the main factors limiting their shelf-life [2]. In recent years, natural antioxidants have been focused on because of the harmful effects of synthetic antioxidants [3]. Salvia officinalis L (Lamiaceae), is an important and rich source of antioxidant used and have wider implications for the dietary intake of natural antioxidants [3]. Turkey is an important country for Salvia species. The flora of Turkey includes 88 species of the genus Salvia. The 70% methanol and water extracts were prepared from the aerial parts of S. virgata Jacq. collected from Bursa, Turkey. All the extracts were analyzed by HPLC and in vitro antioxidant assays. The 1,1-diphenyl-2-picrylhydrazin (DPPH)’, 2,2’-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) (ABTS−) radical scavenging activity and β-carotene bleaching methods were used. Total phenolic compounds and reductive activity of the extracts were also analyzed. BHT, BHA, ascorbic acid, gallic acid and rosmarinic acid were used as positive controls. Phenolics rich extract of aq. methanol showed more scavenging activity on DPPH− than water extract whereas water extract more scavenged the ABTS− radical. The aq. methanol extract more reduced the ferric(III) to ferro(II) in a certain proportion than water extract. All extracts were inhibited linoleic acid peroxidation in β-carotene bleaching test and shown more activity than rosmarinic acid. Rosmarinic acid was found as the main component and caffeic acid, ferulic acid and luteolin-7-O-glycoside were identified in the extracts. References: 1. Kumaran A, Joel Karunakaran R (2006) Food Chem 97: 109 – 114. 2. Pizzale L et al. (2002) J Sci Food Agric 82: 1645 – 1651. 3. Kintzios SE (2000) Sage The Genus Salvia. Harwood Academic Publishers, 27 – 53 and 185 – 192.

**PL43**

Impact of nitrogen nutrition on growth and plant quality of Centella asiatica

Müller V, Lankes C, Hunsche M, Noga G

Institute of Crop Science and Resource Conservation – Horticultural Sciences, University of Bonn, Auf dem Hügel 6, 53121 Bonn, Germany

Due to its bioactive triterpene saponins, Centella asiatica (L.) Urb. has been used as a medicinal herb since ancient times and its economical importance is still rising [1,2,3]. Up to now plants are collected spontaneously which implicates a large variation in plant and product quality depending on the origin, genotype and time of harvest of the plants [1 – 4]. To assure high plant quality, it will be necessary to encourage cultivation of Centella. To our knowledge, there are scarce investigations on cultivation techniques, especially on mineral nutrition of Centella plants. The aim of this study was to investigate the effects of nitrogen on growth and saponin biosynthesis of Centella asiatica and to find appropriate parameters to evaluate quality of plant material by non-destructive measurements in situ. Plants were grown for eight weeks in rock wool cubes in a greenhouse and fed with five nutrient solutions differing in their nitrogen concentrations. Number of leaves and stolons, length of stolons, assimilation rate and leaf green intensity were monitored weekly. Non-destructive measurements were conducted with a portable optical sensor (Multiplex® Research, Force-A, France) recording the fluorescence signature which is associated with plant constituents. Fresh and dry weight of leaves and stalks, leaf area and specific leaf weight were ascertained biweekly, while examination of leaf chlorophyll and leaf nutrient content was carried out once at the end of the study. Leaf samples for determination of triterpenoid content were harvested four times. Analyses of asafoetidin and asiatic acid by HPLC are in progress and will be discussed. Acknowledgements: Regionale 2010, State of North Rhine-Westphalia (Germany); Institute of Systematic Botany, The New York Botanical Garden (USA), A.N. Nicolas; Institut Malacée de Recherches Appliquées (Madagascar), D. Randriamampionona; National Center for Natural Products Research, University of Mississippi (USA), B. Avula; Unité d’Analyse Chimique et Physico-Chimique des Médicaments et Pharmacognosie, Université Catholique de Louvain (Belgium), M.H. Rajamana. The project was supported by the Institute of Nutrition, the University of Bonn (Germany), B.F. Zimmermann. References: 1. Thomas MT et al. (2010) Ind Crop Prod 32: 545 – 550. 2. Devkota A et al. (2010) Biochem Syst Ecol 38: 12 – 22. 3. Randriamampionona D et al. (2007) Fitoterapia 78: 482 – 489. 4. Sritongkul J et al. (2008) Acta Hort 804: 367 – 372.

**PL45**

Antioxidant Properties and Phenolic Composition of Alchemilla mollis from Turkey

Çerück S, Seker Karatoprak G, Kusar M

Erciyes University, Faculty of Pharmacy, Department of Pharmacognosy, Kayseri, Turkey

Alchemilla mollis (Buser) Rothm. (Rosaceae) is also known as Lady’s Mantle and native to southern Europe and grown throughout the world as an ornamental garden plant (Evenor et al., 2001). In folk medicine, lady’s mantle was also used to soothe infections of the mucous membranes of mouth and throat. The leaf tea and dewdrops from the leaves of the living plant are most commonly employed to help female conditions such as menorrhagia, menopause and painful periods. Lady’s mantle was also used traditionally for treating blood sugar control diseases, although no evidence exists to support its usefulness (Kisilova et al., 2006; Shivrastava et al., 2007). Air-dried A. mollis herb material (100 g)
was powdered and sequentially extracted with hexane, ethyl acetate, methanol, and n-butanol using a Soxhlet apparatus for 8 h for each. Thereafter, the extract was filtered and evaporated to dryness in vacuo at 40 °C. All the extracts were analyzed in in vitro antioxidant assays. The free radical scavenging activity of the extracts were investigated using 1,1-diphenyl-2-picrylhydrazine (DPPH) and 2,2’-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS+) radicals. Total phenols, flavonoids and flavonoids, and reductive activity of the extracts were also analyzed. BHT, BHA, sorbic acid, and gallic acid were used as positive controls. Polar fractions were found to be more active as antiradical assays. These active fractions were contained more tannins, especially galloyl tannins. Chemical composition and antiradical activity results of the A. mollis are the first report in the same research. References: 1. Evenor D et al. (2001) Plant Cell Tiss Org 65: 169 – 172. 2. Shivastava R et al. (2007) Phytother Res 21: 369 – 373. 3. Kiselova Yet al. (2006) Phytother Res 20: 961 – 965.

The plants are one of the attractive sources of novel antitumor compounds. Isolation of pharmaceuticals from plants is difficult due to their extremely low concentrations. To extend the research to human clinical studies, we needed to find a reliable supply of plant material, produced target compounds. As part of our ongoing program on the optimization of Linum species, cell cultures of L. leonii F.W.Schultz, were examined. We have established several callus and suspension cultures and checked for the occurrence of lignans. The main component in cell cultures of L. leonii was isolated and analyzed by means of GC-MS and NMR. The EI-MS of the isolated compound showed an ion at m/z 364 and mass fragmentation, which is consistent with the data for an arylaphthalene lignan. the 1H NMR spectrum showed that the isolated compound is justicidin B. Justicidin B produced by in vitro cultures of Linum leonii was tested for cytotoxic activity and induction of apoptosis in MDA-MB-231 and MCF-7 breast cancer derived cell lines. The tested ligand evoked strong, concentration-dependent cytotoxicity in both cell lines, whereby MCF-7 proved to be more sensitive. The 24 h treatment of both cell lines increased the level of apoptotic DNA fragmentation; however the proapoptotic activity is completely inhibited if the cells are co-incubated with the non-selective pan-caspase inhibitor, Boc-Asp(Dme)-fluoromethyl ketone (PC(1)), which implies that justicidin B, activates PC(1) via caspase-dependent mechanisms. Exposure of MDA-MB-231 cells with justicidin B leads to concentration-dependent decrease in the NFκB expression; strong NFκB expression is observed in MCF-7 cells. Acknowledgement: Financial support from Ministry of Science, research, Education, and technology, Bulgaria (grant D002 – 128/08 I. Ikonova) is acknowledged.

Fungal endophytes are widespread in plants and colonize living internal tissue of their hosts symptomlessly [1]. They are well known for their beneficial effects for their hosts, providing increased tolerance against abiotic and biotic stresses, enhancing inter alia resistance to insect pests and fungal or microbial infection. This is largely due to their production of bioactive secondary metabolites [2]. Recently, Zingiberaceae species were studied and several bioassays successfully developed to test for antifungal activities [3]. These tests were now applied to the analysis of Vitis plants and their endophytic fungi, including routinely: a) competition tests against Cladosporium sphaerospermum, to investigate the dominance of the endophyte compared to its competitor in vitro [4]; b) thin layer bio-autography with the crude endophytic extract and subsequent determination of inhibition halos, caused by separated compounds after spraying with conidiospores of C. sphaerospermum; c) establishing species-specificity by cultivation of the endophytic fungus on media containing the crude extract of the respective plant; d) performing a modified ELISA with the fungal crude extract to determine the median effective concentration (EC50) for inhibition of the growth of C. sphaerospermum. Several fungal endophytes have so far been isolated from Vitis vinifera L cultivars, and respective results will be presented. The methods described provide tools not only for testing for antifungal activities, but also for subsequent isolation of bioactive compounds, and eventually for their practical applications in pest control. Acknowledgement: The financial support of "Society for the Advancement of Plant Sciences" is gratefully acknowledged. References: 1. Petrini O (1991) Microbial Ecology of Leaves, Springer Verlag, New York. 2. Gao F et al. (2010) Afr J Microbiol Res 4(13): 1346 – 1351. 3. Zahradník C (2010) Pilzendophyten aus Alpinia malaccensis und Curcuma sp.: Kultur, Seukurzstoffsprofil und Bioaktivität. Diploma thesis, Univ. of Vienna. 4. Yuen TK (1999) Micro Ecol 37: 257 – 262.
Rhus coriaria L., commonly known as sumac (also spelled sumach), grows wild in the region extending from the Canary Island over the Mediterranean coastline to Iran and Afghanistan. It is native to the Mediterranean and southeast Anatolian region of Turkey [1]. The fruits are red colored and contain one seed. It’s dried and ground leaves have been used as a tanning agent due to their high tannin content. Previous phytochemical studies of this plant reported that it contained flavones, tannins, anthocyanins, and organic acids [2]. In this study gallic acid and cyanidin-3-O-glucoside contents of Water and MeOH %70 extracts of sumac were investigated using with HPLC ESI/MSMS MRMR method.

The genus Cistus (Cistaceae) is represented by 21 species on worldwide and 5 species in Turkish flora. The genus Cistus has widespread utilization in Turkish folk medicine such as against rheumatism, for hemorrhoids, to cure sterility, kidney and urinary inflammations, as a hemostatic, antipyretic, expectorant, sedative, and for peptic ulcer, as well as diabetes mellitus. Anti-inflammatory, anti-helicobacter pylori, anti-hypertensive, anti-microbial activities and cytotoxic effects of Cistus species were also reported. In this study, pharmacognostical investigations have been carried out on Cistus salvifolius L. The aerial parts of the plant were extracted with n-hexane and MeOH respectively. The methanolic extract was partitioned with petroleum ether and n-hexane and MeOH, respectively. The methanolic extract yielded three flavonoids: C-3; Quercetin 3-O-galactopyranoside and C-7: Myricetin 3-O-galactopyranoside, C-5: Quercetin 3-O-a-arabinopyranoside, C-6: Quercetin 3-O-b-galactopyranoside and C-7: Myricetin 3-O-b-galactopyranoside. The structures of these compounds were elucidated using spectroscopic methods (UV, IR, 1H-NMR, 13C-NMR, 2D-NMR and MS).

Free Radical Scavenging Activities of Flavonoids from Cistus salvifolius L.

Geranium thunbergii Siebold & Zucc. (Geraniaceae) is a traditional herb with anti-diarrhetic, anti-inflammatory, and anti-oxidative effects. This study investigated the anti-obesity properties of a extract of Geranium thunbergii (GTE) in high-fat diet-induced obese mouse. GTE treatment significantly reduced body weight, adipose tissue mass, adipocyte size, serum triglyceride, total cholesterol, and low density lipoprotein-cholesterol levels in obese mice relative to the high-fat diet-fed mice. It also decreased serum leptin levels and increased adiponectin levels. The serum levels of aspartate transaminase, alanine transaminase, blood urea nitrogen, and creatinine were not significantly changed in GTE-treated mice compared to their levels in normal diet and high-fat diet-fed mice. Furthermore, GTE suppressed the mRNA levels of sterol regulatory element-binding protein 1c, peroxisome proliferator-activated receptor γ, adipocyte fatty acid-binding protein, and fatty acid synthase in the adipose tissues of obese mice. These results suggest that GTE ameliorated high-fat diet-induced obesity by altering adipokine levels, and downregulating the expression of transcription factors and lipogenic enzymes involved in lipid metabolism.
Antimicrobial, antioxidant and phytochemical investigations of sea buckthorn (Hippophae rhamnoides L.) organs
Michel T, Destandau E, Elfakir C
Institute of Organic and Analytical Chemistry (ICOA),
University of Orléans-CNRS UMR 6005, BP 67059, 45067 Orléans cedex 2, France

Hippophae rhamnoides L. (Elaeagnaceae), commonly known as sea buckthorn, is a thorny bush with orange berries naturally distributed in Asia and Europe. Fruits of H. rhamnoides have been used by Chinese, Mongolian and Tibetan medicines for decades, and possessed considerable medicinal and nutritional values like antimicrobial, antitumoral, antioxidant and dermatological effects [1,2]. However the therapeutic potential and phytochemical diversity of the other H. rhamnoides parts remain unexplored. In this work we present the phytochemical and bio-activities screening of seed, leaf, stem and root of H. rhamnoides. The crude extracts were obtained by Pressurised Liquid Extraction (PLE) using ethanol. Each extract was then partitioned by liquid-liquid extraction using three solvents of different polarities: aqueous, ethyl acetate and hexane. The antimicrobial effect, the total phenolic content, the reducing power (FRAP), and the free radical scavenging activity (DPPH) of crude extracts and their fractions were evaluated. H. rhamnoides organs have all antibacterial values and that the most antioxidant potential was found in root and seed extracts. Furthermore, the antimicrobial and the antioxidant activities were found in the aqueous fraction. High Performance Thin Layer Chromatography (HPTLC) analyses of aqueous fractions showed that they were mainly constituted of sugar and polyphenolic compounds. The bio-activities were consequently attributed to the polyphenolic compounds present in active fractions of seed, leaf, stem and root of H. rhamnoides. References: 1. Guliev VB et al. (2004)J Chromatogr B 812: 291 – 307 2. Zeb A (2004) J Bio Sci 4: 687 – 693

Bioactive compounds from Phyllanthus atropurpureus Bojer cultivated in Egypt
Sarg T, El Sayed A, Zayed R, Al Sayed M
Department of Pharmacognosy, Faculty of Pharmacy,
Zagazig University, 44519 Zagazig, Egypt.

From the ethyl acetate soluble fraction of the Phyllanthus atropurpureus, six compounds were isolated and identified based on spectral data (IR, UV, Mass (FAB, EI), 1H-NMR and 13C-NMR). Four compounds are isolated for the first time from the plant, the structures were established as di [3, 4, 5-trihydroxy-phenyl] ether, 5, 6, 8, 4’-tetrahydroxy isoflavone, Robustaside A, 6-’(4’- hydroxy cinnamoyl) arbutin, and 6-’(3”, 4”-dihydroxy cinnamoyl) arbutin. The other two isolated compounds are Demethoxy Sudachitin, and quercetin-7-O-glucoside. Concerning the cytotoxic activities were evaluated on all isolated molecules. The selectivity towards parasites was determined. The attempt for the access to the target of active molecules on Plasmodium falciparum is discussed.

The stem-bark is traditionally used for the treatment of fevers and as tonic. Screening based on inhibitory activity against the chloroquine-resistant strain FcB1 of Plasmodium falciparum allowed the selection of this plant for a phytochemical investigation. Selective acid-base extraction with gradient of pH performed on methanol extract from the powdered stem-bark, yielded a crude alkaloid and EtOAc extracts. Both extracts were active in vitro on P. falciparum with an IC50 value of 1.6 and 6.5 µg/ml, respectively. The bioassay-guided fractionation of EtOAc extract by combined chromatographic methods (preparative TLC, CC (SiO2, Al2O3), Sephadex LH 20 gel, MPLC, preparative HPLC) led to isolation of indole along with vobasinyl-iboga bisindole alkaloids as active constituents. Their structures were elucidated by spectrometric techniques (IR, UV, ESI-MS, 1D and 2D NMR) (OSY, HSOQC, HMB, NOESY). Six compounds are new among the thirteen isolated1, 2. NMR spectra at low temperature allowed the characterization of bisindole alkaloids whose 1H NMR spectra were not resolved at room temperature. A hypothesis of biogenesis is proposed. The antiparasitic on Plasmodium falciparum, Trypanosoma brucei brucei and Leishmania donovani and cytotoxic activities were evaluated on all isolated molecules. The selectivity towards parasites was determined. The attempt for the access to the target of active molecules on Plasmodium falciparum is discussed.


New vobasinyl-iboga bisindole alkaloids with antiparasitic activities from Muntafara sessilifolia
Cirando M1, Deregnacourt L1, Deville A1, Dubost L1, Joyeau R1, Allorge L1, Rasoamivbo P2, Mambo L1
1UMR 7245 CNRS-MNHN Communication Molecules and Adaptation of Micro-organisms, National Museum of Natural History. Box 54, 57 rue Cuvier, 75005 Paris, France.; 2Laboratory of Pharmacognosy applied to infectious diseases, Malagasy Institute of Applied Research. PO Box 3833, 101 Antananarivo, Madagascar.

Muntafara sessilifolia (Baker) Pichon or Tabernaemontana sessilifolia is an endemic plant of Madagascar which belongs to the Apocynaceae family.
The tubers of *Corydalis cava* Schweigg. & Kort. were extracted with ethanol and the summary alkaloid extract was fractionated in silica gel chromatography column using step gradient elution with hexane, chloroform and ethanol. Repeated column chromatography, preparative TLC and crystallization led to the isolation of fifteen isosquillamine alkaloids. The chemical structures of isolated compounds were determined on the basis of spectroscopic techniques and by comparison with literature data. Isolated alkaloids were tested on ability to inhibit human erythrocyte acetylcholinesterase, serum butyrylcholinesterase (IC$_{50}$) and for its free-radical scavenging activities (EC$_{50}$). Cholinesterase inhibitory activities were determined in vitro by modified spectrophotometric Ellman's method [1]. (+)-canadaline inhibited acetylcholinesterase as well as butyrylcholinesterase in a dose-dependent manner with IC$_{50}$ values 20.1 ± 1.1 μM and 85.2 ± 3.2 μM, respectively. (+)-canadine with an IC$_{50}$ value 12.4 ± 0.95 μM was the most potent inhibitor of acetylcholinesterase, whilst (+)-corycavidine and (+)-bulbocapnine were effective inhibitors of butyrylcholinesterase with IC$_{50}$ values 46.2 ± 2.4 μM and 67.0 ± 2.1 μM. Other isolated alkaloids were considered inactive (IC$_{50}$ > 100 μM). Free-radical scavenging activities of isolated alkaloids were tested in vitro by means of the DPPH test [2]. The highest activities exhibited (+)-scouline, (+)-sinocatindine and (+)-bulbocapnine with EC$_{50}$ values 102 ± 6.2 μM, 209 ± 8.1 μM and 279 ± 16.7 μM, respectively. Other isolated alkaloids were considered inactive (EC$_{50}$ > 1000 μM). Acknowledgement: The study was supported by grants of GA UK No. 122309 and SVV-2010 – 263 – 002. References: 1. Ellman L, Courtney D, Andres V, Featherstone R (1961) Biochimie Pharm 7: 88 – 95. 2. Polšček M, Škalá P, Opletal L, Jehoš J (2004) Anal Bioanal Chem 379: 754 – 758.

**Biodiversity of high mountain flora as a source of new medicines – Dinaric Alps (W. Balkan)**

**Redzic S**
Dep.of Botany, Fac. of Science University of Sarajevo

The biodiversity of high mountain flora is very rich [1]. It still pharmacologically poorly investigated. This is especially true in areas that are rich in endemic species like this Dinarides (Western Balkans). This is an important resource in getting new drugs [2]. The aim is to make identification of potentially endemic medicinal plants and their biochemical background. In order to achieve these results, the following methodology was applied: field research on different profiles, including ethnobotanical interviews, followed at the end by comparative taxonomic-biochemical method. In the mountainous zone of the western Balkans was found 2500 species [3]. Very small number used in the official pharmacy and medicine. As potentially are 1500 species of medicinal plants. On the basis of their taxonomic similarity is expected and biochemical similarity, the pharmacological activity, as well. As a real or potential sources of alkaloids are the species of the genera: Onosma, Moltkaea, Colchicum, Senecio, Cyanus, Arastragus, Oxytropis, Vicia, Papaver, Euphorbia, Erdfrau, Campanula; heterosides are species of genera: Arctous, Ferulago, Atamancha, Pancira, Bupleurum, Seseli, Genista, Gentianella, Gentiana, Frangula, Rhamnus; saponosides are: Verbascum, Scrophularia, Primula, Solidanella, Dianthus, Silene, Arenaria, Minuartia, Knautia, Scabiosa, Viola, etc.; tannins are: Geum, Potentilla, Sibirea, Cretaegus, Dryas, Saxifraga, Geranium, Asplenium, etc.; terpenoids are species of genera: Centaurea, Hiriacium, Hipochoeris, Ambaphorcos, Petasites, Homogyne, Stachys, Satureja, Micromeria, Scutellaria, Euphrasia, Pedicularis, Veronica, Iris, Pinus, etc.; carbohydrates are: Orchis, Gymnadenia, Dactylorhiza, etc. and lipids are species of genus Linum. Sources of alkaloids are the species of the genera: Onosma, Moltkaea, Gentiana, Pedicularis, Veronica, Iris, Petasites, Homogyne, Stachys, Satureja, Chelidonium, Trochodendron, Dendrocalyx, Kafikovska Panoevka, Kafandzieva, Kulevanova. Other isolated alkaloids were considered inactive (EC$_{50}$ > 1000 μM). Free-radical scavenging activities of isolated alkaloids were tested using hole-plate method. The broth dilution method was used for testing the minimal inhibitory concentration (MIC) of the essential oil. The needle essential oil was confirmed to have significant antimicrobial activity, especially against Gram positive bacteria such as *Streptococcus pneumoniae*, *Streptococcus pyogenes* and *Streptococcus agalactiae* with minimal inhibitory concentrations 15.26 μg/ml, 7.5 μg/ml and 31.25 μg/ml, respectively.

**Basic pharmacognostic research of *Gentiana cruciata* L. species from Bosnia and Herzegovina (W. Balkan)**

**Tuka M, Redzic S, Babić A**

1Private Pharmaceutical institution “Apotheke VITA”，
Kiseljak, Bosnia and Herzegovina; 2Dept. of Biology of the Faculty of Science University, 33 – 35 Zmaja od Bosne St., 71 000 Sarajevo, Bosnia and Herzegovina

*Gentiana cruciata* L. (Gentianaceae) is a widely distributed species in the area of Dinarides. [1]. It is used in traditional medicine in some mountainous areas of Bosnia [2]. As related species of *Gentiana lutea* L. is endangered, [3] similar uses of the other species of *Gentiana* in modern phytotherapy are investigated. *Gentiana cruciata* has such capabilities. As a precondition for its use, we planned to conduct basic botanical and pharmacognostic research. The material was sampled 2007 at the mountains of Sarajevo (800 to 1500 m). All studies were carried out in accordance with the European Pharmacopoeia IV monograph. The leaves have dorsi-ventral histological structure. Stomata have 5 – 6 pores. Type of stoma is anomocytic. Root has the primary and later secondary structure. Use of spectrophotometric analysis and paper chromatography confirmed the ratio between chlorophyll a and b 2:1. Chlorophyll a is 0.6 mg/g; chlorophyll b is 0.383 mg/g and carotenoids 0.285 mg/g. The proportion of plant pigments indicates the presence of antioxydative activity of this plant. The roots of this species has been prepared for chemical analyses. For the separation of metabolites was used thin layer chromatography method. Standard analysis showed the presence of sucrose and amarangetin. The method of micro-sublimation proved the presence of gentisine. Preliminary and basic results suggest that the roots and aerial parts of *Gentiana cruciata* could be a useful replacement for the very popular and highly endangered species *Gentiana lutea* L. subsp. *symphandra* (Murb.) Hayek. References: 1. Redzic S (2006) Proc. 1st IFOM Intern. Conf. Organic Wild Production 117 – 141. 2. Redzic SS (2007) Coll Antropol 31: 869 – 890. 3. Redzic S et al. (2009) Planta Med 75: 902 – 902.

**Chemical characterization and antimicrobial activity of the needle essential oil of *Pinus mugo* (Pinaceae) from Macedonian flora**


1Faculty of Pharmacy, Department for Pharmacognosy, Skopje, The Former Yugoslav Republic of Macedonia; 2Faculty of Medicine, Department for Microbiology, Skopje, The Former Yugoslav Republic of Macedonia

*Pinus mugo* Turra (Pinaceae) or Mountain pine is low and shubby conifer which can be found in Republic of Macedonia in very huge population, only in central part of the country, on Karadzica Mountain. This location is the southernmost extensive point for this plant. The needle essential oil was obtained by hydrodistillation in Clevenger apparatus after removing the needles from the branches and was yielded from 0.15 to 0.65%. The chemical composition of the essential oil was analyzed by gas chromatography/mass spectrometry and the most abundant components were monoterpenes alpha-pinene (6.2 – 12.9%), beta-pinene (1.3 – 3.3%), delta-3-carene (10.1 – 18.7%), limonene + beta- phellandrene (3.1 – 5.7%), alpha-tepinone (2.1 – 3.0%) and bornyl acetate (2.0 – 3.7%) and sesquerpenes trans-caryophyllene (5.7 – 6.4%), germacrene D (2.4 – 11.8%), bicyclogermacrene (3.0 – 6.8%), delta-cadinene (4.0 – 6.6%), tau-murolol + tau-cadinol (2.5 – 4.4%) and alpha-cadinol (3.4 – 5.0%). The essential oil was screened for antimicrobial activities against 13 bacterial isolates representing both Gram positive and Gram negative bacteria and one strain of *Candida albicans* using plate-diffusion method. The broth dilution method was used for testing the minimal inhibitory concentration (MIC) of the essential oil. The needle essential oil was confirmed to have significant antimicrobial activity, especially against Gram positive bacteria such as *Streptococcus pneumoniae*, *Streptococcus pyogenes* and *Streptococcus agalactiae* with minimal inhibitory concentrations 15.26 μg/ml, 7.5 μg/ml and 31.25 μg/ml, respectively.
PL61

Elemental compositions of Echinacea purpurea, E. pallida radix and herb cultivated in Turkey

Kan Y1, Çoksan G1, Guner ST2, Kose YB3, Demirci F4
1Selçuk University, Agriculture Faculty, Department of Field Crops, Konya, Turkey; 2Research Institute for Forest Soils and Ecology, Eskisehir, Turkey; 3Department of Pharmaceutical Botany, Faculty of Pharmacy, Anadolu University, Eskisehir, Turkey; 4Department of Pharmacognosy, Faculty of Pharmacy, Anadolu University, Eskisehir, Turkey

Echinacea sp. (Asteraceae) are one of the most popular medicinal plants used in phytotherapy. In this present study, E. purpurea (L) Moench and E. pallida (Nutt.) Nutt. were successfully cultivated under controlled conditions in experimental fields in Konya. Dried aerial parts and the roots of 36 cultivation samples were investigated for their macro (N, P, K) and micro (Ca, Mg, Na, Fe, Mn, Zn, Cu) trace elemental compositions using various techniques. N was determined by the dry combustion method using elemental analyses, P was measured by a colorimetric method, whereas K and Na by flame photometry. Finally Ca, Mg, Fe, Cu, Zn and Mn was detected and quantified by atomic absorption spectroscopy (AAS). All experiments were performed qualitatively and quantitatively with statistical data comparison to a certified reference plant material, respectively. The total dry matter content, including those of the areal parts of the crops ranged 25 – 30%. The results of elemental analyses showed that N ranged 0.54 – 1.69%, P ranged 1100 – 2600 ppm and K ranged 9990 – 29585 ppm. To the best of our knowledge, this is the first report on micro and macro elements of cultivated Turkish Echinacea sp. As a conclusion, the elemental composition and the nutritional value E. purpurea and E. pallida are worthwhile to investigate with comparison to other Echinacea sp. used medicinally.

PL62

Bioassay-guided fractionation and cytotoxic activity of flavonoids from Echinococcus crus-galli (L. Barnyard Grass)

El Hefawy HM1, Gad El Molla SC2, Abdel Motaa A3, El Fishawy AM4
1Pharmacognosy Department, Faculty of Pharmacy, October 6 University, Central Axis Part 1/1, 6th of October, Egypt.; 2Pharmacognosy Department, Faculty of Pharmacy, Cairo University, Kasr-El-Ainy St., Cairo11562, Egypt.

Echinococcus crus-galli L. (Family Poaceae) is a problematic summer weed found in rice fields and moist soil and is well known as Barnyard Grass [1]. Bioassay-guided fractionation of the seeds of Echinococcus crus-galli L. led to isolation of two cytotoxic flavonoids after screening against four human cancer cell lines: MCF-7 (breast cells), HCT-116 (colon cells), HELA (cervical cells) and HEPG-2 (liver cells) using the sulforhodamine B (SRB) colorimetric assay [2]. Different extracts showed a comparison to other Echinacea sp. used medicinally.

PL63

Plant pigments in some medical plants of family Lamiaceae (Bosnia and Herzegovina, W. Balkans)

Redzic S1, Kurtagic H2, Sedjic N3, Pulic A4
1Dept. Of Biology, Fac. Of Sci. Univ. Sarajevo, 33 – 33 Zmaja od Bosne St., 71 000 Sarajevo, Bosnia and Herzegovina; 2Federal Institute Of Agriculture, Butmir, Sarajevo, Bosnia and Herzegovina

Plant pigments chlorophyll and carotenoids are very important group of primary and secondary metabolites, resp. Besides their role in process of photosynthesis and plant protection from extensive radiation, they have huge appliance in pharmaceutical industry, cosmetology and dietetic. Plant pigments are also given significant role in anti-oxidant activity [1, 2]. Goal of these studies has been qualitative – quantitative analysis of main and side pigments in selected medicinal species of wild flora in BiH, including endemic species. Plant materials were gathered during different seasons. They were transported fresh to the laboratory where qualitative (paper and thin layer chromatography) and quantitative (spectrophotometric) analyses took place. Results (Table 1) showed significant presence of chlorophyll a, chlorophyll b and carotenoids. Ratio between chlorophyll a and chlorophyll b was rarely 3:1, as stated in classical literature but rather close to 3:2 and more, which makes these species even more medicinal and gives them higher potential for anti-oxidant capacity [3].

Marketed Flavonoid Compounds

H. Nyberg1,6, L. Nyberg2,3,4,5, H. Sundh6,7
1Pharmaceutical Toxicology, 06100, Sıhhiye, Ankara, Türkiye; 2Hacettepe University, Faculty of Pharmacy, Department of Pharmacognosy, 06100, Sıhhiye, Ankara, Türkiye; 3Hacettepe University, Faculty of Pharmacy, Department of Pharmaceutical Toxicology, 06100, Sıhhiye, Ankara, Türkiye

There are 33 Sedum species growing in Turkey and some Sedum species have been employed in folk medicine for their anti-inflammatory, wound healing, and diuretic properties as well as treatment of chronic viral hepatitis. The crude MeOH extract prepared from the aerial parts of Sedum acaule (Cav.) DC. and its EtOAc, n-BuOH, H2O subextracts were screened for their protective effect against H2O2 induced cytotoxicity at different concentrations in human red blood cell. The ETOAc subextract were found to be the most protective one and its chemical composition was further analysed. Five polyphenolic secondary metabolites including gallic acid (1), kaempferol 3-α-rhamnopyranoside (2), quercetin 3-O-β-glucopyranoside (3), quercetin 3-O-α-rhamnopyranoside (4), myricetin 3-O-α-rhamnopyranoside (5) were isolated from the ETOAc extract by successive chromatographic methods. The structures of the isolated compounds were elucidated by 1D- and 2D-NMR techniques. This is the first phytochemical work on S. acaule. The protective effect of the isolates against H2O2 induced cytotoxicity in human red blood cells also evaluated at 5 and 10μg/ml and kaempferol 3-α-rhamnopyranoside was shown most protective effect. Acknowledge-
Fagopyrin and its derivatives in buckwheat (Fagopyrum sp.)
Tavcar E, Stojilkovski K, Krste S
Faculty of Pharmacy, University of Ljubljana, Ljubljana, Slovenia

Fagopyrin and its derivatives are dimerized anthraquinone polyphenolic substances from buckwheat. They act as photosensitizers upon excitation with visible light (540–610 nm), which causes phototoxic effect after ingestion of large amounts of buckwheat called fagopyrin (1). According to known structures and transformations of hypericin derivatives from St. John’s wort and their similarity to fagopyrin, several forms of fagopyrin derivatives have been postulated as pseudofagopyrin and protofagopyrin (2). It was shown that pre-forms of fagopyrin transform to fagopyrin in plant extract under daylight exposure (3). We optimized the extraction method of fagopyrin from plant material. We developed a HPLC method coupled with fluorescence detector (excitation wavelength 330 nm, emission 590 nm) for separation and detection of those compounds. The HPLC method yielded several chromatogram peaks with close retention times presenting different forms of fagopyrin. Various buckwheat products available on the market were analyzed using this method. The highest amount of fagopyrins was found in buckwheat herb samples and less in fruit samples, which contain most of the compounds in peels. Buckwheat herb was used for further observation of the nature of fagopyrins in plant extract. As described previously (4), we observed that the content of different forms of fagopyrin varied due to different extraction conditions (time, temperature, solvent). Since the differences were observed even if the conditions changed after the end of the extraction (removal of the herbal substance), we assume that this change was due to transformations of fagopyrins and not only due to extraction efficacy. The transformations were at least partly reversible. References: 1. Chick H, Ellinger P (1941) Physiol 212–230. 2. Brockmann H, Lackner H (1979) Tetrahedron Letters 18: 1575–1578. 3. Habermann B (2000) Arch Farm, Farm Med Chem 333, Suppl. 2. 4. Hinneburg, Neubert Reinhard HH (2005) J Agric Food Chem 53: 3 – 7.

Seasonal variation of lipophilic constituents in roots of Echinacea purpurea and E. pallida
Thomson MD, Green K, Christensen UV
1Department of Food Science, Faculty of Science, Aarhus University, Aarslev, Denmark; 2Institute of Chemical Engineering, Biotechnology and Environmental Technology, University of Southern Denmark, Odense, Denmark

Echinacea purpurea (L.) Moench and E. pallida (Nutt.) Nutt. are widely used for the unspecific enhancement of the immune system. The plants origin from North America and are grown all over the world as garden flowers or as medicinal plants. Lipophilic constituents such as alkaloids and ketoalkenes/ketoalkynes are believed to be among the active meta-bolites in E. purpurea and E. pallida, respectively, with the highest concentrations being found in the roots. Most investigations on roots have been conducted on plants younger than one or two seasons and few have investigated the seasonal variation of lipophilic constituents in roots of Echinacea species. From early winter 2009 to fall 2010 five to 4 – 5 year old E. pallida and seven 3 – 4 year old E. purpurea roots from the same population of plants were collected throughout one year (i.e. before and after soil freeze in winter, mid spring, at high soil temperature in the summer and mid fall). Lipophilic constituents were extracted from milled freeze dried roots with EOH-H2O (70:30) and analyzed by HPLC-PDA and LC-MS/MS. The highest concentration of alkaloids in E. purpurea roots was found when soil temperature was just above 0 °C after winter and during summer, when the soil temperature was high. In the first case dodeca-2E,4E-diene-8,10-diydic acid isobutylamide was the major alkalide and in the latter case dodeca-2E,4E,8Z,10E/Z-tetra-enoic acid isobutylamides were the major constituents. For E. pallida roots the highest concentration of 2-ketoalkenes and –alkynes were found when the soil temperature was just above 0 °C after winter and here the major constituent was pentadeca-8Z,13Z-dien-11-yn-2-one.


Study of content and composition of anthocyanins in selected plants species
Labun P1, Fejér J2, Salamon P2, Ragács P3
1Department of Ecology, FHNS, Presov University, 01, 17th November St., SK-081 16 Presov, Slovakia; 2Excellence Centre of Human and Animal Ecology, Presov University, 01, 17th November St., SK-081 16 Presov, Slovakia; 3Medicoproduct, Co., Kap. Nalepka St., 02, SK-082 01 Lipany, Slovakia

Anthocyanins are heteroglycosides composed of aglycone – anthocyanine and sugar moiety. They are the final product of flavonoid production in secondary metabolism of plant cells. They are characteristic with antioxidant effects, through which they have positive effect on human organism. There is a large number of anthocyanidins, out of which only six are of the greatest importance, those with hydroxyl group at C-3 location. They are cyanidine, pelargonidine, peonidine, delphinidine, petunidine, malvidine. These are present in large amounts in plant species Vitis vinifera, Vaccinium corymbosum and Sambucus nigra. In the berries of Vitis vinifera they are accumulated in hypodermal cell layer of peel, or in some cultivars. Except for pelargonidin these anthocyanins contain all important anthocyanidines, with predomination of malvidine. Total content of anthocyanins in fresh berries ranged from 0,50 to 4,99 g·kg⁻¹ and in peels from 2,07 to 66,6 mg·g⁻¹ of peels dry matter. In the anthocyanins of Vaccinium corymbosum there were identified cyanidine, delphinidine, malvidine and peonidine. Their total content varies significantly depending on variety. Total determined content of anthocyanins ranged from 290,16 to 1343,08 mg·dm⁻². Sambucus nigra contains five important anthocyanidines: cyanidine 3-sambubiosid-5-gluco-side, cyanidine 3,5 diglucoside, cyanidine 3-sambubioside, cyanidine 3-glucoside and cyanidine 3-rutinoside. The content of identified anthocyanins in fruits of this species range from 602,9 to 1265,3 mg·100 g⁻¹. The amount of accumulated anthocyanins pigments depends on variety, ecological conditions standard of agricultural technology, and particularly on the temperature and solar radiation. Acknowledgement: The participation is supported by the Ministry of Education, Science, Research and Sport of the Slovak Republic, the project: 00162 – 0001 (MS SR-3634/2010 – 11).
Antioxidant activity of *Jasminum malabaricum* – A medicinal plant from Western Ghats

Hrudakale PJ, Gadkar SS, Hegde HV

1Department of Pharmaceutical Biotechnology, KLE University College of Pharmacy, Nehrunagar, Belgaum-590 010, Karnataka, India; 2Department of Pharmaceutical Biotechnology, KLE University College of Pharmacy, Nehrunagar, Belgaum-590 010, Karnataka, India; 3Regional Medical Research Centre, ICMR, Belgaum-590 010, Karnataka, India

*Jasminum malabaricum* Wight belonging to the family Oleaceae is endemic to Western Ghats of India. It is a climber with white flowers and fragrance, which is known for its ethnomedicinal importance as blood purifier and anti-tumor properties. The extensive exploitation of this species has led to reduction of its natural population. In the present study the leaves and stems were subjected for continuous shaking extraction (CSE) and microwave assisted extraction (MAE) using methanol. The antioxidant studies were performed with various concentrations of methanolic extract by DPPH and FRAP radical scavenging assay. The leaf extract showed significant activity (i.e. 92.86 ± 0.08% for CSE and 80.21 ± 0.01% for MAE) for DPPH method and for FRAP method the extract showed significant activity (i.e. 125 ± 31.25). The results showed significant activity (i.e. 84.80 ± 2.9% for CSE and 6.31 ± 0.01% for MAE) at concentration 0.2% which was compared with the standard ascorbic acid. References: 1. Mann HH (2008) J Linnear Soc (London) Botany 45:302: 155 – 8. 2. Thangavelu NR, Thomas S (2010) Int J Biol Med Res 1(4): 188 – 192

Antioxidant potential of Brazilian plants

Cruz CB, Campaña PR, Silva AP, Silva CG, Almeida VL

1División de Ciencias Farmacéuticas, Fundación Ezequiel Días, Belo Horizonte, CEP 30510 – 010, MG, Brasil; 2División de Ciencias Farmacéuticas, Fundación Ezequiel Días, Belo Horizonte, CEP 30510 – 010, MG, Brasil; 3Laboratório de Fitogênova, Faculdade de Farmácia, UFMG. Av. Antônio Carlos, 6627, Belo Horizonte, CEP 31270 – 090, MG, Brasil; 4Empresa de Pesquisa Agropecuária de Minas Gerais – Av. José Cândido da Silveira, 1647, Belo Horizonte, CEP: 31170 – 495, MG, Brasil.

Oxidative stress, the imbalance between free-radical formation and elimination, is part of the pathophysiology of many diseases. Exogenous antioxidants, such as those from plants, can help to restore the normal redox state of tissues. The aim of this study was to evaluate the antioxidant potential of plant species found in the Brazilian cerrado. The ethanolic extracts of different anatomical parts of 22 plant species (13 botanic families) were evaluated in vitro using two distinct approaches: the DPPH free-radical scavenging method (determination of EC_50_) and the β-carotene bleaching test (125; 62.5 e 31.25 μg/mL), both performed in microplates and with pyrogallol and quercetin as antioxidant standards. In this study, the sample was considered active when showed EC_50_ < 30 μg/mL in the DPPH method and inhibition of 50% of β-carotene oxidation at 31.25 μg/mL. All the 46 extracts evaluated showed concentration-dependent responses and 19 of them were considered active.


Phytochemical investigation of the Neotropical plant *Strychnos aff. darienensis*

Travasarou A, Vougioukanopoulou K, Fokialakis N, Cantrell C, Skalsounis AL

1Department of Pharmacognosy and Natural Products Chemistry, Faculty of Pharmacy, University of Athens, Zografou, Athens 15771, Greece; 2Natural Products Utilization Research Unit, USDA/ARS, P.O.Box 8048, University of Mississippi, 38677, USA

*Strychnos darienensis* Seem. of the family Loganiaceae, initially identified by Seemann [1], is widely distributed in the region of Central and South America [2] and it was used in the preparation of curarizing arrow poison from the South American Indian hunters. Generally, Strychnos species are rich in alkaloids, whereas the content of other secondary metabolites is in many cases notable. In continuation of our investigation of plants from the Amazonia [3], S. aff. darienensis that has not been previously investigated was collected from Peru. The evaluation of the best extraction procedure in order to recover a vast range of metabolites was of great importance. Profiling of the extracts with TLC revealed that the best approach was the maceration of the stem bark with EtOAc-EO-H2O-NH3 (96:3:1) and percolation with EtOAc and then with MeOH [4]. We report herein the investigation of the EtOAc and MeOH extracts, which has lead to the isolation of flavonoids and alkaloids. The isolation procedure was performed using Medium Pressure Liquid Chromatography (MPLC) with stationar and nonstationary phases. Two monomeric flavonoids and one bioflavonoid were identified, namely luteolin (1), 3-methoxyquercetin (2) and strychnobiflavone (3) and the monoterpene alkaloid venoterpin. Compound 2 is for the first time reported in the genus and strychnobiflavone (3) is isolated for the second time from a plant extract [5].

Figure 1: Isolated flavonoids of Strychnos aff. darienensis
Phenolic acids and free radical scavenging activity of Bulgarian endemic - *Alchemilla jumrukczalica* Pawl.

Nikolova MT, Dincheva P, Vitkova AA, Badjakov I

1Department of Plant and Fungal Diversity and Resources, Institute of Biodiversity and Ecosystem Research, Sofia, Bulgaria; 2AgroBiolInstitute, Sofia, Bulgaria

In the phytotherapy *Alchemilla vulgaris* L. complex is widely used as astringent, diuretic, anti-inflammatory agents, characterized by the presence of phenolic acids, flavonoids, tannins, triterpenes, etc. [1]. *Alchemilla jumrukczalica* Pawl. is a rare plant, unstudied for chemical constituents and biological activity until now. The present study aims to establish the antiradical potential and phenol content of *A. jumrukczalica* (cultivated materials) and *A. vulgaris* complex. The antioxidant activity of the methanol extracts was evaluated by the scavenging effect on 1,1-diphenyl-2-picrylhydrazyl radical (DPPH); radicals. The extracts showed significant antioxidant activity with IC\textsubscript{50} Values of 12.05 and 19.62 mg/ml respectively for *A. jumrukczalica* and *A. vulgaris* complex. Commercial antioxidant butylated hydroxytoluene (BHT) and syringic acid were used as positive controls and their IC\textsubscript{50} values are respectively 12.65 and 4.40 µg/ml. The methanol extracts of the studied samples were examined before and after acid hydrolysis for free and bounded phenolic acids. Ten free and seventeen bounded phenolic acids were identified and quantified by performed of gas chromatography mass-spectrometry (GC-MS). The extracts of the both samples contain phenolic acids in comparable amounts. Among the identified free phenolic acids gentisic, protocatechuic, salicylic and caffeic acids are represented in the greatest quantity. Salicylic, protocatechuic, caffeic, trans-cinnamic, gentisic and vanillic acids were the major bounded phenolic acids in the studied extracts. The present study revealed the extract of *A. jumrukczalica* as potential source of antioxidant activity. Acknowledgement: The authors are grateful for the financial support provided by the Bulgarian National Science Fund, Ministry of Education, Youth and Science (Project DTK-02/38). References: 1. Nikolov S (ed.) (2007) Specialized Encyclopedia of the Medicinal Plants in Bulgaria. Publishing House Trud, Sofia

NMR and UHPLC-MS-based metabolicomics for the discrimination of different resistant Vitis vinifera L. cultivar woods


1Laboratory of Pharmaceutical and Nutritional Products, Chemistry and Laboratory of Pharmaceutical Chemistry, School of Pharmacy, Panepistimiopolis, Zagorofia, 15771, Athens, Greece.; 2School of Pharmaceutical Sciences, University of Geneva, University of Lausanne, CH-1211 Geneva 4, Switzerland; 3Agroscope Changins-Wädenswil ACW, 1260 Nyon, Switzerland

Vitis cultivars exhibit different susceptibility to pathogens such as botrytis or downy mildew and the selection of resistant species is important for a sustainable production without use of harmful pesticides. In order to highlight biomarkers that can be related to Vitis resistance to common diseases, woods of resistant Vitis cultivars were profiled by NMR and UHPLC-TOF-MS [1] and analysed based on differential metabolomics [2]. Three different samples of Vitis wood, one resistant to botrytis, one resistant to downy mildew and one susceptible to both phytopathogenic microorganisms were used in this study. The wood samples of specific specimens were divided in 18 groups (6 per cultivar) and extracted separately with EtOAc to offer statistical confidence. Different sample preparation protocols were developed and applied in parallel for the NMR (600 MHz) and UHPLC-TOF-MS analysis of the extracts, respectively. Multivariate data analysis using both supervised (PLS-DA) and unsupervised (PCA) methods and different scaling methods revealed a clear distinction between the three groups as well as in the discrimination between the two different resistant species. A high convergence of the discrimination pattern was confirmed by both NMR and UHPLC-TOF-MS data was obtained. The NMR and MS variables derived from the loading plots were attributed to specific biomarkers. This statistical model could be efficiently applied for the determination of resistant cultivars of Vitis as well as for the identification of novel biomar-
The objects of present study are protected, endemic and rare high-mountain medicinal plant in Bulgaria – *Alchemilla achtarowii* Pawl., *A. mollis* (Buser) Rothm., *Gentiana lutea* L. ssp. *symphandra* (Murb.) Hayer, *Arnica montana* L. The species are widely used in modern phytotherapy, they are demand raw materials on national and international markets. This requires their cultivation by conventional and biotechnological methods. The purpose of present study is to evaluate antiradical capacity and total phenols of plants which were grown in situ (A. achtarowii, G. lutea), ex situ (A. mollis) and ex vitro (A. montana). The methanol extracts of examined species were estimated using a 2,2-diphenyl-1-picylhydrazyl (DPPH) and Folin–Ciocalteu assays. The extracts of aerial parts of *A. mollis* and *A. achtarowii* showed significant antiradical activity with IC$_50$ values below 50 mg/ml. The lowest activity was found of extract of *G. lutea* > 200 mg/ml. The extracts of folia and flowers of ex vitro plants of *A. montana* revealed high radical scavenging activity too, their IC$_50$ values are 64.01 and 85.73 mg/ml respectively. Commercial antioxidant butylated hydroxytoluene (BHT) was used as positive control and its IC$_50$ value is 12.65 mg/ml. The antiradical properties of the studied extracts positively corresponded with their total phenol content. The results obtained showed high antiradical qualities of the examined species. It is especially important that the ex situ and ex vitro grown plants kept their valuable properties. These results will be basis for a future comparative analysis of antioxidant capacity and the content of active components of these species. Acknowledgement: The authors are grateful for the financial support provided by the Bulgarian National Science Fund, Ministry of Education, Youth and Science (Project DTK-02/2008).
lanthus atropurpureus Bojer, revealed that its extracts is quite similar to silymbin. In both of them improve the parameters of CCl₄-induced liver injury including serum AST and ALT. Among the extracts tested, root extract showed maximum activity as compared with aerial part extract relative to silymbin.

**Response of germination and seedling growth of,** hyssop (Hyssopus officinalis) and Marigold (Chrysanthemum x superbum) as medicinal plants to water stress

Rezvani Moghaddam P1, Ehyai H2, Amiriz M2, Fallahi F1, Aghhavani Shajari M3
1College of Agriculture, Ferdowsi University of Mashhad, Iran.; 2Department of Crop Physiology, College of Agriculture, Ferdowsi University of Mashhad, Iran.; 3Department of Agroecology, College of Agriculture, Ferdowsi University of Mashhad, Iran.

In order to study the effects of five levels of water stress (0, -2, -4, -6 and -8 bar) on germination characteristics and seedling growth of two medicinal plants (Hyssopus officinalis L. and Chrysanthemum x superbum Bergmans ex Ingram); two experiments were conducted at physiology laboratory of Faculty of Agriculture Ferdowsi University of Mashhad as a Completely Randomized Design with four replications. The results showed that the effects of different levels of water stress were significant in all of the studied characteristics of two plants. Germination percentage was decreased and mean germination time were increased by increasing in water stress levels and, germination percentage was zero in levels of -6 bar in two types of plants. It is suggested that decrease in seed germination and depression in seedling growth under drought conditions related to limited hydrolysis of food reserves from storage tissues as well as due to impaired translocation of food reserves from storage tissue to developing embryo axis [3]. Root length of Hyssop and Mariguerite respectively, were increased and decreased by increasing in water stress levels. Plumule length had a decreasing trend in two studied plants but amounts of this trend was less in hyssop and the root length/plumule length were increased in each of plants. Many researches were shown that an increased root/shoot ratio resulting in more efficient water and nutrient uptake [1,2]. Also, dry weight root had an increased trend and dry weight plumule had decreasing trend but root dry weight/plumule dry weight was increased in two types of plants.


**Omididun (corn liquor): an economic solution to xerostomia**

Okoyun OA1, Agbebiyi OA2, Samuel TA1
1Department of Pharmacognosy, Faculty of Pharmacy; 2Department of Preventive Dentistry; 2Department of Biochemistry, College of Medicine, University of Lagos, Idi-Araba, P.M.B 12003, Lagos-Nigeria

Xerostomia is dry mouth resulting from reduced or absent saliva flow associated by dehydration, use of drugs, various syndromes (Plummer-Vinson syndrome) and side effects of radiotherapy and chemotherapy in cancer treatment. It can affect nutrition and dental as well as psychological health. Omididun is the liquor obtained from fermented ground and sieved maize or sorghum. While the ground wet flour obtained is boiled into a semisolid cereal (Ogi) for breakfast in Nigeria. Lactoperoxidase (LPO) presence was confirmed in Omididun obtained from four varieties of fermented corn [Zea mays Linn. (Poaceae) white and yellow varieties and Sorghum bicolor Linn. (Poaceae) white and red varieties] using the principle of LPO decomposition of hydrogen peroxide and the oxidation of colorless 1, 4-phenylenediamine into the purple indophenol. LPO was estimated with a reaction mixture of hydrogen peroxide and potassium iodide solutions, incubated at room temperature to achieve equilibrium and absorbance read at 350 nm in a UV spectrophotometer against a blank without Omididun and procedure repeated for commercially available dry mouth wash and toothpaste. The color intensity was proportional to the LPO’s concentration in the order of yellow corn> white corn>red sorghum>white sorghum. LPO content increases from 3.528±0.451% in white sorghum to 34.713±0.068% in yellow corn. It is proposed that omididun could be used as a mouth rinse or incorporated in tooth paste because of the natural LPO content to treat xerostomia and as well reduce oral bacteria and consequently the acid produced by those bacteria.

**Fungal transformation of pimaradienoic acid and its schistosomicidal activity against Schistosoma mansoni**

Ambrósio SR1, Porto TS1, Filho AA1, Magalhães LC2, Veneziani RC1, Furtado NA2, Simão MR3, Severiano ME4, Melo ME5, Rodrigues V2, Said S2
1University of Franca, Av. Dr. Armando Salles de Oliveira 201, 14404 – 600, Franca-SP, Brazil.; 2University of São Paulo, Av. Café s/n, 14040 – 903, Ribeirão Preto-SP, Brazil.

In the present work, the microbial transformation of pimaradienoic acid (PA, 1) (Figure 1) was performed using submerged shaken liquid culture of Aspergillus ochraceus (1.8 x 106 spores/mL). The microorganism was grown by a two-stage fermentation procedure [1]. PA was added as a dimethylsulfoxide solution (0.1 g/L) and incubated for 3 days. The culture was filtered and the aqueous layer was extracted with ethyl acetate to furnish the extract codified as AoPA. Chemical and NMR studies of AoPA allowed us to isolate and to identify two PA derivatives (Figure 1: Compounds 2 and 3). The in vitro schistosomicidal activity of these metabolites was performed against male and female S. mansoni adult worms [2], and the results denote that PA is very effective with respect to the separation of coupled pairs, mortality, decrease in the motor activity and tegumental alterations. In addition, PA is able to reduce the percentage of eggs number and egg development. In this context, the schistosomicidal effects of PA indicate that ent-pimarane diterpenes could be considered a promising source for discovery of new agents to treat human schistosomiasis.

**Biotransformation of ent-8(14),15-pimaradiene and antimicrobial activity of the obtained derivatives against multi-resistant Gram-positive bacteria**

Ambrósio SR1, Porto TS1, Da Silva JR2, Melo ME3, Martins CH4, Veneziani RC1, Helfno VC5, Furtado NA2, Arakawa NS5, Said S2
1University of Franca, Av. Dr. Armando Salles de Oliveira 201, 14404 – 600, Franca-SP, Brazil.; 2University of São Paulo, Av. Café s/n, 14040 – 903, Ribeirão Preto-SP, Brazil.; 3University of Vale do Paraíba, Av. Shishima Hifumi 2911, 12240 – 000, São José dos Campos-SP, Brazil.

In the present work, the microbial transformation of ent-8(14),15-pimaradiene (Figure 1: 1; PI) was performed using submerged shaken liquid culture of Aspergillus ochraceus (1.8 x 106 spores/mL). The microorganism was grown by a two-stage fermentation procedure [1]. PA was added as a dimethylsulfoxide solution (0.1 g/L) and incubated for 7 days. The culture was filtered and the aqueous layer was extracted with ethyl acetate to furnish the extract codified as AoPI. Chemical and NMR studies of AoPI allowed us to isolate and to identify four PA derivatives (Figure 1: Compounds 2, 3 and 4). The schistosomicidal activity of these metabolites was performed against S. mansoni adult worms [2], and the results show that the effects of different levels of water stress were significant in all of the studied characteristics of two plants. Germination percentage was decreased and mean germination time were increased by increasing in water stress levels and, germination percentage was zero in levels of -6 bar in two types of plants. It is suggested that decrease in seed germination and depression in seedling growth under drought conditions related to limited hydrolysis of food reserves from storage tissues as well as due to impaired translocation of food reserves from storage tissue to developing embryo axis [3]. Root length of Hyssop and Marguerite respectively, were increased and decreased by increasing in water stress levels. Plumule length had a decreasing trend in two studied plants but amounts of this trend was less in hyssop and the root length/plumule length were increased in each of plants. Many researches were shown that an increased root/shoot ratio resulting in more efficient water and nutrient uptake [1,2]. Also, dry weight root had an increased trend and dry weight plumule had decreasing trend but root dry weight/plumule dry weight was increased in two types of plants.

Antioxidant capacity of Matricaria chamomilla L. extract and its effect on neural tube structure in diabetic rat offspring

Namayan F1, Panahi M1, Ahmadpour F2, Darvish A1, Azemi M1, Khodayar M1, Samaei H1
1Medical Plant Research Center, Pharmacognosy Department, School of Pharmacy, Ahvaz Jundishapur University of Medical Sciences; 2Anatomy Department, School of Medicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran

Increased oxidative stress has been suggested to play a role in the pathogenesis of disturbed embryogenesis in diabetic pregnancies also cause several types of histopathologic changes in the placenta (1) The present study was conducted to determine whether Matricaria chamomilla L. extract, a well-known medicinal herb with appropriate antioxidant activity, would reduce the incidence of diabetic embryopathy in the streptozotocin-induced diabetic rat model. Antioxidant capacity of extract was measured using DPPH method. Diabetic and control rats were administered 100,300,500, mg/kg chamomile extract. Mating condition was prepared by putting male rats and diabetic female rats together. Vaginal plug mentioned as a positive sign of pregnancy and treatment started with extract or vehicle from 1th to 17th day of gestation by gastric gavages. Blood glucose was measured during 17 days. Results show levels of blood glucose was reduced about 1.62 fold (p < 0.00) in treated diabetic rats. At 17th day, rats were sacrificed. The fetuses were released from the yolk sacs and surrounding decidua and were neural tube of fetuses was examined by Light microscopy and electron microscopy. Neural tube defect was significantly reduced and only some tube of fetuses was examined by Light microscopy and electron microscopy. Neural tube defect was significantly reduced and only some tube of fetuses was examined by Light microscopy and electron microscopy. Neural tube defect was significantly reduced and only some tube of fetuses was examined by Light microscopy and electron microscopy.


Figure 1: Chemical structures of PI (1) and its derivatives obtained through fermentation for 7 days with A. ochraceus.

Quantitative analysis of rosmarinic acid in Rosmarinus officinalis growing in Turkey by LC-MS/MS

Alitans A1, Göger F1, Duymus HC1, Krammer N1, Başer KHC2
1Anadolu University, Faculty of Pharmacy, Department of Pharmacognosy, 26470 Eskişehir, Turkey; 2King Saud University, College of Science, Botany and Microbiology, P. D. B. XON 2455 – Riyadh 11451- Saudi Arabia

Rosmarinic acid is naturally occurring polyphenolic compound mostly found in Lamiaceae family herbs such as rosemary, perilla, oregano, sweet basil. It exhibits various biological activity like antioxidant, anti-viral, antibacterial and anti-inflammatory. LC-MS/MS has an important role in the studies of identification and characterization of natural compounds, drug metabolism, discovery of new drug candidates because of its both sensitivity and specificity. In the present study, we aimed to quantify of rosmarinic acid in methanol extract of Rosmarinus officinalis leaves collected from Izmir by using a rapid LC-MS/MS method. Therefore we used turbo spray ionization for LC-EI-MS method in negative mode. Rosmarinic acid was characterized by its MS/MS spectrum and LC retention time. The assay performed in different concentrations of rosmarinic acid as standard solutions. The diagnostic fragmentations 358.9/160.9 and 358.9/197.0 of rosmarinic acid were used for the quantification. As a result, 0.31 g rosmarinic acid measured in 100g methanol extract of rosemary leaves. References: 1. Petersen M, Simmonds MSJ (2003) Phytochem 62:121 – 125. 2. Park SU, Uddin MR, Xu H, Kim YK, Le, S (2008) African J Biotechn 7(25): 4959 – 4965.

Chronic anti-inflammatory potential of aqueous extract of Capraria biflora L

Viken I1, Valido R2, Boffill M1, Grau R1, Siverio D1, Gonzalez DM1
1Central University of Las Villas, Road to Camajuaní Km 5, Santa Clara, Cuba; 2Experimental Toxicological Unit, Villa Clara Medical Sciences University, Santa Clara, Cuba

Capraria biflora L is a plant with a long history in the traditional medicine, commonly used in chronic pathologies associated to inflammatory processes. Extracts of this plant were active in models of acute inflammation in studies developed previously but information of its action in models of chronic inflammation does not exist. The present study was designed to investigate anti-inflammatory activity of aqueous extract of Capraria biflora using cotton pellet-induced granuloma method. Male Sprague-Dawley rats weighing 180 – 220 g were used. Six groups of six animals each one were used. Group I served as control (water distilled) and group II as standard (Indomethacin 5 mg/kg). Group III, IV and V received extract at the doses of 200, 400 and 800 mg/kg respectively. The results were analyzed for to SPSS, version 11.5 for Windows, using the tests of Kruskal-Wallis and Mann-Whitney for independent samples. Oral administration of the aqueous extract decreased significantly (p < 0.01) the weight of the granuloma forma

PL90

Medical and aromatic plants in generating new values for the 21st century

Redzic S

Academy of Sciences and Arts of Bosnia and Herzegovina, Bistrik 7, 71 06 Sarajevo, Bosnia and Herzegovina

The population is growing exponentially. The needs for food and medicine is increasing. Regional, and global poverty is increasing. A way to reduce the galloping growth of poverty is sustainable use of medicinal and aromatic plants (MAP). Especially in countries in transition [1]. The biggest global market of MAPs is China, Germany, France, Italy, Japan, Spain, United Kingdom and the United States. The International Council for MAPs has announced that global growth during 2001 and 2002 was 8 – 10% per year. The world market was estimated at 60 billion U.S. $ in 2003. Europe is a major world trader of MAPs. Today at the market are at least 2000 species of MAPs, of which 1200 to 1300 species are associated only to the European continent [3,4]. In current situation needs for herbal products at the international market is increasing. It is high opportunity for generating of sustainable benefit using of natural resources. There are more chances for regional and global economy to improve. Particularly it is great opportunity for global poor (especially in the Third and Fourth World). For the sustainable use of MAPs, it is necessary to develop programs of organic certification [5].


PL91

Systemic Studies on Arctii Fructus

Kang T

Liaooning University of Traditional Chinese Medicine, Dalian, China

Arctii fructus is the dry seeds of Arctium lappa L. and generally used as an herbal medicine in traditional Chinese medicine. The pharmacognosy and anticancer constituents of Arctii fructus as well as the ecological suitability of Arctium lappa and its suitable cultivation regions in China were studied. Our research established a method for distinguishing Arctii fructus from its adulterants. In Chinese patent medicine, the processed Arctii fructus was mostly used. The optimal procedure for the processing of Arctii fructus were studied and the processing principle of Arctii Fructus was determined to be: 1) Protect arctinin from degradation by hydrolytic enzyme in fructus arctii; 2) Extract the active constituents from arctii fructus easier by making the pericarp texture of arctii fructus crispy; 3) Abate the nature of arctii fructus by attenuating the purgative action by a decreasing content of lipids and arctin. Dao-Di-Yao-Cai means the best and highest quality of Chinese medicine materials. It is a unique Chinese medicine in traditional Chinese medicine and the result of long-time clinical experience of practitioners. The suitable cultivation regions for Arctii fructus in China were determined on the basis of its ecological suitability.

PL92

Competition of chemical composition of Artemisia annua volatile oil from Romania

Toth E1, Dezo AC2, Kopas A3, Polko J4, Ichim MC4

1Targu Mures University of Medicine and Pharmacy, Cheorghi Marinescu St., 38, 540139, Romania; 2Sapienita University, Department of Food Science, Libertatii Sq., 1, Miercuri Cu Caz, 530104, Romania; 3Politehnica University of Bucharest, Department of Chemical Engineering, Spiru Independentei, 313, 060042, Bucharest, Romania; 4NIRDS/”Stejarul” Research Centre for Biological Sciences, Alexandru cel Bun St., 6, Patra Neamt, 610004, Romania

The aim of our work was to obtain and compare, quantitatively and qualitatively, the composition of volatile oil from the Artemisia annua L. (sweet wormwood). The fresh plant material, harvested from a Romanian natural population and Anamed (A3) cultivar, was distilled with the classical Clevenger (HD) and the microwave assisted (MWHD) laboratory hydrodistillation [1]. The MWHD apparatus, with original design, was equipped with a microwave oven (750 W), a rotating head and a Clevenger extension. The volatile oil samples were analyzed by thin layer chromatography (TLC) and gas chromatography (GC). The amount of volatile oil obtained was 0.57 respectively 0.58% v/w (HD) and 0.69% v/w (MWHD). The operation time for MWHD was 20 minutes and 180 minutes for HD. With TLC 11 spots were visualised; in case of MWHD 20 spots were visualised. With this method it is necessary to apply international law, particularly the Convention on Biological Diversity and the CITES Convention. Sustainable use of MAP is a prerequisite in generating ecologically sustainable benefit. MAPs are a great opportunity for new medicines and bio-materials [6] in both, developed and developing countries. References: 1. Redzic S. (2006) Proc.Ist IFOAM Intern. Conf. Organic Wild Production, 117 – 141.


PL93

Comparison of chemical composition of Artemisia annua volatile oil from Romania

Toth E1, Dezo AC2, Kopas A3, Polko J4, Ichim MC4

1Targu Mures University of Medicine and Pharmacy, Cheorghi Marinescu St., 38, 540139, Targu Mures, Romania; 2Institut f. Pharmazie, Pharmakognosie, Leopold-Franzens-Universität Innsbruck, A-6020 Innsbruck, Austria

Determination of alkannin/shikonin derivatives in endemic Greek Alkanna species

Assimopoulou AN1, Tappeiner J2, Ganzera M3, Vasiliiou A4, Stuppner H2, Papageorgiou VP5

1Institute of Pharmacognosy, University of Thessaloniki, Department of Chemical Engineering, School of Engineering, 541 24 Thessaloniki, Greece; 2Institut für Pharmazie, Pharmakognosie, Leopold-Franzens-Universität Innsbruck, A-6020 Innsbruck, Austria

Alkannin and Shikonin (A/S) derivatives are optical antipodes of plant origin with a verified wide spectrum of antimicrobial, wound healing, anti-inflammatory and antioxidant activity. Although the aforementioned antipodes were originally introduced as wound healing agents, recent studies on cancer chemotherapeutics revealed that A/S also exhibit antitumor activity. A/S have been found in roots of several Boraginaceae species [1 – 3]. Determination of A/S, their esters and the total A/S content in Boraginaceae roots of different origin was reported in several papers [4 – 7]. Alkanna species grown wild in Greece have been analysed for their A/S esters by LC–PDA–MS previously [5], whereas the total A/S content (A/S and their derivatives) has not been reported. In the present study endemic Alkanna species (tinctoria (L) Tausch, pindicola Hausskn., orientalis (L) Boiss., methana Hausskn., callensis Boiss., graeca Boiss. & Spruner, primuliflora Griseb., stribrnyi Velen., sieberi DC., noneiformis Griseb.) grown in various Greek regions were collected and analyzed for their total A/S content for the first time. A comparison was additionally performed among species and different regions. Quantitative analysis revealed that specific root samples of A. tinctoria, A. pindicola and A. sieberi showed the highest amount of A/S and derivatives (1.41, 1.38, 1.00 mg/100 mg root respectively), but the A/S content varied from one region to another even within the same species. Yet, a significant difference in A/S content was observed among species. With this study it can be concluded that some of the examined Alkanna species of the Greek flora could serve as alternative sources for medicinally valuable A/S derivatives. Keywords: Alkannin, shikonin, Alkanna, Boraginaeae, naphthoquinones, wound healing References: 1. Papageorgiou VP, Assimopoulou AN et al. (1999) Angewandte Chemie, Int. Edition 38(3): 270 – 301. 2. Papageorgiou VP, Assimopoulou AN et al. (2006) Current Organic Chemistry 10(16): 2123 – 2142. 3. Papageorgiou VP et al. (2008) Current Medicinal Chemistry 15(30): 3248 – 3267. 4. Papageorgiou VP, Assimopoulou AN et al. (2006) Current Organic Chemistry 10(5): 583 – 622. 5. Assimopoulou AN et al. (2006) Biomedical Chromatography 20: 1359 – 1374. 6. Pekin G. et al. (2007) Planta Med 73: 267 – 272. 7. Akgun IA et al. (2009) Chromatographia 70: 963 – 967.
Mexican poppy (Argemone mexicana L.) is an annual thorny herb which belongs to Papaveraceae family. It is commonly spread in Mexico and in south-western North America where it is growing in the wasteland. Argemone mexicana belongs to Papaveraceae family. It is commonly spread in Mexico and in south-western North America where it is growing in the wasteland.

In this study quantitative determination of hypericin and qualitative analysis of the phenolic compounds of the flowering aerial parts of five endemic Hypericum species of Turkey, namely, Hypericum kotschyanum Boiss., H. salignum Robson et Hub.-Mor., H. scabroides Robson et Poul., H. thyrsomis Boiss. and H. uniglandulosum Hausskn. ex Borm. were performed by HPLC (1). The results were also compared to each other. It was observed that the H. salignum was the highest hypericin content among the others. In the phenolic compounds side of view, quercetin and isoquercitrin were determined in all species. The rutin was observed in the H. kotschyanum, different from the other species. References: 1. European Pharmacopoeia (2008). Herbal Monographs: St John’s Wort (Hyperici herba), EDQM, Strasbourg, pp: 3839 – 3842.

Recent reports of increased tolerance to artemisinin derivatives, the most recently adopted class of antimalarials, have prompted a need for new treatments. In this context, we tested the antimalarial activity of lamaroflavone, a biflanonoid isolated from the methanol extract of the aerial part of Campnosperma panamense Standl. (Anacardiaceae), an endemic tree species of Colombia (1). Lamaroflavone showed good in vitro antimalarial activity but was inactive in a rodent model. Here, we report the antimalarial activity of several extracts from the aerial part of Argemone mexicana in the Artemia test. The methanol extract of this plant was found to be active against Plasmodium berghei and Plasmodium falciparum in vitro.

Results: The methanol extract of A. mexicana showed in vitro antimalarial activity against P. berghei and P. falciparum. The extract was found to be active against both sensitive and resistant strains of P. falciparum (100% inhibition at 500 µg/mL). The extract was also found to be active against P. berghei (100% inhibition at 1000 µg/mL).

Conclusions: The methanol extract of A. mexicana has potential antimalarial activity. Further studies are needed to investigate the mechanisms of action and to develop this plant as a potential antimalarial drug.

Methods: The methanol extract of A. mexicana was obtained by maceration. The extract was tested against P. falciparum and P. berghei using the in vitro Artemia test. The IC50 values were calculated using GraphPad Prism software.

References:
3. Lanaroflavone showed good in vitro antimalarial activity but was inactive in a rodent model. Here, we report the antimalarial activity of several extracts from the aerial part of Argemone mexicana in the Artemia test. The methanol extract of this plant was found to be active against Plasmodium berghei and Plasmodium falciparum in vitro.

Results: The methanol extract of A. mexicana showed in vitro antimalarial activity against P. berghei and P. falciparum. The extract was found to be active against both sensitive and resistant strains of P. falciparum (100% inhibition at 500 µg/mL). The extract was also found to be active against P. berghei (100% inhibition at 1000 µg/mL).

Conclusions: The methanol extract of A. mexicana has potential antimalarial activity. Further studies are needed to investigate the mechanisms of action and to develop this plant as a potential antimalarial drug.

Methods: The methanol extract of A. mexicana was obtained by maceration. The extract was tested against P. falciparum and P. berghei using the in vitro Artemia test. The IC50 values were calculated using GraphPad Prism software.

References:
3. Lanaroflavone showed good in vitro antimalarial activity but was inactive in a rodent model. Here, we report the antimalarial activity of several extracts from the aerial part of Argemone mexicana in the Artemia test. The methanol extract of this plant was found to be active against Plasmodium berghei and Plasmodium falciparum in vitro.

Results: The methanol extract of A. mexicana showed in vitro antimalarial activity against P. berghei and P. falciparum. The extract was found to be active against both sensitive and resistant strains of P. falciparum (100% inhibition at 500 µg/mL). The extract was also found to be active against P. berghei (100% inhibition at 1000 µg/mL).

Conclusions: The methanol extract of A. mexicana has potential antimalarial activity. Further studies are needed to investigate the mechanisms of action and to develop this plant as a potential antimalarial drug.

Methods: The methanol extract of A. mexicana was obtained by maceration. The extract was tested against P. falciparum and P. berghei using the in vitro Artemia test. The IC50 values were calculated using GraphPad Prism software.

References:
3. Lanaroflavone showed good in vitro antimalarial activity but was inactive in a rodent model. Here, we report the antimalarial activity of several extracts from the aerial part of Argemone mexicana in the Artemia test. The methanol extract of this plant was found to be active against Plasmodium berghei and Plasmodium falciparum in vitro.

Results: The methanol extract of A. mexicana showed in vitro antimalarial activity against P. berghei and P. falciparum. The extract was found to be active against both sensitive and resistant strains of P. falciparum (100% inhibition at 500 µg/mL). The extract was also found to be active against P. berghei (100% inhibition at 1000 µg/mL).

Conclusions: The methanol extract of A. mexicana has potential antimalarial activity. Further studies are needed to investigate the mechanisms of action and to develop this plant as a potential antimalarial drug.

Methods: The methanol extract of A. mexicana was obtained by maceration. The extract was tested against P. falciparum and P. berghei using the in vitro Artemia test. The IC50 values were calculated using GraphPad Prism software.

References:
3. Lanaroflavone showed good in vitro antimalarial activity but was inactive in a rodent model. Here, we report the antimalarial activity of several extracts from the aerial part of Argemone mexicana in the Artemia test. The methanol extract of this plant was found to be active against Plasmodium berghei and Plasmodium falciparum in vitro.

Results: The methanol extract of A. mexicana showed in vitro antimalarial activity against P. berghei and P. falciparum. The extract was found to be active against both sensitive and resistant strains of P. falciparum (100% inhibition at 500 µg/mL). The extract was also found to be active against P. berghei (100% inhibition at 1000 µg/mL).

Conclusions: The methanol extract of A. mexicana has potential antimalarial activity. Further studies are needed to investigate the mechanisms of action and to develop this plant as a potential antimalarial drug.

Methods: The methanol extract of A. mexicana was obtained by maceration. The extract was tested against P. falciparum and P. berghei using the in vitro Artemia test. The IC50 values were calculated using GraphPad Prism software.

References:
Comparison of the antioxidant activity and total phenolic contents in several species of Lamiaceae family.

Lamiud M1, Fatihzadeh I2
1Young Researchers Club, Islamic Azad University, Sari, Iran.; 2Department of Pharmacognosy, University of Medical Sciences, Tabriz, Iran.

Antioxidant compounds in food play an important role as a health protecting factor. In this study, the methanolic extracts of the aerial parts of nine Lamiaceae species: Mentha spicata L., Mentha aquatica L., Mentha piperita L., Stachys byzantina K.Koch., Marrubium vulgare L., Rosmarinus officinalis L., Salvia officinalis L., Thymus vulgaris L. and Melissa officinalis L. were investigated for their antioxidant activity and total phenolic and flavonoid contents. DPPH and Polin-Cioccini reagents and potassium chloride assays respectively. The IC50 of the methanolic extracts ranged between 42.67 – 489.97 µg/ml, total phenolic content were between 38.27 – 59.14 mgGAE/g dw. R. officinalis and M. vulgaris showed the most content of antioxidant activity. There was a direct correlation between total phenol and antioxidant activity which indicates that polyphenols are the main antioxidants.

Comparative analysis of polyphenols and flavonoids in natural populations of Crataegus monogyna from Eastern Carpathians

Toth ET1, Mitroiu C2, Kelenem L1, Ichim MC4
1Targu Mures University of Medicine and Pharmacy, Gheorghe Marinescu St., 38, 540139, Targu Mures, Romania; 2Commercial Society for Medicinal Plant Research and Processing PLANTAVOREL S.A., Caza Voda St., 46, 610019 Piatra Neamt, Romania; 3Gedeon Richter LTD Romania, Caza Voda St., 99 – 105, Targu Mures, Romania; 4NIRDAS/ "Stefan" Research Centre for Biological Sciences, Alexandria cel Buz St., 6, Piatra Neamt, 610044, Romania

Our aim was to obtain and compare, quantitatively and qualitatively, the polyphenols and flavonoids from six natural populations of Crataegus monogyna Jacq. harvested from the spontaneous flora of Eastern Carpathians. The Crataegi fructus and Crataegi folium cum flore samples were collected from Neamt County –Cernegura Hill (no. 1), Batca Doamnei (no. 2), Cheile Bicazului (no. 3) and other three from Harghita County – Praid area (no. 3, 4, 5). The qualitative analysis of polyphenols and flavonoids was performed by TLC and HPLC. The quantitative analysis was performed by UV/VIS spectrophotometry [1] (rutside for flavonoids and chroomic acid for polyphenols). TLC analysis of Crataegi fructus samples has revealed four polyphenols (chlorogenic and caffeic acids and two caffeic compounds) and two flavonoids (hyperoside and vitexine). TLC analysis of Crataegi folium cum flore samples has revealed two polyphenols (chlorogenic and caffeic acids) and three flavonoids (rutin, hyperoside and a 6-0-glicoside of luteolin). Sample no. 6 seems to contain also vitexine. The HPLC analysis of an additional Crataegi folium cum flore sample from Piatra Neamt has revealed UV spectra of the main seven compounds (the chlorogenic acid was identified). The spectrophotometric quification of flavonoids from Crataegi folium cum flore samples has detected a high content (1.0175 % – 1.9175 %) then the one detected in fruit samples (0.1218 % – 0.2801 %). The quantification of polyphenols from Crataegi fructus has identified high amounts (2.11 % – 2.70 %). When the total quantity of polyphenols was compared (flowers, leaves and fruits) the most valuable populations amounts (2.11% – 2.70%). When the total quantity of polyphenols was compared (flowers, leaves and fruits) the most valuable populations amounts (2.11% – 2.70%). When the total quantity of polyphenols was compared (flowers, leaves and fruits) the most valuable populations amounts (2.11% – 2.70%). When the total quantity of polyphenols was compared (flowers, leaves and fruits) the most valuable populations amounts (2.11% – 2.70%). The content of the main seven compounds (the chlorogenic acid was identified). The spectrophotometric quification of flavonoids from Crataegi folium cum flore samples has detected a high content (1.0175 % – 1.9175 %) then the one detected in fruit samples (0.1218 % – 0.2801 %). The quantification of polyphenols from Crataegi fructus has identified high amounts (2.11 % – 2.70 %). When the total quantity of polyphenols was compared (flowers, leaves and fruits) the most valuable populations amounts (2.11% – 2.70%).

Biological effects and phenolic content of felty germander (Teucrium polium L. subsp. polium) Stankovic MS1, Mila I1, Franko B2, Milos M1, Politeo O2
1Department of Biology and Ecology, Faculty of Science, University of Kragujevac, Radoja Domanovica 12, 34000 Kragujevac, Serbia; 2Department of Chemistry and Technology, University of Split, Teslina 10/IV, 21000 Split, Croatia

Felty germander – Lamiaceae is popular species of Teucrium genus in the folk medicine and used for treatment of appetite loss and gastrointestinal ailments [1]. In the present study, antioxidative and anti-acetylcholinesterase activity, total phenolic content as well as flavonoid concentration of methanolic, acetone and ethyl acetate extracts were investigated. Ferric reducing/antioxidant power (FRAP) [2] was assayed and values were between 235 and 846 µmol Fe 2+ eq/l. The antioxidant capacity have been evaluated using the Briggs-Rauscher oscillating reaction method [3], expressed as a time required for regeneration of oscillations in minutes and obtained values were: 43.5 for methanolic, 2.0 for acetone, while ethyl acetate extract did not show activity. The ability to scavenge DPPH radicals [4] was determined and expressed as IC50 values that ranged from 59.37 to 622.96 µg/ml. Acetylcholinesterase inhibition was measured using slightly modified Ellman’s method [5] and results indicate a weak inhibitory activity of extracts. Total phenolic content was determined using Folin-Ciocalteu reagent and the values ranged from 26.6 to 124.62 mg of GA/g of extract. The content of flavonoids in extracts ranged from 47.76 up to 78.82 mg of RU/g of extract. Methanolic extract was most active in comparison with other extracts for all measurements. That indicates that the methanol, as a polar solvent, is the very effective for phenolic compounds extraction from T. polium subsp. polium. Based on the obtained results, T. polium subsp. polium extracts are rich sources of phenolic compounds and promising candidates for further development as natural antioxidant agents. Acknowledgement: Ministry of Science and Education, Republic of Serbia (III4010). References: 1. Sharififar F et al. (2008) Food Chem 112: 885 – 888. 2. Benzie IFF & Strain JJ (1996) Analytical Biochem 239: 70 – 76. 3. Briggs TS & Rauscher WCJ (1973) Chem Educ 50: 496. 4. Stankovic SM et al. (2010) J Med Plant Res 5. Ellman GL et al. (1961) Biochem Pharmac 7: 88 – 95.

Antitubercular activity of pimarane and kaurene diterpenes against Mycobacterium tuberculosis

Helena VC1, Martins CG2, Cabral MW3, Silva AN4, Matos PM5, Sozza MC6, Veneziani RS7, Ambrosio SR8
1Universidade de Franca, R. Dr. Armando Salles Oliveira, 201, 14404 – 600 Franca-SP, Brazil

Tuberculosis (TB) is still a public health problem and causes millions of deaths every year [1]. According to the World Health Organization, 8.8 million new TB cases occurred in 2007 [2]. Since the increase of bacterial resistance and the emergence of new infections are common problems [3], the search for new antibacterial or antitubercular agents is a urgent matter. In the course of our investigation about diterpenes and their biological activities, we have performed some antimycobacterial assays against Mycobacterium tuberculosis H37Rv, ATCC 27294 with five diterpenes. All of them showed at least moderate activity a MIC of <= 31.25 µg/ml, that can be classified as promising, as according to Cantrell et al. [4] a MIC value below 64 µg/ml. For isolated compounds can be considered to be of interest. In the present work, compound 1 was the less active with a MIC of <= 250 µg/ml. Compounds 2 and 4 are of moderate activity each with a MIC of 125 µg/ml. Most active compounds were 3 and 5 with a MIC of <= 31.25 µg/ml. Further assays will be performed to determine detailed final MIC values for compounds 3 and 5 and other diterpenes of both classes will be investigated in the test system. Acknowledgement: FAPESP (Proc. 2008/01949 – 1), CAPES, CNPQ. Refer-
Comprehensive Analysis of Artemisia scoparia herba from different growing areas
Scherrer R,9*; Orland A,9 Demirci B,9 Knoes W,9
Bayer MK,10; Franc G,11; Hervé J
1University of Regensburg, Institute of Pharmacy,
Pharmazeutische Biologie, Universitätstraße 31, 93040
Regensburg, Germany; 2Federal Institute for Drugs
and Medical Devices (BfArM), Kurt-Georg-Kiesinger-Allee 3,
53175 Bonn, Germany; 3Anadolu University Faculty of
Pharmacy, Department of Pharmacognosy, 26470 Eskişehir,
Turkey; 4King Saud University, College of Science, Botany
and Microbiology Department, 11451 Riyadh, Saudi Arabia

Artemisia scoparia herba (Yinchens) is used in Traditional Chinese Medicine (TCM) for treatment of hepatic diseases like jaundice. Since TCM is getting popular in Europe, monographs are developed for the German and European pharmacopoeias [1,2]. Moreover the Bavarian State Research Center for Agriculture (BfArM) successfully cultivates Chinese medicinal plants to ensure authenticity of the plant material and quality of the preparations thereof. Plant material provided from suppliers of Chinese origin and the BfArM showed after morphological analysis a significant different habitus between German and Chinese plants. Furthermore, phytochemical analyses by TLC and GC also revealed a different spectrum of polyphenols and volatile compounds between the two geographical sources. To examine the variation of the essential oil in more detail the water-distilled essential oil from the aerial parts of Artemisia scoparia Waldst et KIT was analyzed with GC-FID and GC-MS for both provenances revealing capillone as one characteristic differentiating compound. PCR-analysis of the internal transcribed spacer (ITS) authenticated from the genetic point of view that despite all morphological and phytochemical differences both sources of Artemesia scoparia herba belong to the same source plant named Artemisia scoparia.

Acknowledgement: The BfArM (Kurt-Georg-Kiesinger-Allee 3, 53175 Bonn, Germany) is gratefully acknowledged for financial support. The Bavarian State Research Center for Agriculture (Am Gereuth 8, 85354 Freising, Germany) is gratefully acknowledged for providing samples and support.


PL04

Contribution of two agronomic characteristics to yield and oil content of safflower germplasm in eastern Algeria
Maleouadi R, Liyamine M, Aboulfoutouh OE, Mostapha MS, Mourad B
1Department of Biology, University 20 Aout 1955, Skikda, 21000 Algeria; 2Department of Cultivation and Production of Medicinal and Aromatic Plants, NRC, Dokki, Cairo, Egypt; 3Genetics, Biochemistry and Plant Biotechnologies Laboratory, University Mentouri, Constantine, 25000 Algeria

Safflower (Carthamus tinctorius L.) is one of humanity’s oldest crops, but generally it has been grown on small plots for the grower’s personal use and it remains a minor crop. Since safflower is a drought tolerant crop, the objective of this research was the investigation of the seed yield and oil content under semi-arid conditions in eastern Algeria. The results showed that plant height (PH) and plant dry matter weight (PDMW) showed 63.37 – 107.63 cm and 65.35 – 123.04 g of variation respectively. Safflower varieties gave the highest PDMW (132.04 g) and yield oil (YO) (420.53 g/m2). While Finch variety gave the highest PH (107.63 cm). Considering the yield of the fixed oil (YO) (% of seeds), Gila variety produced the highest percentage (38.47%). The research revealed that the most suitable safflower variety, under semi-arid conditions of eastern Algeria was Syria variety which was provided by ICARDA (International Center for Agricultural Research in Dry Areas, Syria). Analyses of variance showed highly significant differences among the varieties for yield agronomic traits and oil content. Correlation coefficients between variables (traits) are calculated, and the cluster analysis of observations (varieties) is also used to clarify the clustering pattern of genotypes tested.

PL05

Pharmacognostic study of two medicinal species of Rytigynia (Rubiaceae): Rytigynia nigerica (S. Moore) Robyns and Rytigynia umbellulata (Hiers) Robyns
Ajaoyi GO, Kadiri AR, Egbedi ME, Oyeyemi OO
Departments of Pharmacognosy and Botany, University of Lagos, Lagos, Nigeria

Micromorphological and phytochemical studies were carried out on the leaves of Rytigynia nigerica (S. Moore) Robyns and Rytigynia umbellulata (Hiers) Robyns. The epidermal cells of both the adaxial and the abaxial surfaces have irregular shape and the anticlinal cell wall patterns are either curved or undulate. Remarkable diagnostic features of the two plants which in a way justify their grouping in the same genus are paracytic stomatal type, hypodermal leaf and centrally located vascular bundles in the midrib and spatial deposition of crystals of calcium oxalate in the perivascular tissue. But the distinctive features of each species include higher epidermal cell number in R. nigerica than R. umbellulata. Thin cell wall of 1.0 (1.6 ± 0.2) 3.0 μm on the abaxial surface of R. nigerica. Higher stomatal size of 6.0 (12.4 ± 1.2) 20 μm in R. umbellulata and long and tip bent trichomes reported on the abaxial surface of R. nigerica and multicellular glandular type on the adaxial layer of R. umbellulata. Phytochemical screening showed that in both R. nigerica and R. umbellulata, bioactive compounds such as alkaloids, tannins, saponins, reducing sugar, glycosides, flavonoids and terpenes were present; whereas anthraquinones, cardiac glycosides, cyano-gegetic glycosides and phlobatannins were absent. However, only the extracts of R. nigerica were positive for steroids. These bioactive compounds found in the leaves of these plants play a major role in their medicinal potentials. The two species are well known plants used in folkloric medicine in Nigeria.

PL106

Bioassay-guided fractionation of a hepatoprotective and antioxidant extract of pea by-product
Seida AA1, El Tanbouly ND1, Islam WT1, Eid HH2, El Maraghy SA1, El Senousy AS2
1Department of Pharmacognosy, Faculty of Pharmacy, Cairo University, Cairo, Egypt; 2Department of Biochemistry, Faculty of Pharmacy, Cairo University, Cairo, Egypt

Fruits and vegetables waste products offer a cheap and practical source of potent antioxidants that could be used as functional ingredients. The hydroalcoholic extract (PE) of pea (Pisum sativum L) waste (husks) was evaluated for hepatoprotective and antioxidant activities, using CCl4-induced oxidative stress and hepatic damage in rats. PE significantly inhibited CCl4-induced elevation of serum ALT and AST by 45.3, 17.8% respectively and normalized the levels of serum total protein and albumin in hepatotoxic rats. It afforded 31.2% protection against hepatic lipid peroxidation, recovered hepatic glutathione and protein thiols levels by 161.3, 55.9%, respectively, restored the glutathione-peroxidase activity (by 42.7%) and significantly increased the glutathione-S-transferase activity (by 10%). PE also inhibited CCL4-induced elevation of serum ALT and AST by 45.3, 17.8%, respectively and normalized the levels of serum total protein and albumin in hepatotoxic rats. It afforded 31.2% protection against hepatic lipid peroxidation, recovered hepatic glutathione and protein thiols levels by 161.3, 55.9%, respectively, restored the glutathione-peroxidase activity (by 42.7%) and significantly increased the glutathione-S-transferase activity (by 10%). PE also inhibited CCL4-induced elevation of serum ALT and AST by 45.3, 17.8%, respectively and normalized the levels of serum total protein and albumin in hepatotoxic rats. It afforded 31.2% protection against hepatic lipid peroxidation, recovered hepatic glutathione and protein thiols levels by 161.3, 55.9%, respectively, restored the glutathione-peroxidase activity (by 42.7%) and significantly increased the glutathione-S-transferase activity (by 10%).

**PL107**

Biological Study and Phytochemical Screening of Several Aloe Species Cultivated in Egypt

El Fiki NM, Shemina IA, Ibrahim TA, Slem AA, Shoukry MA
1Pharmacognosy Department, Faculty of Pharmacy, Cairo University, 11562, Cairo, Egypt.; 2Pharmacognosy Department, Faculty of Pharmacy, King Abd El Aziz University, Jeddah, Saudi Arabia.; Pharmacognosy Department, Faculty of Pharmacy, Cairo University, 11562, Egypt.; 3Pharmacognosy Department, Faculty of Pharmacy, King Saud University, 11495, Riyadh, Saudi Arabia.; Pharmacognosy Department, Faculty of Pharmacy, Cairo University, 11562, Egypt.; 4Pharmacology Department, National Research Center, Dokki, Giza, Egypt.

Aloe, has long been used in traditional medicine for the treatment of digestive system diseases, skin troubles, wounds and burns. Recently it was proved to have antitumor activity. About 19 species were cultivated in Egypt whereas Aloe arborescens Mill., Aloe ciliaris Haw., Aloe eru Berger and Aloe grandidentata Salm-Dyck were found to be most abundant. They were subjected to biological and phytochemical investigations. The biological study includes toxicological (LD₅₀) and pharmacological investigation for ethanolic extracts. Acute anti-inflammatory activity was done using paw edema method in rats with standard indomethacine. Chronic anti-hyperglycemic activity was carried out using standard metformine. Antitumor activity was investigated using available human cell lines, U251 (brain), MCF7 (breast), H460 (lung), HELA (cervix), HCT116 (colon) and HEPG2 (liver). Antimicrobial activity was investigated on the bacteria tested against the bacteria. Phytochemical screening of the ethanolic extracts indicated that carbohydrates and/or glycosides, sterols and/or triterpines, combined and free anthraquinones are the main constituents present.

**PL108**

Biosynthesis of Bioactive Secondary Metabolites in Herbs

Chen W
Department of Pharmacy, Changzheng Hospital, Second Military Medical University, No. 415, Fengyang Road, Shanghai, 200003, P. R. China; Modern Research Center for Traditional Chinese Medicine, Second Military Medical University, No. 325, Guohe Road, Shanghai, 200433, P. R. China

Plant secondary metabolites are the major source of bioactive compounds of Herb. Metabolic engineering has opened a new promising perspective for the improved production of these valuable secondary metabolites in plant cell factory. Apparently, the key to metabolic engineering is the detailed knowledge of pathways of interest. We have developed RACE (rapid amplification of cDNA ends) method for the isolation of genes involved in certain biosynthesis pathway or crucial regulation process [1], which prompted the possibility of a key gene-based metabolic engineering for the synthesis of active compounds. In addition, we have successfully developed several plant cell culture systems such as hairy root, suspension cell as well as Saccharomyces cerevisiae cultures [2], which not only facilitated gene manipulation such as transformation and knockout, but also feasible for the industrial production of desired compounds in the near future. In our study, several metabolic engineering strategies have been successfully used to channel metabolites into pathways leading to desired products, including overexpression of rate-limiting enzyme genes, suppression or knockout of competitive enzyme genes, regulation of signal molecular pathway, and transformation of important transcription factors or transporters, etc [3]. Furthermore, for the unidentified secondary metabolites we are now using isotope tracing and 2-dimensional electrophoresis technology to explore them [4]. The identification and isolation of the enzymes involved will certainly help us to elucidate the whole biosynthesis pathway(s), and ultimately enable the possibility of metabolic engineering for the production of specific bioactive secondary metabolites in herbs. Acknowledgement: This research was financially supported by National Natural Science Foundation of China (20527230, 30800786) and Modernization of traditional Chinese medicine foundations (0802119502), Shanghai Science and Technology Committee. References: 1. Xiao Y et al. (2009) Mol Biol Rep 36: 2019 – 2029. 2. Huang BB et al. (2011) Metabolomics 7: 134 – 146. 3. Xiao Y et al. (2009) Physiol Plantarum 137: 1 – 9. 4. Xiao Y et al. (2010) Biosci Rep 30: 33 – 40.

**PL109**

Effect of Ailanthus altissima (Mill.) Swing and Ailanthus excelsa Roxb. stem bark extracts on Streptozotocin Induced Diabetes

Abd Elheem Said A, Nabih Rashed K, Ho Kim C
1Department of Pharmacognosy, National Research Centre, Dokki, Cairo, Egypt.; 2Cheorl Ho-Kim, Department of Biochemistry, College of Oriental Medicine, Dongguk University, Kyongu, Korea

The inhibitory effects of melatonin (70%) extracts of Ailanthus altissima (Mill.) Swing and Ailanthus excelsa Roxb. stem bark on streptozotocin (ST) – induced diabetes mellitus were studied using ST – treated diabetic model. When the effects of the extracts on ST-induced ATP/ADP ratio of islets were assayed, the extracts were effective in restoring of ATP/ADP ratio and when the islets (200/condition) were treated with ST (5 mM for 30 min.) and then the extracts were added to the ST-treated cells, the extracts concentration (200 μg/ml) showed increased insulin production in pancreatic islet cells. Keywords: Ailanthus excelsa, Ailanthus altissima, stem bark, Antidiabetic activity

**PM1**

Antibacterial potential of essential oil of medicinal plant Satureja bachtiarica Bunge against human pathogenic bacteria

Ahanjan M, Ghaffari J, Nasalohi M, Mirabi AM, Mohammadpour C
1Microbiology Department, Mazandaran University of Medical Sciences, Sari, Iran.; 2Biology Department, Islamic Azad University, Sari, Iran.

Keywords: satellite; Bunge, S. bachtiarica; essential oil, antioxidant activity of the oil and methanolic extracts of A. arborescens and A. grandidentata

**PM2**

Composition of volatile oil and antioxidant activity of the oil and methanolic extracts of Ferula microcolea Boiss

Amiri H, Dehsiri M, Zarei A, Mehria M, Servat Z
1Department of Biology, Lorestan University, Khoramabad, Iran.; 2Department of Biology, Islamic Azad University, Broujerd Branch, Broujerd, Iran.; 3Research Center of Agricultural and Natural Resources of Lorestan Province, Khoramabad, Iran

The essential oils of Ferula microcolea Boiss. collected from west of Iran during the flowering stage, were obtained by hydrodistillation and analyzed by gas chromatography (GC) and gas chromatography/mass spectrometry (GC-MS). Under the optimum distillation and analysis conditions, 22 constituents (mainly monoterpens) were identified in Ferula microcolea which represented 93.6% of the oil. The main constituents were α-pinene (27.3%), β-pinene (16.4%), nonanal (8.7%), β-caryophyllene (8.5%) and thymol (6.7%). The samples were also subjected to...
screening for their possible antioxidant activity by using 2,2-diphenyl-1-picrylhydrazyl (DPPH) and β-carotene-linoleic acid assays. In the first case, the free radical scavenging activity of polar sub-fraction of methanol extract was superior to all other extracts (IC$_{50}$= 34.3 ± 0.3 μg/ml), nonpolar sub-fraction of methanol extract exhibited stronger activity than the essential oil. In the case of the linoleic acid system, oxidation of the linoleic acid was effectively inhibited by the polar sub-fraction of methanol extract, while the oil and non polar sub-fraction of methanol extract were less effective. Keywords: Ferula micrococa, Antioxidant activity, Essential oil.

PM3

Targeted modification of trilobolide and search for related sesquiterpenes with immunobiological properties

Harmatha J$^{1}$, Budešinský M$^{1}$, Vokac K$^{1}$, Kmoníčková E$^{2}$, Zidek Z$^{2}$

$^{1}$Institute of Organic Chemistry and Biochemistry, Academy of Sciences, 166 10 – Prague, Czech Republic; $^{2}$Institute of Experimental Medicine, Academy of Sciences, 142 20 – Prague, Czech Republic

Trilobolide and its analogues belong to guaianolide type of sesquiterpene lactones, widely distributed within families Asteraceae and Apiaceae [1]. Trilobolide (1), structurally related to thapsigargin (4), is quite specific in its structure and biological activities [2]. Certain guaianolides evoked attention for their promising anti-inflammatory, anticancer, anti-infectious and SERCA inhibitory activities. However, due to their allodynic capabilities, they are generally toxic. Search for compounds with significant immunobiological properties, but with minor cytotoxicity is a challenge for immunopharmacological research also in our case [3]. (Fig.1) We extended investigation of the immune interventions of trilobolide [2, 3] also on related guaianolides (2 - 7) isolated either from Laser trilobum (L.), Borkh., or from related Laserpitium sier (L.). For the structure-activity relationship study, we included also a series of structurally related exomethylene lactones (e.g. well recognised helenalin) [1]. For better relationship evaluations, additional series of transformed deacetyl derivatives were prepared, either by alkaline hydrolysis or by PM3

**Keywords:** sesquiterpenes, guaianolides, trilobolide, structure-activity relationship, immunomodulation, Laser trilobum

![Figure 1](image)

Acknowledgement: Supported by GACR grant No 305/07/0061

PM4

Evaluation of inhibitory effects of some Iranian plants against Plasmodium falciparum

Bahraminejad S$^{1}$, Saed A$^{1}$, Sayyed Mohammad M$^{1}$, Saed T$^{2}$

$^{1}$Agronomy and Plant Breeding, Campus of Agriculture and Natural Resources, Razi University, Kermanshah, Iran; $^{2}$Plant Protection, Campus of Agriculture and Natural Resources, Razi University, Kermanshah, Iran

Crude aqueous and methanolic extracts of 121 plant species belonging to 41 families collected from the west of Iran were screened for anti-fungal activity against mycelial growth of Phytophthora drechsleri. Bioassay was performed based on paper disc diffusion method with four replicates. Thirty eight of 121 (about 31%) plant species showed inhibitory activity against this phytopathogenic fungus, among which 23 species measurably inhibited the growth of Phytophthora drechsleri. Results indicated that methanolic extract of Xanthium strumarium L. showed the maximum activity (17.79 ± 1.35 mm) against P. drechsleri followed by Glycyrrhiza glabra L., Hypericum perforatum L., Centaurea depressa M.Bieb., Lamium amplexicaule L., Haplophyllum perforatum (M.B.) Vved. The investigation on the effect of plant parts on mycelial inhibition of tested fungus using paper disc method indicated that inflorescence and fruits of cocklebur (Xanthium strumarium) has significantly more inhibitory effect against P. drechsleri. The study on the antifungal activity of two common species of cocklebur grown around city of Kermanshah, X. strumarium and X. spinosum L., showed that both of them have inhibition on mycelial growth of tested fungus, but the X. strumarium showed significantly more inhibitory effect against P. drechsleri than X. spinosus.


PM5

Structure-Activity Relationship of 9,10-Anthraquinones Analogues from Renellia elliptica and Their Antiplasmodial Activity

Osman C$^{1}$, Ahmad R$^{1}$, Widya Waryanti A$^{1}$, Ismail N$^{1}$

$^{1}$Faculty of Applied Sciences, Universiti Teknologi MARA, 40450 Shah Alam, Selangor, Malaysia; $^{2}$Department of Pharmacognosy and Phytochemistry, Faculty of Pharmacy, Airlangga University, Jalan Dharmawangsa Dalam, Surabaya 60286, Indonesia

Anthraquinones isolated from the roots of Renellia elliptica Korth. demonstrated interesting antiplasmodial activity. The activity however, varies depending on substitution pattern of the anthraquinone skeleton which warrants further investigation. This paper reports preliminary structure-activity relationship of a series of 9,10-anthraquinones and their antiplasmodial activity. The natural anthraquinones were isolated from roots extract of R. elliptica. The analogues of bioactive anthraquinones were synthesized through Friedel-Craft reaction between phthalic anhydride and various benzene derivatives in the presence of eutectic mixture of aluminium chloride and sodium chloride. The antiplasmodial activity was inhibited in the control Plasmodium falciparum (3D7) growth in vitro. Combination of methyl and hydroxyl substituents at different positions on the anthraquinone skeleton caused strong antiplasmodial activity. The ortho-arranged substituents at C1, C3 positions exhibited strongest activity with IC$_{50}$ value of 0.08 μg/ml followed by the compound with substituents at 1,2 positions. The para-arranged (1,4) and meta-arranged (1,3) substituted anthraquinones showed less potent activity. On the other hand, analogues of dihydroxyanthraquinones displayed a reverse order of activity with the strongest inhibition shown by 1,3-dihydroxyanthraquinone. The hydroxy-methyl anthraquinones and dihydroxyanthraquinones were substituted with additional methyl group at C-6-C-7 on ring A showed similar pattern of activity but much weaker than those substituted only on ring C. Protection of hydroxyl group via methylation reaction caused significant variation in antiplasmodial activity. Anthraquinones substituted at C-2 and C-3 and anthraquinones substituted at C-1, C-2 and C-6-C-7 promotes antiplasmodial activity. Structural differences due to different substitution pattern affects antiplasmodial activity of 9,10-anthraquinones. Keywords: Anthraquinones, Antiplasmodial, Structure-Activity Relationship, Renellia elliptica

PM6

Assessment of Anti-angiogenic and Anti-tumoral Potential of Origanum onites L. Essential Oil

Bostançoloğlu R$^{1}$, Kırkıçoğlu M$^{1}$, Başer KHC$^{2}$, Kopral AT$^{1}$

$^{1}$Anadolu University, Faculty of Sciences, Department of Biology, 26470-Eskişehir, Turkey; $^{2}$Anadolu University, Faculty of Pharmacy, Department of Pharmacognosy, 26470-Eskişehir, Turkey; $^{3}$Botany and Microbiology Dept., College of Science-King Saud University, P.O. BOX 2455, Riyadh 11451, Saudi Arabia

Medicinal plants and culinary herbs with anti-angiogenic and little toxicity properties have gained importance in the last decade. Non-toxic anti-angiogenic phytochemicals are useful in combating cancer by preventing the formation of new blood vessels to support the tumor growth. We have investigated the essential oil of Origanum onites L., which is commonly used as a condiment, with reported antibacterial, antifungal, antioxidant, insecticidal and anti-carcinogenic activities for a possible anti-angiogenic activity. Essential oil of Origanum onites L. was
analysed by gas chromatography (GC) and gas chromatography-mass spectrometry (GC/MS). The antiproliferative activities (by MTT assay, 3-(4,5-dimethyl-2-thiazol)-2,5-diphenyl-2-H-tetrazolium bromide), the anti-angiogenic activities (by matrigel tube formation assay), cell migration inhibiting capability (migration assay) and apoptotic potential (DAPI staining) of the Origanum onites essential oil (OOEO) were evaluated on rat adipose tissue endothelial cells (RATECs) and SRP7 (c-H-ras transformed rat embryonic fibroblasts). Our experimental results revealed that OOEO could markedly inhibit cell viability and induced apoptosis of SRP7 cells and also could block in vitro tube formation and migration of RATEC. These results imply that OOEO having anti-angiogenic activity might be useful in preventing angiogenesis-related diseases and combating cancer. Keywords: Essential oil, Origanum onites L, antiangiogenesis, cytotoxicity, apoptosis, cancer

Neuropharmacological Activity of Pimenta Pseudocaryophyllus (Gomes) L. R. Landrum Faygrove RJ, Luis MF, Fereira BA1, Abadia PJ1, Redimo PPF, Al CE2
1Instituto de Ciências Biológicas, Universidade Federal de Goiás, (131) Brazil; 2Faculdade de Farmácia, Universidade Federal de Goiás, (131) Brazil; 3Universidad estatal de Goiás, Andropolis, (459) Brazil

Pimenta pseudocaryophyllus (Gomes) L. R. Landrum (Pp) is popularly used as a tranquilizer in the treatment of emotional tension in the city of Campos do Jordão, São Paulo, Brazil. The aim of this study was to evaluate behavioral changes induced by ethanol extract of the Pp leaves (PePp), seeking to identify the most active fraction. PePp was obtained by soaking the dried leaf powder in ethanol (95%;1:5). The hexane (HF), dichloromethane (DF), ethyl acetate (ACF) and aqueous (WF) fractions were prepared through PePp Fractionation with solvents of different polarities. Swiss male mice (25–35 g) were treated orally with PePp at 1 g/kg; HF160 mg/kg; DF260 mg/kg; ACF420 mg/kg, or WF 640 mg/kg in proportion to their respective yield. After 1hr of treatment, anxiolytic effect was evaluated in sleep induced by sodium pentobarbital (50 mg/kg; HF160 mg/kg; DF260 mg/kg; ACF420 mg/kg, or WF 640 mg/kg). The PpEE prolonged sleep time by 62%; increased the number of squares crossed and time spent on the open arm of EPM were also increased by 32% and reduced sleep latency by 20%. DF prolonged sleep duration by 32% and 42% respectively. These results suggest the presence of compounds with anxiolytic activity in the DF. Keywords: Pimenta pseudocaryophyllus, Anxiolytic effect, Medicinal plant, Elevated plus maze

Reduction toxicity by doxorubicin entrapped in liposomes nanocapsules Mezbah L1, Mohamed A1, Gillian B2
1Tahbol Medlab Laboratory of Molecular Toxicology, University of jijel, 18000, Jijel, Algeria; 2Alyane Mohamed Laboratory of Molecular Toxicology, University of jijel, 18000, Jijel, Algeria; 3Barrett Gillan Umr Curs 8612. Centre d’études Pharmaceutiques. 92296 Chatenay Malabry, France

Doxorubicin induced an irreversible congestive heart failure, renal and hematological toxicity that are often fatal. The molecular mechanisms involved are only partially known and are complex and different from the anticancer mechanism involving oxidative stress. Encapsulation of doxorubicin in liposomes was elaborated in order to prevent the toxicity observed with the free form. The study was conducted in vivo by treatment of Wistar rats with doxorubicin encapsulated in liposomes or naked at different doses (10, 20 and 30 mg/kg) and in vitro on H9c2 cells. In order to evaluate the toxicity, different mitochondrial parameters (CR, Swelling, bioenergy...) were evaluated. The study is complemented by MTT and LDH tests. In vivo doxorubicin causes oxidative stress more pronounced than liposomal doxorubicin. A activity inhibition cytochrome c oxidation, depletion of tissue glutathione concomitant with increased production of ROS, swelling of mitochondria are observed. The mitochondrial dysfunction at origin of the cardiotoxicity is confirmed by the MTT assay and LDH test. We observed also renal dysfunction and aplasia in blood, spleen and bone marrow more serious with naked doxorubicin than with the encapsulated one. In conclusion, doxorubicin would be responsible for cytotoxicity by damaging the mitochondria. Theses disorders may be prevented by its encapsulation in liposomes. Keywords: Cardiotoxicity, Nephrotoxicity, Haematoxicity, Doxorubicin, Liposomes, Oxidative stress References: 1. Plassat V et al. (2007) Int J Pharm 118 – 127. 2. Leite A et al. (2007) Life Sciences 80: 1327 – 34. 3. Lahouel M et al. (1987) Drugs Exp Clin Res 10: 593 – 599.

Total phenolic content, flavonoids and Superoxide radical scavenging activity of some Citrus peels Golfakhrabadi F1, Shabpoosh A2, Javdani F3, Hassanzadeh A4
1Department of Pharmacognosy and Medicinal Plants Research Center, Faculty of Pharmacy, Tehran University of Medical Sciences, P.O. Box: 14155 – 64117, Tehran, Iran; 2Department of Pharmacognosy and Medicinal Plants Research Center, Faculty of Pharmacy, Ahwaz University of Medical Sciences, P.O. Box: 61357 – 15794, Ahwaz, Iran.; 3Department of Medical Research Center, Faculty of medicine, Ahwaz University of Medical Sciences, P.O. Box: 61357 – 15794, Ahwaz, Iran.

The Genus Citrus is a shrub from Rutaceae family. The main constituents of Citrus are phenolic compounds, acids, volatile oil, pectin, carote- noids, flavonoids and vitamin C. Polyphenols of citrus have antioxidative effect to prevent lipoperoxidation, increase serum antioxidant capacity and decrease oxidative stress in geriatrics. In this study three different extracts (methanolic extraction, chloroform and flavonoids fraction) from peel of 8 species (orange, mandarin, sour orange, citron, grape fruit, lemon, sweet lime, lime) of citrus were prepared. The total phenolic content, inhibition of superoxide radical capacity and antioxidant activity of citrus were evaluated using the Folin-Ciucalteu method, NBT, DPPH and FRAP assays. In NBT assay Citron flavonoids fraction (IC50= 0.035 mg/ml) has the the highest capacity of inhibition and were comparable with Vitamin C (IC50= 0.058 ug/ml), in DPPH assay lime metha- nolic extraction (IC50= 0.94 mg/ml) and in FRAP assay Lime methanolic extraction have the highest capacity of inhibition. In DPPH assay Vit C has (IC50= 0.072 ug/ml). The maximum amount of phenolic compound was observed in grapefruit flavonoids fraction (142.47 mg acid tannic)
1 g extraction). The maximum amount of flavonoids compounds was observed in Lime methanolic extraction (12.91 mg Rutin/1 g extraction).

Methanolic extract in DPPH and NBT method have the same results for flavonoids. The initial antibacterial activity was exerted against P. aeruginosa by ethyl acetate extract (MIC 0.625 μg/ml), followed by its bacteriostatic activity against E. faecalis (MIC 0.625 μg/ml). Conversely, the methanol extract showed bacteriostatic activity against P. aeruginosa (MIC 1.25 μg/ml) and bactericide activity against E. faecalis (MIC 1.25 μg/ml). Keywords: Citrus, NBT, DPPH, Folin-Ciucalteu, FRAP, Antioxidant, Flavonoids

Acknowledgement: This research has been supported by Abwaz University of Medical Sciences.

**PM11**

**Antibacterial Activity of Different Extracts of Clidemia hirta (L.) D. Don leaves**

Dania R., Ramasyamy K., Ab Rahman N. 1Faculty of Pharmacy, University Teknologi MARA Pulau Pinang, Seberang Perai, 13500 Pulau Pinang, Malaysia; 2Faculty of Pharmacy, Universiti Teknologi MARA, Pancak Alam Campus, 42300 Selangor, Malaysia

Clidemia hirta (L.) D. Don (Malastomataceae), locally known as “senduduk bulu” by a local tribe in Malaysia, has been used traditionally to stop bleeding [1] and in the treatment of venom fever [2]. The use of this species as traditional medicine for several bacterial infections has also been recorded in several references [3, 4]. Thus, this study was conducted to investigate the antibacterial properties of this species and determine its MIC towards several bacteria, Escherichia coli, Enterococcus faecalis, Pseudomonas aeruginosa, and Staphylococcus aureus. Several extracts of the plant were collected: a cold extract (cold extraction) the leaves with different polarity of solvents such as hexane, ethyl acetate and methanol, respectively. The initial antibacterial property identification was done by using disk diffusion method. The active extract was further investigated for its mechanism and MICs value by dilution technique. Only the hexane extract was not showing any antibacterial property against selected bacteria. Meanwhile, none of these extracts showed activity against E. coli. Interestingly, bactericidal activity was exerted against P. aeruginosa by ethyl acetate extract (MIC 0.625 μg/ml), followed by its bacteriostatic activity against E. faecalis (MIC 0.625 μg/ml). Conversely, the methanol extract showed bacteriostatic activity against P. aeruginosa (MIC 1.25 μg/ml) and bactericide activity against E. faecalis (MIC 1.25 μg/ml). Keywords: Clidemia hirta, Melastomataceae, antibacterial References: 1. Musa N (2007) The Forgotten Jungle Medicine of Taman Negara Pahang. Malaysian Pharmaceutical Association. Penang. 2. Kamarudin MS, Latiff A (2002) Tumbuhan Ubatan Malaysia. of Taman Negara Pahang. Malaysian Pharmaceutical Association. Penang, Malaysia; 3. Franca F Lago EL, Marsden NG, 2. Kamarudin MS, Latiff A (2002) Tumbuhan Ubatan Malaysia. of Taman Negara Pahang. Malaysian Pharmaceutical Association. Penang, Malaysia; 4. Franca F Lago EL, Marsden NG, 2. Kamarudin MS, Latiff A (2002) Tumbuhan Ubatan Malaysia. of Taman Negara Pahang. Malaysian Pharmaceutical Association. Penang, Malaysia.

**PM12**

**Preventive Role of Cactus (Opuntia ficus-indica) Cladodes on methotrexate-induced Biochemical, Hematological and oxidative damage in rat liver**

Lazar Z, Akacha A, Mohamed A 1Research Unit BMG, Faculty of Sciences, Gafsa University, Gafsa, Tunisia

Methotrexate is widely used in the therapy of various types of malignancy as well as in the treatment of various inflammatory diseases. Its use can cause severe side effects including bone marrow suppression and severe hepatotoxicity. The aim of this work was to investigate the antihyperglycemic potential of the aqueous extract from Cactus cladodes with the MTX induced a reestablishment of hematological and oxidative parameters, and levels of serum biochemical enzyme activities. While the mixture of the extract at 20 mg/kg MTX with Cactus cladodes (PI14 extract) for the 10th day rats were sacrificed by decapitation, blood samples were collected for hematological and serum biochemical parameters measurement. Our results clearly showed that MTX treatment significantly decreased hematocrit, hemoglobin, white blood cells, and increased the most of biochemical serum parameters. While the mixture of the extract of cactus cladodes with the MTX induced a reestablishment of hematological, parameters, and levels of serum biochemical enzyme activities. In conclusion, it appears that cactus cladodes extract protects against methotrexate-induced oxidant organ injury and it may become a promising treatment in the prevention of undesired side effect of MTX. Keywords: Opuntia ficus indica, Methotrexate, Biochemical and Hematological Parameters, Prevention, Cladodes of Cactus

**PM13**

**Antihyperglycemic effect of Derris reticulata Crab extract in alloxan-induced diabetic rats**

Kumkrai P, Ramanonwanisit S, Chadapongse N 1School of Biology, Institute of Science, Suranaree University of Technology, Nakhon Ratchasima 30000, Thailand

The global prevalence of diabetes mellitus (DM), a metabolic disorder characterized by chronic hyperglycemia, has been estimated to be increasing worldwide [1]. It is known that the present synthetic drugs available for treatment of DM can cause several undesirable side effects. As recommended by WHO, the quest for effective and safer antidiabetic plant drugs is an important topic [2]. In Thailand, Derris reticulata Crab (DC) which belongs to Leguminosae family has been traditionally used for diabetic treatment. However, limited scientific data are available. The aim of this work was to investigate the antihyperglycemic potential of the aqueous extract from Derris cladodes in alloxan-induced diabetic rats. The result showed that administration of DC extract at the daily dose of 250 mg/kg for 15 consecutive days significantly decreased fasting blood glucose levels compared with diabetic control group. More gross pathological lesions were found on the pancreas of diabetic control rats than that of DC-treated rats. An association between antioxidant property and antihyperglycemic activity of plant extract has been reported [3]. Total phenolic content and the IC50 of antioxidant potential against DPPH radicals of the Derris extract showed antioxidant effect of the extract was increased with a mixture of Derris and MTX (20 mg/kg). In conclusion, the DC extract exerts antidiabetogenic effect which may be associated with its antioxidant-mediated pancreatic protection. Keywords: diabetes mellitus, Derris reticulata, antioxidant. References: 1. Wild S et al. (2004) Diabetes Care 27:1047 – 1053. 2. Gupta S et al. (2009) Journal of Ethnopharmacol 123: 499 – 503. 3. Alarcon-Aguilar FJ et al. (2010) Journal of Ethnopharmacol 132: 400 – 407.

**PM14**

**Echinocystic acid inhibits acute-lung injury by inhibiting TLR4/LPS complex formation**

Joh E, Lee I, Kim D 1Department of Life and Nanopharmaceutical Sciences, Kyung Hee University, Seoul 130 – 701, Korea

Orally administered lancemaside A isolated from Codonopsis lanceolata Traur. (Campanulaceae) showed anti-inflammatory effects in vivo and produced 3 metabolites by the incubation with human intestinal microflora in vitro [1, 2]. Among lancemaside A and its 3 metabolites, echinocystic acid most potently suppressed the production of the pro-inflammation cytokines, TNF-α and IL-1β, as well as the activation of their transcription factor NF-κB in LPS-stimulated alveolar macrophages. Echinocystic acid also down-regulated the production of inflammatory markers, including inducible nitric oxide synthase and cyclooxygenase-2, as well as the inflammatory mediators, nitric oxide and prostaglandin E2 in LPS-stimulated macrophages. Echinocystic acid also inhibited the activation of IL-1 receptor-associated kinase, the phosphorylation of IKK-β and IκB-α, the nuclear translocation of NF-κB. Furthermore, echinocystic acid potently inhibited the interaction between LPS and TLR4. Echinocystic acid suppressed LPS-induced acute-lung injury in mice, as well as the expression of pro-inflammatory cytokines such as IL-1β and TNF-α, and the activation of their transcription factor, NF-κB. When lancemaside A was orally administered for mice, its metabolite echinocystic acid alone was detected in the blood. Based on these findings, echinocystic acid may express anti-inflammatory effects by inhibiting the binding of LPS to TLR4 on alveola macrophages in vitro and in vivo. Acknowledgement: This study was supported by a grant from World Class University Program through the National Research Foundation of Korea funded by the Ministry of Education, Science and Technology (R33 – 2008 – 000 – 10018 – 0). References: 1. Joh EH et al. (2010) Int J Cytotoxic Dis 25: 545 – 551. 2. Joh EH et al. (2010) Chromatogr B Analyst Technol Biomed Life Sci 878: 1875 – 1880.
Antioxidant and antibacterial activities of the extract of *Aquilaria crassna* leaves

Kamonwannasit S, Kumkrai P, Nantapong N, Kappayamani S, Chudapon N
School of Biology, Institute of Science, Suranaree University of Technology, Nakhon Ratchasima 30000, Thailand

*Aquilaria crassna* Pierre ex Leecomte (Thymelaeaceae) or Krisana agarwood has long been used for the production of high valued incense, cosmetic and pharmaceutical products in Asia [1]. In Thailand, leaves of young *Aquilaria crassna* are used to produce commercial herbal teas. In addition to its aroma, it is believed that Krisana leaves possess many interesting medicinal properties, such as antiinflammatory, antidiabetic and antibacterial activities. However, scientific study on its pharmacological activity is very limited. The aims of this study were to investigate the safety and antibacterial activity of the aqueous extract of *Aquilaria crassna* leaves. Acute toxicity test showed that even at high dose (15,000 mg/kg) the extract did not cause death or overt signs of toxicity when observed for 14 consecutive days in mice. It was found that the extract exhibited antibacterial activities against *Staphylococcus aureus* and *Streptococcus epidermidis* with MIC of 12.0 and 4.0 mg/ml, respectively. Since the correlation between antioxidant and antibacterial activities of plants has been reported [2], the total phenolic compound (TPC) and antioxidant property were also examined. The TPC of the extract was 162.4 ± 0.3 mg gallic acid equivalent/g. The extract showed strong antioxidant activity against DPPH radical with IC50 of 6.04 ± 0.18 mg/ml. It is concluded that the aqueous extract of *Aquilaria crassna* leaves may be beneficial for treatment of diarrhea caused by *Staphylococcus aureus* and skin infection associated with *Streptococcus epidermidis*. Keywords: *Aquilaria crassna*, Acute toxicity, Antibacterial, Antioxidant Acknowledgement: This study is funded by the Office of the Higher Education Commission, Thailand. References: 1. Eurlings MCM et al. (2010) Forensic Sci Int 197: 30 – 34. 2. Bajpai VK et al. (2009) Food Chem Toxicol 47: 1876 – 1883.

Potential antibiotic and anti-infective effects of rhodomyrtone from *Rhodomyrtus tomentosa* (Alston) Hassk. on *Streptococcus pyogenes* as revealed by proteomics

Kayser O, Limsuwan S, Hesseling Meinders A5, Voravutkhunichai SP, Van Djil JM4
1Techical University Dortmund, Technical Biochemistry, 44227 Dortmund, Germany; 2Faculty of Traditional Thai medicine and Natural Products Research Center, Faculty of Science Prince of Songkla University, Hat Yai, Songkhla 90112, Thailand; 3Department of Molecular Genetics, Groningen Biomolecular Sciences and Biotechnology Institute, University of Groningen, Nijenborgh 2, 9747 AG Groningen, the Netherlands; 4Department of Microbiology and Natural Products Research Center, Faculty of Science Prince of Songkla University, Hat Yai, Songkhla 90112, Thailand; 5Department of Medical Microbiology, University Medical Center Groningen (UMCG) and University of Groningen, Hanzeplein 1, 9700 RB Groningen, the Netherlands

Rhodomyrtone from *Rhodomyrtus tomentosa* (Alston) Hassk. leaf extract has a strong antibacterial activity against the bacterial pathogen *Streptococcus pyogenes*. Our previous studies indicated that the bactericidal activity of rhodomyrtone might involve intracellular targets. In the present studies we followed a proteomics approach to investigate the mode of action of rhodomyrtone on *S. pyogenes*. For this purpose, *S. pyogenes* was cultivated in the presence of 0.35 µg/ml rhodomyrtone, which corresponds to 50% of the minimal inhibitory concentration. The results show that the amounts of various enzymes associated with important metabolic pathways were strongly affected, which is consistent with the growth-inhibiting effect of rhodomyrtone. Additionally, cells of *S. pyogenes* grown in the presence of rhodomyrtone produced reduced amounts of known virulence factors, such as the glycerolaldehyde-3-phosphate dehydrogenase, the cAMP factor, and the streptococcal pyrogenic exotoxin C. Taken together, these findings indicate that rhodomyrtone has both antimicrobial and anti-infective activities, which make it an interesting candidate drug. Keywords: glycolysis, proteomics, rhodomyrtone, Rhodomyrtus tomentosa, Streptococcus pyogenes, two-dimen-sional gel electrophoresis Acknowledgement: We thank Jan Arends, and members of the Department of Medical Microbiology and Department of Molecular Genetics for strains and technical support. We thank Assoc. Prof. Dr. Wilawan Mahabursarakam and Mr. Asadhawat Hirarannt for rhodomyr-tone isolation. The work was funded by the Thailand Research Fund through the Royal Golden Jubilee, Ph.D. Program (PHD/0029/2548). Funding was furthermore provided by the National Research University Project of Thailand’s Office of the Higher Education Commission, the Van Leer am-bonds, The Netherlands (VLEF/DA/3689), and the CEU projects LSHM-CT-2006 – 019064 and LSHC-CT-2006 – 037469. References: [1] Limsuwan S, Hesseling-Meinders A, Voravutkhunichai SP, van Djil JM, Kayser O (2011) Phytomedicine, in press [2] Limsuwan S et al. (2009) Phytomedicine 16: 645

In vivo evaluation of an herbal remedy for antimarial activity

Olufunwanya AA, Adelolu EA, Adejimi AS4, Morohunfolu AJ4, Olugbenga IA2
1Department of Pharmacognosy, Obafemi Awolowo University, Ille-Ife, Nigeria; 2Drug Research and Production Unit, Obafemi Awolowo University, Ille-Ife, Nigeria; 3Department of Pharmacology, Obafemi Awolowo University, Ille-Ife, Nigeria

The antimarial activity of an herbal remedy (HR) formulated, based on ethnomedical claims followed by observational experiences, was investigated in *Plasmodium berghei* NK 65- infected mice. The decoction of the HR was prepared, concentrated in vacuo and freeze-dried. Evaluation of the antimarial activity involved the use of early malarial (4 -day test) and established infection models [1, 2]. The HR was tested at 15 – 240 mg/kg, the standard drug, amodiaquine (AQ) was tested at 1.25 – 10 mg/kg. For the established infection test, the HR was tested at 60 – 240 mg/kg with AQ (10 mg/kg) as positive control. Distilled water was used as negative control in both test. The HR and AQ gave ED50 of 40 and 3.8 mg/kg respectively, while for the established infection test, the highest dose of 240 mg/kg gave 54.45% clearance on day 5. The HR showed higher suppressive than curative activity. Keywords: Herbal remedy, *Plasmodium berghei*, Amodiaquine Acknowledgement: Prof. G. A. Ademowo, Department of Pharmacology and Therapeutics, University of Ibadan, Ibadan, Nigeria for access to the Plasmodium berghei NK 65 parasitic References: 1. Peters W (1965) Exp. Parasitol 14: 80 – 87 2. Buley J, Peters W (1971) Am J Trop Med Parasitol 84: 209 – 211

Procyanidins of *Nelia meyeri* SCHWANT. elicit endothelium-dependent relaxation in porcine coronary arteries by activation of the PI3/Akt signalling pathway

Kaufeld JM, Peretz HH, Kolodziej H
FU Berlin, Institute of Pharmacy, Koenig-Luise-St. 2+4, Berlin, Germany

*Nelia meyeri* Schwant. (Mesembryanthemaceae) is a South African succulent known to contain procyanidins [1]. The aim of this study was to examine the molecular mechanism by which the extract from leaves of this plant elicits blood vessel relaxation. For this, a highly purified fraction comprised of bi- to tetrameric flavan-3-ols was applied to porcine coronary arterial rings suspended in organ chambers containing Krebs-Henseleit solution maintained at 37 °C. In endothelium-intact rings pre-contracted with the thromboxane A2 mimetic U46619, the sample produced a concentration-dependent relaxation that was abolished by mechanical removal of the endothelium. Concentration-response curves to the defined procyanidin fraction were shifted to the right in the presence of L-NAME (NG-nitro-L-arginine methyl ester), an inhibitor of eNOS. The observed relaxation was also abolished by wortmannin, an inhibitor of PI3K (phosphoinositide 3-kinase). However, the relaxant response to the *Nelia* extracts remained unaffected in the presence of ICI 182,780, an estrogen receptor antagonist, and pertussis toxin, an inhibitor of Gi proteins. These observations confirm the essential role of EDRF in the relaxant response to *Nelia* procyanidins. In addition, relaxation to the *Nelia* fraction was abolished by MnTMPyP®, a cell permeable mimetic of superoxide dismutase but not by tiron, a superoxide anion scavenger. The relaxation was insensitive to charybdotoxin plus apamin (Ca2+-activated K+ channel blockers) but was abolished by the combination of charybdotoxin plus apamin plus L-NAME. Taking together, these findings suggest that the endothelium-dependent relaxation induced by *Nelia* procyanidins is mediated by EDHF and EDRF following activation of PI3/Akt. Keywords: *Nelia meyeri*, procyanidins, endothelium relaxation, EDHF, EDRF References: 1. Kolodziej H (1984) Phytochemistry 23: 1745 – 1752
**PM19**

*In vitro* antileishmanial activity of resveratrol appears associated with cell cytotoxicity rather than antiparasitic properties

Lucas IK*, Leube U*, Kolodziej H*t

*Freie Universität Berlin, Institute of Pharmacy, Koenigin-Luise-Str. 2+4; tRobert Koch-Institut, Mycology/Parasitology FG 16*

Recently, we reported the antileishmanial activity of resveratrol against *Leishmania major* GFP in infected BMMs. In parallel we observed host cell cytotoxicity in a concentration-dependent manner, contrasting with claimed cell tolerability [1]. This apparent discrepancy prompted the present study using the reported resveratrol-tolerable J774-G8 cell line. When *L. major* GFP-infected J774-G8 cells were exposed to resveratrol (5–75 μg/mL), the resulting GFP signal was similarly reduced from 95% to 5% as a reflection of antileishmanial activity. However, host cell cytotoxicity invariably increased with sample concentrations as assessed by FACS analysis following staining with propidium iodide and apparent changes in cell morphology (Diff-Quick Staining). The MTT-assay provided an IC50 of 96 μM (22 μg/mL) and 83 μM (20 μg/mL) of resveratrol for non-infected J774-G8 and BMMs, respectively. This finding provides evidence for similar cytotoxicity of the test compound in both cell lines. Its antileishmanial activity appears to be associated with cytotoxic effects on host cells at concentrations of 25–40 μg/mL rather than selective antiparasitic properties. Having in mind that J774-G8 is a murine macrophage-like cancer cell line, the observed pronounced cytotoxic effects are in line with reports on anticancer/chemopreventive properties of resveratrol [2]. Current studies include staining techniques to discriminate between apoptotic and necrotic effects. Preliminary results suggested the induction of apoptosis, consistent with reports on apoptosis-associated proteins [3]. Keywords: resveratrol, antileishmanial, cytotoxicity, BMMs, J774-G8 cells References: 1. Kedzierski L et al. (2007) Parasitol Res 102: 91 – 97 2. Bhat et al. (2001) Antioxidants & Redox Signaling 3: 1041 – 1064 3. Li, G. et al. (2011) Phytomedicine 18: 1094 – 1064 4. G. et al. (2011) Phytomedicine doi:10.1016/j.phymed.2010.11.015

**PM20**

Targeting enteric digestive enzymes by natural products: synergistic effect of flavonoids

Habtemariam S

Pharmacognosy Research Laboratories, School of Science, University of Greenwich, Chatham-Maritime, Kent ME4 4TB, UK

Diabetes is a common metabolic disorder that is caused by either inherited and/or acquired deficiency in insulin secretion or due to decreased responsiveness to insulin. One of the common approaches in the treatment of diabetes is decreasing postprandial hyperglycemia by inhibiting key enzymes for the hydrolysis of carbohydrates in the small intestine. In our laboratories, the effects of various natural products on key digestive enzymes, α-glucosidase and α-amylase, are routinely assessed [1]. It was found that some flavonoids including kaempferol-3-O-rutinoside (KR, Fig. 1) are potent inhibitors of these enzymes. A synergistic enzyme inhibitory effect: e.g. between KR and flavonoid aglycones, was observed for some flavonoids. The structure activity relationship established from the study and potential therapeutic implications are discussed.

**Figure 1:** KR Structure Structure of exemplary flavonoid glycoside with potent α-glucosidase inhibitory activity.

**PM21**

The effect of Boswellia serrata on *Giardia duodenalis*

Hehn I*, Abitscher I*, Kolodziej H*

*Freie Universität Berlin, Institute of Pharmacy, Koenigin-Luise-Str. 2+4, 14195 Berlin, Germany; tRobert Koch-Institut, Mycology/Parasitology FG 16, Nordufer 20, 13353 Berlin, Germany*

*Giardia* duodenalis is a parasite that colonizes the small intestine of various mammalian hosts especially in humans. The common treatment for giardiasis includes metronidazole, furazolidone and benzimidazole-based drugs which cause many side effects besides an increasing resistance problem [1,2]. Having in mind that *Boswellia serrata* Roxb. is used for the treatment of chronic inflammatory disorders and that this parasite is known to facilitate these conditions [3], the gum resin of this plant source was tested for antigiardial effects. A crude extract standardized to 85% boswellic acids reduced the viability of the parasite by ca. 65% at a concentration of 20 μg/mL. At the highest concentration tested (80 μg/mL), the antigiardial effect was ca. 80% based on the metabolic conversion of resazurin [4]. Metronidazole (50 μg/mL) served as a positive control. In search for the active principle, the extract was subjected to HPLC separation showing two major peaks at Rt 12.3 and 14.7 min, respectively. The former, comprising a complex mixture of boswellic acids, exhibited pronounced antigiardial activity at 20 μg/mL, as evident from a ca. 80% reduction in parasite viability. HPLC analysis showed also the presence of oleanolic acid. Preliminary analyses proved this triterpenoid only moderately active (parasite viability ca. 65% at 45 μM corresponding to 20 μg/mL). This finding suggested boswellic acids as the active principle. Owing to the complexity of the fractions, the isolation of distinct boswellic acid members for antigiardial activity studies is still in progress. This is the first report on antigiardial effects of boswellic acids. Keywords: *Boswellia serrata*, antigiardial, boswellic acids, oleanolic acid References: 1. Gardner B and Hill D (2001) Clin Microbiol Rev 14: 114 – 128 2. Upcroft P and Upcroft J (2000) Clin Microbiol Rev 14: 128 – 144 3. Brit J Rheumatol 37: 581 – 583 4. Bénére E et al. (2007) J Microbiol Methods 71: 101 – 106

**PM22**

Anti-ulcerogenic Activity of the Standardized Water Extract of *Phyllanthus emblica* Linn jajoy K*, Soonthornchareonmon N*, Panthong A*, Sireetawong S*

*Department of Pharmacology, Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand; tDepartment of Pharmacognosy, Faculty of Pharmacy, Mahidol University, Bangkok, Thailand; Division of Pharmacology, Department of Preclinical Science, Faculty of Medicine, Thammasat University, Pathumthani, Thailand*

*Phyllanthus emblica* Linn. (synonym: *Emblica officinalis* Gaertn.), Family Euphorbiaceae is native to the tropics of South and Southeast Asia. It is also called Emblic, Emblic myrobalan, Indian Gooseberry, Malacca tree and Myrobalan. In Thailand, it is known as Ma-kham-pom. *P. emblica* is an herbal plant commonly used in various traditional medicine systems for treatment of many disorders including anorexia, indigestion, and anemia (1, 2). The fresh or dry fruit is used in traditional medicine for the treatment of diarrhea, jaundice and inflammatory disorder (2, 3). The *P. emblica* water extract was prepared according to the Thai Herbal Pharmacopoeia and standardized on the phytochemical study, the *P. emblica* water extract contained tannins about 42.51%. The HPLC analysis of *P. emblica* water extract showed the presence of 20.48% gallic acid. Preliminary study, *P. emblica* water extract elicited the inhibitory effect on both COX-1 and COX-2 enzymes. Thus, the gastric ulcer may be one of the potential side effect of *P. emblica* water extract caused by its inhibitory effect on COX-1 enzyme. The oral administration of the *P. emblica* water extract at the dose of 600 mg/kg did not produce gastric lesions. On the contrary, the extract at the doses of 150, 300 and 600 mg/kg reduced ulcer formation in all tested acute gastric ulcer models i.e. EtOH/HCl-, indomethacin-, and stress-induced gastric lesions. These results indicate that *P. emblica* water extract possess anti-ulcerogenic effect. Keywords: Anti-ulcerogenic, *Phyllanthus emblica* Linn Acknowledgement: Royal Golden Jubilee Ph.D. Program and the National Research Council of Thailand. References: 1. Santisuk T et al. (2005) Floral of Thai-
Inhibition of angiogenic factors by laserolide, a sesquiterpene lactone from Laser trilobum Borkh. ex Gaertn. Kromickova E., Harmatha J., Zidek z
Institute of Experimental Medicine, Academy of Sciences of the Czech Republic, Videdliska 1083, 14220 Prague 4, Czech Republic; Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic, Flemingovo nam. 2, 16610 Prague 6, Czech Republic

Sesquiterpene lactones (SLs) are plant secondary metabolites, widely distributed within the families of Asteraceae and Apiaceae. SLs have received ever increasing attention for their beneficial effects in pathologies etiologically associated with angiogenesis, such as chronic inflammation and cancer. We have investigated the interference of SLs isolated from the non-pharmacopoeia C/E. European plant Laser trilobum Borkh. ex Gaertn., i.e. laserolide, isolaserolide, eudeslaserolide, archangelolide and 2-deangeloyl-archangelolide with production of angiogenic factors such as nitric oxide (NO), prostaglandin E2 (PGE2) and cytokines vascular endothelial growth factor (VEGF), interleukins IL-1β and IL-6. The recognized SL costunolide was included for comparative purposes. The cytotoxic effects of the compounds were evaluated as well. The immunobiological experiments were done under in vitro conditions using rat resident peritoneal cells which were cultured at a density of 2 x 10^5/mL in RPMI-1640 medium for 24h. The production of NO, PGE2 and cytokines were triggered by lipopolysaccharide (1 μg/mL). Formation of NO was assayed using Griess reagent. Concentrations of PGE2 and cytokines were determined by ELISA. In contrast to costunolide, the SLs from L. trilobum, are devoid of cytotoxic effects up to a concentration of 50 μM. Laserolide may be considered as a promising candidate for further preclinical investigations because its immunosuppressive effectiveness is very close to that exhibited by costunolide. It inhibits the cytokine (including the major angiogenic factor VEGF), PGE2 and NO production at IC50 of approximately 5 – 10 μM. Keywords: Laser trilobum; laserolide; nitric oxide; prostaglandins; cytokines

Acknowledgement: The work was supported by the grant 305/07/0061 from GACR.

PM24
Participation of citral in the relaxation of isolated rat tracheal smooth muscle induced by ginger oil
Chudapongse N., Mangprayoon T., Kuppatyanant S
School of Biology, Institute of Science, Suanarom University of Technology, Nakorn Ratchatams, Thailand

Ginger (Zingiber officinale) Roscoe is a common food plant that has been used as alternative medicine for a number of ailments. The rhizome of this plant is well known for the treatment of gastrointestinal tract disorders, such as dyspepsia, nausea and vomiting, as well as respiratory illnesses. Its hydroethanolic extract has been shown to inhibit hyperreactivity and remodelling, and lung inflammmation. The inhibitory effects of aqueous and methanolic crude extracts of ginger oil were tested. In the relaxation study, participation of citral in the relaxation of isolated rat tracheal smooth muscle induced by ginger oil was observed. Citral was found to relax the tracheal smooth muscle in a concentration-dependent manner. The IC50 of citral in the relaxation of isolated rat tracheal smooth muscle was 4.87 μM. The relaxation of isolated rat tracheal smooth muscle by citral was potentiated by the addition of 6-farnesyl-3',4',5,7-tetrahydroxyflavanone (MT1), a metabolite of ginger. The IC50 value of MT1 was 0.01 μM. The combination of citral and MT1 caused a synergistic effect in the relaxation of isolated rat tracheal smooth muscle. Keywords: Ginger, Zingiber officinale, Citral, Tracheal smooth muscle, Relaxation


PM25
Screening on cytotoxicity, antioxidant and antimicrobial of stem bark from Malaysian Vatica odorata and Vatica bella (Dipterocarpaceae)
Wan Mohd Zain W2, Ahmad N1, Latip F1, Mat Soir S2, Daud S1
1Faculty of Applied Sciences, Universiti Teknologi MARA, 40450 Shah Alam, Selangor, Malaysia; 2School of Chemistry and Food Technology, Universiti Kebangsaan Malaysia, 43600 Bangi, Selangor, Malaysia

Dipterocarpaceae have proven to be rich sources of variety biological activities (Zain et al. 2010). In our continuous investigation on this family we wish to report the screening on three biological activities: cytotoxicity, antioxidant and antimicrobial of methanol and acetone extract from Vatica odorata (Griff) Symington and Vatica bella Slooten. The antioxidant activity were evaluated by 1,1-diphenyl-2-picrylhydrazyl (DPPH). Total Phenolic Content (TPC), Ferric reducing power and Thiobitiorubic acid (TBA) method. The cytotoxicity activities were screened against Chang and HepG2 cells line (Mackeen et al. 1997). Meanwhile antimicrobial activity was conducted against six types of bacteria (Escherichia coli, Aeromonas hydrophila, Bacillus subtilis, Trichophyton mentagrophyte, Trichophyton tonsurans, Microsporum gypseum) and Candida glabrata) by disc diffusion method (Barry et al.1979). The results indicated that acetone extract of Vatica bella displayed moderate activity against Chang cell and HepG2 cell with IC50 values of 14 ± 0.50 μg/mL and 20.5 ± 2.68 μg/mL respectively. Both the extract displayed total phenolic content with range of 331.54 – 482.31 mg/g GAE and are weak DPPH scavenger as compared to standard with the range between 35.60 – 66.2% in TPC and TBA test, the Vatica extracts exhibited antioxidiant potential with percent inhibition between the ranges 26.60 – 88.36%. The antimicrobial screening showed that both the crude extract inhibited moderately S.aureus except for acetone extract of Vatica bella. Meanwhile methanolic extract of Vatica bella gave the active result where it inhibited moderately against Trichophyton mentagrophyte, Trichophyton tonsurans and Microsporum gypseum. Keywords: Vatica bella, Vatica odorata, Dipterocarpaceae, oligostilbenoids, cytotoxicity, antimicrobial, antioxidant Acknowledgement: We wish to thank to Ministry of Higher Education Malaysia for financial support via FRGS grant (01H000070006) and UTM for all the support References: 1- Zain WAZRM, Ahmad N, Nawi L & Jusoh, K (2010) World Applied Science Journal 8(9):1050 – 1055. 2- Mackeen MM et al. (1997) Int J Pharmacognosy 35: 174 – 178 3- Barry AL et al. (1979), Journal of Clinical Microbiology 10: 885 – 889.

PM26
Isolation, cytotoxic and antiplasmodial activities of 6-farnesyl-3',4',5,7-tetrahydroxyflavanone from the flower of Macaranga triloba
Zakaria I, Ahmad N
Faculty of Applied Sciences, Universiti Teknologi MARA, 40450 Shah Alam, Selangor, Malaysia

The genus Macaranga is one of the largest genera of the Euphorbiaceae, with approximately 300 species [1]. Macaranga triloba Müll.Arg. locally known as “Mahang merah” is a tree endemic to Southeast Asia and its constituents on tracheal smooth muscle in vitro. Chemical compositions of ginger oil were determined by GC-MS. Citral and camphene appeared to be its major components. For the relaxation study, compositions of ginger oil were determined by GC-MS. Citral and camphene, but not camphene, were found to reverse the Ach-induced airway contraction in concentration-dependent manner. This result indicates that citral is, at least partly, responsible for the antimicrobial activity of MT1. The antiplasmodial property of MT1 was evaluated using MT1 assay. 6-Farnesyl-3',4',5,7-tetrahydroxyflavanone (MT1) was found to exhibit a strong antiplasmodial activity with an IC50 value of 0.06 μM. This study
indicates the potential of MT1 as anti-cancer and anti-plasmodium agents. Keywords: *Macaranga triloba*, 6-farnesyl-3',4',5, 7-tetrahydroxyflavanone, farnesyl, cytotoxic, antimalarial

Figure 1: MT1

Table 1: Cytotoxic and antimalarial activities of MT1

<table>
<thead>
<tr>
<th>Cytotoxicity</th>
<th>Antiplasmodium</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIC60 (µM)</td>
<td>MCI7 (µM)</td>
</tr>
<tr>
<td>6.71</td>
<td>11.38</td>
</tr>
<tr>
<td>Hela (µM)</td>
<td>Plasmodium falciparum (µM)</td>
</tr>
<tr>
<td>2.64</td>
<td>0.06</td>
</tr>
</tbody>
</table>


**PM28**

The role of rice bran extract on acetyl CoA carboxylase in liver of rats fed a high-fat diet Charkhonpunya C1, Sireratawong S1, Komindr S2, Lerdvuthisopon N3

Institute of Clinical Science, Faculty of Medicine, Thammasat University, Pathumthani 12120, Thailand; 2Faculty of Science, Ramathibodi Hospital, Mahidol University, Bangkok 10400, Thailand; 3Department of Biochemistry, Faculty of Medicine, Thammasat University, Pathumthani 12120, Thailand

Rice bran water extract (RBE) was shown to reduce fat mass in rats fed a high-fat diet [1]. The involvement of RBE in metabolic alteration was investigated in 7 groups of Sprague Dawley rats, 8 rats each. High-fat fed group 3 to 7 were either co-treated daily with RBE (2205 ± 4.41; 15.10; 38.20 mg/kg) and a 20% FCS (0.06 vs. 0.05, n = 3) at the same concentration. Positive control (5% FCS) showed 63.0% closure. There was no effect of cell proliferation. In conclusion, fatty acid flux might induce fat synthesis in liver as an initial step in increasing fat accumulation [2]. Both RBE and metformin were able to reduce the alteration. Keywords: Rice bran, acetyl CoA carboxylase, high-fat diet

**PM27**

Antifungal activity of the extract of *Alpinia officinarum* Hance rhizomes on Candida albicans Klaian K, Nantapong N, Chudapongse N

School of Biology, Institute of Science, Suranaree University of Technology, Nakhon Ratchasima, 30000 Thailand

*Alpinia officinarum* Hance, known as lesser galangal, is a pungent and aromatic plant which is used as spice for flavoring food throughout Asian countries [1]. This plant has also been used as traditional medicine for several purposes such as relieving stomachache and pain, treating colds, invigorating the circulatory system, and reducing inflammation and swelling [2]. *Candida albicans* is a major causative microbe of Candida infections, immunosuppression, malignant disorders and AIDS [3]. Nowadays, choices of antifungal against candidiasis are quite limited due to drug toxicity and resistance. The crude extract of the rhizomes of *Alpinia officinarum* has been shown to possess antibacterial activity [4], however, antifungal activity of this plant has not been reported. In the present study, we found that lesser galangal exhibited antifungal activity against *Candida albicans*. The minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) value were 1.2 mg/ml and 2.0 mg/ml respectively. The assessment of cell damage produced by the crude extract of *Alpinia officinarum* rhizomes was conducted through scanning electron microscope (SEM) observation. SEM analysis showed that the extract induced deformation of *Candida albicans*. The treated cells had coarse surface and changed from oval to rounder shape. The result suggested that the extract damaged cell wall, causing *Candida albicans* to form spheroplast. This postulated mechanism may contribute to the antifungal activity of the crude extract of the *Alpinia officinarum* rhizomes against *Candida albicans*. Keywords: *Alpinia officinarum* Hance, *Candida albicans*, minimum inhibitory concentration, minimal fungicidal concentration, scanning electron microscope.


**PM29**

Wound healing potentials of a herbal homeopathic remedy on NIH 3T3 fibroblasts in vitro Hostanska K, Rostock M, Sailer R

University Hospital Zürich, Institute for Complementary Medicine, Zurich, Switzerland

This study was aimed to investigate the effect of the commercial herbal homeopathic remedy Similasan® Arnica plus Spray (Similasan AG, Jönigen, Switzerland), which is an ethanolic (22% m/m) preparation of *Arnica montana* L. D4, *Callendula officinalis* L. D4, *Hypericum perforatum* L. D4 and *Symphytum officinale* L. D6 (0712 – 2), on wound healing in cultured NIH 3T3 fibroblasts. Wound healing requires the coordination of complex cellular and molecular interactions. Therefore we investigated the cell proliferation, migration and wound closure promoting effect of the preparation and its potentized hydroalcoholic solvent (0712 – 1) using BrdU uptake, well chamber assay and wound healing scratch assay, respectively. All assays were performed in a controlled, blinded manner at least in three independent experiments. The preparation (0712 – 2) exerted a stimulating effect on cell migration 31.7% vs. 15% solvent (0712 – 1) at 1:100 dilutions (p < 0.05, n = 3). Mean wound closure achieved 59.5% by preparation in comparison to 22.1% by solvent (p < 0.05, n = 3) at the same concentration. Positive control (5% FCS) caused 63.0% closure. There was no effect of cell proliferation. In conclusion, the Similasan® Arnica plus homeopathic remedy showed wound healing activity in the NIH 3T3 fibroblasts scratch assay, which may be partly a result of fibroblasts migration and does not seem to be related to mitotic activity.

Figure 1: Wound closure of 3T3 fibroblasts by Similasan Arnica plus preparation.

Representative microphotographs of wound healing effect of preparation (1:100) on 3T3 fibroblasts after 24 h treatment. Indicated percentages of wound closure are normalized to the untreated control (medium).

Keywords: wound healing, 3T3 fibroblasts, homeopathic preparation
Antioxidant capacity of *Pistacia lentiscus* and *Fraxinus angustifolia* extracts and their fractions

**PM30**

Oxidative stress is thought to be the main cause of several pathologies; that is why research is focussing on the characterization of bioactive natural substances with antioxidant activity to replace synthetic molecules. The antioxidant activity of extracts of *Fraxinus angustifolia* Reut. ex Nyman and *Pistacia lentiscus* L., two medicinal plants used to treat inflammatory-related disorders was determined. The results indicated that aqueous chloroform extract of *Pistacia lentiscus* exhibited a great reducing power of 657.86 ± 35.25 mg Ascorbic Acid Equivalent/g of the extract, compared to that of the aqueous extract from *Fraxinus angustifolia* (260.73 ± 22.38 mg Ascorbic Acid Eq/g of extract). Extracts issued from both plants showed an outstanding capacity in scavenging the (DPPH) 2, 2-diphenyl-1-picrylhydrazyl (90%) and (ABTS) 2,2-azobis-ethylbenzothiazoline-6-sulphonic acid (85%) radicals at a concentration of 100 μg/ml, even higher than that of the standards: (BHA) butylhydroxyanisol and quercetin. Moreover, *Pistacia lentiscus* extracts showed relatively high scavenging activity (64.86% at 100 μg/mg) against hydro-peroxide, better than that of ascorbic acid (40.09%) and caffeic acid (45.59%) at the same concentration, while *Fraxinus angustifolia* extracts showed significant activity only at high doses (400 μg/ml). Column chromatography associated with thin layer chromatography analysis of plant extracts allowed the recovery of fractions responsible for high antiradical activity. Determination of total phenols and tannins plead for a major role of these compounds in the observed high antioxidant activity and may well explain the use of these plants in traditional medicine. **Keywords:** *Fraxinus angustifolia*, *Pistacia lentiscus*, Phenolic compounds, antioxidant, active fractions **Acknowledgement:** We wish to thank the Ministry of Education and Scientific Research of Algeria for sponsoring this work, Grant number: F00620070022. References: 1- Re et al. (1999) Free Rad Biol Med 26: 1231 – 1237 2- Huang et al. (2002) Food Chem 50: 4437 – 4444. 3- Fee and Teitelbaum (1972) J Immunol Methods 65: 55 – 63.

Antioxidant activity of crude extract from Algerian chemical olive leaves and application in stored meat

**PM31**

In this study an aqueous crude extract and oleuropein have been obtained from Algerian olive leaves (variety chemlal). The antioxidant effect of these compounds was determined in stored meat by TBA-RS methods. These compounds were added to meat chunk at 500 mg/kg. All meat samples have been stored in the aerobic conditions at 4 ± 2°C for one week. The results showed that compounds of olive leaves have a remarkable antioxidant activity throughout the storage phase. However, crude extract showed higher activity on lipid oxidation. The sensory analysis showed that meat "off-odour" added to these compounds reduced by 50% as compared to the control. **Keywords:** olive leaves, crude extract, oleuropein, Antioxidant activity, stored meat **Acknowledgement:** We are grateful to Ministerio de Asuntos Exteriores y Cooperación de Spain (AECID) and Minister de l’Enseignement Supérieur et de la Recherche Scientifique of Algeria for financial assistance to this work within the Programa de Co-operación Interuniversitaria e Investigación Científica PCI/MED Algeria-Spain (grant ALI A/01170/07; A/019342/08; A/023356/09; A/033506/10) and CNPBU (FR05S20090025, respectively).

Antioxidant potential, cytotoxic activity and phenolic content of *Clematis flammula* leaf extracts

**PM32**

Five fractions of *Clematis flammula* L., a plant widely used in the Mediterranean traditional medicine, were isolated from the leaves using a selective extraction procedure and their total antioxidant capacity was measured by both the ABTS and ORAC tests. Furthermore, their capacities to inhibit microsomal lipid peroxidation and to scavenge the hydroxyl radical were assessed. The cytotoxic potential of the crude ethanolic extract and the aqueous fraction obtained from chloroform was also evaluated on three human hepatoma cell lines CHL, PLC and HuH7. The results showed a stronger antioxidant capacity for the two aqueous phases obtained from ethyl acetate and chloroform concerning ABTS (7.9 and 10.5 mmoles Trolox eq/g of plant extract, respectively), ORAC (487 and 387 mmoles Trolox eq/g of plant extract, respectively) and hydroxyl radical scavenging activity (IC50= 56.5 and 48.4 µg/mL, respectively), compared to their organic counterparts which, however, inhibited microsomal lipid peroxidation more efficiently (IC50= 390.7 and 523.5 µg/mL, respectively). The ethanol crude extract exhibited a fairly good cytotoxic potential on the two cell lines CHL and PLC (IC50= 58.5 and 47.3 µg/mL, respectively), in contrast to the aqueous phase obtained from chloroform (IC50= 457.7 and 304.9 µg/mL, respectively). A positive correlation was also found between the phenol content and the different activities. These results provide experimental support for the therapeutic virtues of *Clematis flammula* leaf extracts. **Keywords:** Clematis flammula, anti-cancer, antioxidant, phenolic compounds **Acknowledgement:** We wish to thank the Ministry of Education and Scientific Research of Algeria for sponsoring this work, Grant number: F00620070022. References: 1- Re et al. (1999) Free Rad Biol Med 26: 1231 – 1237 2- Huang et al. (2002) Food Chem 50: 4437 – 4444. 3- Fee and Teitelbaum (1972) J Immunol Methods 65: 55 – 63.

Involvement of serotonergic system in anxiolytic effect of dichloromethane fraction of *Pimenta pseudocaryophyllus* (Gomes) Landrum

**PM33**

The purpose of this study was to verify the putative anxiolytic-like activity of dichloromethane fraction (DF) prepared from the leaves of *Pimenta pseudocaryophyllus* (Gomes) Landrum (Pp) and the mechanism of action involved using the elevated plus maze (EPM) and light-dark box (LDB) tests. Male Swiss mice (25 – 35 g) were treated orally with the vehicle (10mL/Kg), DF (125,250 and 500 mg/kg p. o.) or positive controls diazepam (1 mg/Kg) and buspirone (10 mg/Kg) 1 h before behavioral evaluation in the EPM and LDB. A treatment of DF significantly increased the percentage time spent and the number of entries into the open arms of the EPM as well as latency, number of transitions and time spent at the light part of the LDB in a dose dependent manner. The effects of DF in 250 mg/kg were antagonized by the 5-HT1A receptor antagonist NAN-190 (0.5 mg/kg i.p.). However, the effects could not be blocked by the benzodiazepine antagonist flumazenil (2 mg/kg i.p.). These results indicate an effective anxiolytic activity of Pp mainly mediated via the Serotonergic system without compromising motor function of the mice. Although there is need for the isolation of the less polar constituent responsible for this effect, further clinical investigations are necessary for its possible application as an alternative for the treatment of anxiety disorders to other medications currently in use. **Keywords:** Anxiolytic effect; Benzodiazepines, Elevated plus maze, light-Dark box, *Pimenta pseudocaryophyllus*, GABA receptor, 5-HT1A receptor **Acknowledgement:** CNPq, CAPES, FAPEG, FUNAPE/UFG
**PM34**

**Hypoglycemic properties of banana pseudo-stems**

Kreidyehy SI, Jaber HM, Baydoun EA  

Department of Biology, Faculty of Arts & Sciences, American University of Beirut, Beirut, Lebanon

Water extract of banana (Musa sapientum L.) pseudo-stems has been claimed by Lebanese herbalists to be efficient in the treatment of diabetes mellitus. This work aimed at verifying the alleged effect and elucidating its possible mode of action. Administration of the extract in replacement of drinking water to streptozotocin-induced diabetic rats, did reduce significantly blood glucose levels. The mechanism of action of the extract was studied by investigating its involvement in intestinal glucose absorption and its effect on the Na+/K+ ATPase and glucose transporters SGLT1 and GLUT2 in the rat jejunum. Rat jejunum were perfused in situ with Krebs Ringer buffer containing [14C]-3-0-methyl-D-glucose, and the activity of the Na+/K+ ATPase in jejunal homogenates was assayed in vitro, by measuring the amount of inorganic phosphate released in presence and absence of inhibitors of the ATPase. The extract induced a significant reduction in glucose transport and Na+/K+ ATPase activity, but did not affect the protein expression of SGLT1 and GLUT2 glucose transporters. It was concluded that the extract acts by reducing the Na+/K+ ATPase activity and consequently the sodium gradient required for sugar transport by SGLT1. Reduced activity of SGLT1 leads to a decrease in intracellular glucose and in the number of apical GLUT2, which contributes to the observed hypoglycemic effect. The current on-going work focuses on identifying the active ingredient(s) in the extract. Keywords: Banana, Glucose absorption, Na+/K+ ATPase, SGLT1, GLUT2  

Acknowledgement: This work was supported by a grant from the University Research Board. References: I. Kellet GL et al (2000) Biochem J 350:155 – 162

**PM35**

**Radical scavenging activity and phenolic components in different plant parts of Saraca asoca**

Pandey AK, Ojha V, Sahu SK, Yadav S  

Tropical Forest Research Institute, Jabalpur, Madhya Pradesh, India

The therapeutic properties of herbal drugs depend on certain chemical constituents (secondary metabolites) which varies according to age and maturity of the plant. Phenolic compounds have multiple biological properties as antioxidants that protect the human body against damage by reactive oxygen species. Medicinal plants have been focused for antioxidant compounds because of safety concerns associated with synthetic antioxidants. Saraca asoca (Roxb.) Wilde (Faba-ceae) an important medicinal tree has been well known for its effectiveness in menorrhagia and dysmenorrhea. Its bark has stimulating effect on the endometrium and ovarian tissue and has been used traditionally for gynecological disorders. Different plant parts: bark, leaves and twigs of various aged group trees of Saraca asoca were evaluated for their total phenols (TP), total flavonoids (TF), tannins (T), phenolic acids contents and radical scavenging activity. TP varied from 5.27 – 8.65%, TF from 0.16 – 0.28%, T from 20.88 – 51.17%. This is the first study in which different phenolic acids were estimated in Saraca asoca; vanillic acid varied from 2.34 – 5.07%, caffeic acid from 1.37 – 7.15%, chlorogenic acid from 8.51 – 25.59%, gallic acid from 0.17 – 0.46% and catechin from 4.78 – 7.95 mg/100 g. Radical scavenging activity showed significant variation among different girth classes and IC50 values ranged between 2.29 – 4.82 mg/ml. Bark was found to contain maximum concentration of active ingredients. The results revealed that the optimum girth class to obtain quality produce is 61 – 90 cm as it contains maximum concentration of active ingredients and possess high radical scavenging activity. Thus it can be used for making various formulations containing natural antioxidants. Keywords: Antioxidants, phenolic acids, harvesting age, Saraca asoca Acknowledgement: The authors are thankful to the Director, Tropical Forest Research Institute for providing necessary facilities to carry out the research work. The work was supported by a grant from the National Medicinal Plant Board (NMPB), Govt. of India, New Delhi.

**PM36**

**Decrement of body fat and hypolipidemic effect of 3,4,5-trihydroxybenzaldehyde isolated from Geum japonicum in high fat diet-induced obese rats**

Cho K, Kang S  

1National Academy of Agricultural Science, Suwon, Korea; 2Seoul University of Venture and Innovation, Seoul, Korea

3,4,5-trihydroxybenzaldehyde (THBA) is isolated from the aerial part of Geum japonicum Thunb., a Korean herb belonging to the family of Rosaceae [1]. In a recent study we have shown that the ethyl acetate fraction of G. japonicum inhibited NO production by LPS-activated RAW 264.7 cells and the natural THBA showed a remarkable scavenging activity on the DPPH radical[2]. Being on these findings, we determined the preventive effect of 3,4,5-trihydroxybenzaldehyde (THBA) for adiposity and dyslipidemia using high fat diet-induced obese rats. As a result of the investigation for lipid and leptin metabolism in obese rats, body weight, adipocyte cell size and visceral fat mass was significantly reduced by feeding with THBA. The concentration of triglyceride and leptin in serum was also significantly reduced whereas the fasting insulin level the was significantly increased. These results suggest that THBA isolated from G. japonicum is beneficial for the suppression of diet-induced obesity and hyperlipidemia. Keywords: Geum japonicum, 3, 4, 5-trihydroxybenzaldehyde. hyperlipidemia. References: 1. Kim J et al. (2006) Food Drug Analy14(2): 190 – 193. 2. Kang S et al. (2006) Nutritional Science 9(2): 117 – 123

**PM37**

**Cytotoxicity activities of Dicranopteris linearis extracts and fractions**

Mat Desa N1, Ramasamy K2, Ahmed N2, Zakaria Z2  

1Faculty of Pharmacy, Universiti Teknologi MARA, 42300 Puncak Alam, Selangor, Malaysia; 2Faculty of Applied Sciences, Universiti Teknologi MARA, 40450 Shah Alam, Selangor, Malaysia; 3Department of Biomedical Sciences, Faculty of Medicine and Health Science, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

Cancer is currently a second leading cause of death in the world [1]. Therefore the newer anticancer drugs from natural product are needed to replace the synthetic drug from the chemical compound. Based on the traditional medicinal value, Dicranopteris linearis (Burm.f.) Underw. from the Gleicheniaceae family was shown to possess pharmacological potential such as cytotoxic activity. For initial investigations, the dried leaves of D. linearis were extracted with aqueous, chloroform and methanol. The extracts were tested against HL 60 (acute promyelocytic leukemia cell lines) and WRL 68 (normal liver cell line) using MTT assays. The methanol extract (Table 1) showed the promising cytotoxic activity against HL 60 (IC50 = 7.9μg/ml). The methanol extract was further partitioned in sequence with n-hexane, chloroform and methanol to determine which extract contain the most active constituents. The result showed the methanol fraction to be significantly active against HL 60 with the value IC50= 12.88 μg/ml and non toxic to normal cell. The methanol fraction was then subjected to the bioassay-guided fractionation. The fractionation by vacuum liquid chromatography (VLC) gave eleven fractions labeled as F1, F2, F3, F4, F5, F6, F7, F8, F9, F10 and F11. The fraction F7 (Table 2) demonstrated cytotoxic activity with the best value (IC50 = 25.12 μg/ml). In addition, it was also found to be non-toxic against normal cell. Further work involving the isolation of active compounds in this potent ferns would be necessary to elucidate the actual source of the observed bioactivities. Keywords: Dicranopteris linearis, Gleicheniaceae, MTT assays, acute promyelocytic leukemia cell lines, normal liver cell line Acknowledgement: This study was supported by the research grant (02 – 01 – 01-SF0182) from the Ministry of Science, Technology and Innovation, Malaysia. References: [1] Pratt WB, Ruddon RW, Ensminger WD, Maybaum J (1994) The Anticancer Drugs. 2 Eds. Oxford University Press: New York; 3 – 16.
The aims of the present study were to develop the in vivo antinociceptive profile of methanol crude extracts of Muntingia calabura L. leaves and its fractions. The antinociceptive activity of orally-administered test solutions was assayed using the formalin test in rats. Based on the data obtained, the methanol extract of M. calabura (MEMC) exhibited the most effective (P<0.05) antinociception in the 1st and 2nd phases of the assay in a dose-dependent manner followed by the aqueous and chloroform extracts. The MEMC was then partitioned with petroleum ether (PEP) followed by ethyl acetate (EAP) and the remaining residue was dissolved in distilled water (AQ). Following subjectation to the formalin test, the PEP (100, 500 and 1000 mg/kg) exerted the most effective antinociception (P<0.05) in both phases of the assay in a dose-dependent manner followed by the EAP and AQ. The PEP was subjected to the fractionation processes and yielded 7 types of fractions labelled as FA, FB, FC, FD, FE, FF and FG. All fractions, in the dose of 300 mg/kg, were subjected to the assay and only fractions FC, FD, and FF demonstrated significant (P<0.05) antinociception at least in the 2nd phase of the formalin test. In conclusion, the antinociceptive activity of M. calabura involved modulation of central and peripheral pain mechanisms and attributed to the presence of flavonoids. Keywords: Muntingia calabura; Elaeocarpaceae; Antinociceptive activity; Formalin test; Flavonoids.

Neural mechanisms and sedative effect of different fractions of Holarrhena floribunda (Apocynaceae) stem bark in mice

Holarrhena floribunda G. Don (Apocynaceae) stem bark is used locally in the treatment of mental illness in Nigeria. This work examined the neural mechanism and the sedative properties of the crude extract and its fractions. Acute toxicity studies were carried out to determine the acute toxicity of the crude extract and its fractions by oral and intraperitoneal administration using Lorke (1983) method. The effect of the crude extract of Holarrhena floribunda and the fractions; hexane, chloroform, ethylacetate, butanol and aqueous was examined on novelty-induced rearing and grooming behaviors and on pentobarbital-induced sleeping time in mice. The results showed that the crude extract and the fractions were not toxic with the exception of butanol fraction. The crude extract and fractions reduced novelty-induced rearing and grooming behavior in mice. The inhibitory effect of the crude extract and the fractions were not reversed by atropine, dicyclohexylamine, yohimbine and naloxone; however the crude extract and the fractions blocked the facilitating effect of flumazenil. This suggests that the crude extract and the fractions appear to facilitate GABA-ergic transmission. The crude extract, aqueous, butanol and chloroform fractions prolonged pentobarbital-induced sleeping time in mice which were blocked by flumazenil a GABA antagonist, indicating that the crude extracts, aqueous, butanol and chloroform fractions contain GABA agonist. The result suggests that the stem bark of Holarrhena floribunda possess sedative effects which may be mediated through GABA-ergic neurotransmission. Keywords: Holarrhena floribunda, Rearranging, Grooming, Sedative, GABA References: Lorke D (1983) Arch Toxicol 54: 275 – 287.

Hepatoprotective effects of Artemisia monosperma and silymarin on carbon tetrachloride-induced hepatic damage in rat

The hepatoprotective effect of aqueous ethanol extract of Artemisia monosperma Delile aerial parts was investigated against carbon tetrachloride-induced acute hepatotoxicity in rat. The hepatoprotective activity of A. monosperma was evaluated by determination of liver enzymes marker in the serum (aspartate amino transferase AST; serum alanine transaminase ALT and alkaline phosphatase ALP). The histopathological studies were also carried out to confirm the acute carbon tetrachloride-induced pathological changes of the liver and kidney. Oral administration of A. monosperma (100 and 200 mg/kg) markedly reduced the elevated values of marker enzymes caused by CCl₄ – treatment. Glutathione (GSH) significantly decreases in the group treated with CCl₄. The two doses of A. monosperma and silymarin (25 mg/kg) significantly increased GSH values when given in combination with CCl₄. However, silymarin normalized liver enzymes and increased GSH levels than A. monosperma (two doses) when compared with the control group. A comparative histopathological study of liver of rat treated with A. monosperma exhibited almost normal architecture, compared to CCl₄-treated group. Image analysis of liver revealed a marked reduction damage area after treatment with A. monosperma (100 or 200 mg/kg) and silymarin compared with CCl₄-treated group. Phytochemical study of A. monosperma resulted in the isolation of a quercetin 3-O-β-glucopyranoside; quercetin 5-O-β-glucopyranoside; isorhamnetin 3-O-β-glucopyranoside; 5, 4’-dihydroxy flavone; 3, 4’-dihydroxy flavone; 5, 3’-trihydroxy flavone; 5, 3’, 4’-trihydroxy flavone; quercetin and isorhamnetin. Hepatoprotective effect of A. monosperma is probably due to combined effect of flavonoids. Keywords: Artemisia monosperma, Silymarin, CCl₄, Liver damage, Histopathology, Phytochemistry.
monosperma, Carbon tetrachloride, Hepatoprotective activity, Flavoroids

KM42

Sugar A1, Ramsamy K1, Ahmad N2, Zakaria Z3
1Faculty of Pharmacy, Universiti Teknologi MARA, Puncak Alam Campus, 42300 Kuala Selangor, Malaysia; 2Faculty of Applied Sciences, Universiti Teknologi MARA, 40450 Shah Alam, Selangor, Malaysia; 3Faculty of Biomedical Sciences, Faculty of Medicine and Health Science, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia

Hepatocyte apoptosis is a central feature of many liver diseases, leading to liver inflammation and fibrosis. In this study, we screened potent drugs inhibiting hepatic stellate cell (HSC) migration induced by hepatocyte apoptotic bodies (ABs) and evaluated the in vivo therapeutic effects in a rat model of hepatic fibrosis induced by thioacetamide (TAA). Rat HSCs were exposed to UV-irradiated hepatocyte ABs or TNF-α to investigate the anti-fibrotic effects of kaerophyllin. Liver fibrosis was induced by TAA injection into rats twice weekly for 6 weeks. Kaerophyllin (10 or 30 mg/kg) or curcumin (150 mg/kg, as a positive control) was given by gavage twice daily for 4 weeks starting 2 weeks after TAA injection. Kaerophyllin [(α-(trans-3,4-dimethoxybenzylidene)-β-(3,4-methylenedioxy-benzyl)-γ-butyrolactone, a lignan isolated from a Chinese herb Bupleurum scorzonerifolium by bioactivity-guided fractionation) attenuated ABs- and TNF-α-induced HSC migration, protein levels of collagen I and α-SMA, and the mRNA levels of ICAM-1, MCP-1 and IL-1β genes, but elevated PPAR-γ luciferase activity. Furthermore, kaerophyllin reduced TNF-α and ABs-induced NF-κB luciferase activity with decreased phosphorylation and p65 nuclear translocation. In TAA rats, kaerophyllin and curcumin treatment significantly protected liver from injury by reducing serum AST and ALT levels, and improved the histological architecture and fibrosis score. In addition, kaerophyllin treatment suppressed α-SMA protein expression, and mRNA levels of collagen I, TIMP-1, TNF-α, IL-1β and MCP-1 genes in TAA rats. Our results demonstrated that kaerophyllin protected the rat liver from TAA-caused injury and fibrogenesis by suppressing hepatic inflammation and inhibiting HSC activation. Keywords: hepatic stellate cells, liver fibrosis, kaerophyllin, inflammation, Bupleurum scorzonerifolium. Acknowledgement: This work was supported by the National Science Council of Taiwan and the National Research Institute of Chinese Medicine in Taiwan. References: [1] Friedman SL (2008) Gastroenterology 134: 1655 – 1669. [2] Marra F (2002) Front Biosci 7: d1899 – 1914. [3] Canbay A et al. (2003) Hepatology 38: 1188 – 1198.

PM43

Bioassay-guided Isolation of Cytotoxic Fractions from Muntingia calabura Leaf
Sufian A, Ramesamy K1, Ahmad N2, Zakaria Z3
1Faculty of Pharmacy, Universiti Teknologi MARA, Puncak Alam Campus, 42300 Kuala Selangor, Malaysia; 2Faculty of Applied Sciences, Universiti Teknologi MARA, 40450 Shah Alam, Selangor, Malaysia; 3Faculty of Biomedical Sciences, Faculty of Medicine and Health Science, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia

M. calabura L. or locally known as “Kerukup Siam”, belongs to the family Elaeocarpaceae [1]. This plant is native to American continent and is widely cultivated in warm areas of Asian region, including Malaysia [2]. The leaf is used to provide relief from gastric ulcers and to reduce swelling of the prostate gland as reported in Peru folkore medicinal uses. The aim of the present study is to determine the in vitro cytotoxic activity of Muntingia calabura leaf against cancer (HL60 and MCF-7) and normal (WRL68) cell lines using MTT [3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyltetrazolium bromide] assay as described by Mosmann [3] but with slight modifications. The crude methanolic extract of M. calabura (MCME) was suspended in distilled water to afford an aqueous MeOH solution and then partitioned with petroleum ether and EtOAc to give petroleum ether, EtOAc and aqueous extracts. The EtOAc extract showed significant cytotoxic activity when tested against HL60 (IC50 27.48 ± 3.60 μg/ml) and was further fractionated using vacuum liquid chromatography with gradient mixture of hexane-EtOAc (from 9:1 to 1:9) as solvent systems. Seven fractions were obtained (F1-F7), and subjected to cytotoxic activity against HL60, MCF7 and WRL68 cell lines. The IC50 values of the M. calabura extracts and fractions are shown in Table 1. Fraction 5 tested against HL60 showed strong inhibition (IC50 3.98 ± 0.09 μg/ml) as compared to the other cell lines as well as other fractions. Fraction 5 will be isolated further and the bioactive compounds responsible for the activity will be determined in the future study.

Table 1: IC50 values of the M. calabura extracts and fractions

<table>
<thead>
<tr>
<th>Partitions</th>
<th>HL60</th>
<th>MCF7</th>
<th>WRL68</th>
</tr>
</thead>
<tbody>
<tr>
<td>--</td>
<td>IC50 (μg/ml)</td>
<td>IC50 (μg/ml)</td>
<td>IC50 (μg/ml)</td>
</tr>
<tr>
<td>MCME</td>
<td>30.90 ± 4.73</td>
<td>36.56 ± 5.40</td>
<td>&gt; 100</td>
</tr>
<tr>
<td>Petroleum ether</td>
<td>29.46 ± 3.95</td>
<td>43.07 ± 3.25</td>
<td>&gt; 100</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>27.48 ± 3.60</td>
<td>40.72 ± 6.18</td>
<td>&gt; 100</td>
</tr>
<tr>
<td>Aqueous</td>
<td>&gt; 100</td>
<td>&gt; 100</td>
<td>&gt; 100</td>
</tr>
</tbody>
</table>

F1 32.14 ± 1.88 > 100 > 100
F2 35.89 ± 3.69 35.65 ± 4.41 43.93 ± 6.06
F3 84.49 ± 2.26 > 100 37.07 ± 4.66
F4 34.22 ± 5.83 30.40 ± 3.60 33.75 ± 2.04
F5 5.98 ± 0.99 35.47 ± 2.51 32.29 ± 4.32
F6 9.99 ± 9.5 25.72 ± 6.5 47.05 ± 6.16
F7 28.13 ± 3.59 42.16 ± 4.34 36.60 ± 1.29


PM44

Pharmacokinetics of linalool and linalyl acetate, the two main constituents of silexan, an essential oil from Lavandula angustifolia flowers, in rats
Nölinders M1, Germers S2, Koch E1
1Preclinical Research, Dr. Willmar Schwabe GmbH & Co. KG, 76227 Karlsruhe, Germany; 2Analytical Development, Dr. Willmar Schwabe GmbH & Co. KG, 76227 Karlsruhe, Germany

Silexan is the active ingredient in Lasex®, which has recently been approved for the treatment of restlessness and mild anxiety in Germany. The naturally occurring enantiomers R-(+)-linalool and R-(+)-linalyl acetate (LA) are the main constituents of silexan representing 70–80% of the total oil. We investigated the bioavailability and organ distribution of L and LA in rats by headspace GC-MS after administration of silexan or the single constituents. The peak concentrations of L after 100 mg/kg silexan were 77 ng/ml in plasma, 228 ng/g in liver, 670 ng/g in kidney, 2085 ng/g in fat and 164 ng/g in brain tissue. LA was only measured in the brain (31 ng/g). Administration of 28.9 mg/kg L which corresponds to the amount contained in 100 mg silexan, resulted in peak concentrations of 33 ng/ml in plasma, 218 ng/g in liver, 541 ng/g in kidney, 1140 ng/g in fat and 43 ng/g in brain tissue. The gavage of 36.8 mg/kg of LA, the equimolar amount to 28.9 mg L resulted in peak L concentrations of 10 ng/ml in plasma, 274 ng/g in liver, 255 ng/g in kidney, 244 ng/g in fat and 0 ng/g in brain tissue. LA itself was only found in the brain and in fat tissue. The results indicate that the bioavailability of L is generally higher when applied as total oil in comparison to the application of the single constituents. Interestingly, LA is very rapidly metabolized into L and can only be detected in the brain and in fat tissue. Keywords: Pharmacokinetics, Lavandula, Silexan, Bioavailability

PM45

Antiviral Activity of Aloe hijazensis against Some Haemagglutinating Viruses Infection and its Phytoconstituents
Abu Gabal N1, Abd Allah HA2, Hassan AZ2, Shalaby NM1, El Sofy MM1
1Scientific Department, Girls Faculty, King Abdul-Aziz University, Jeddah, Saudi Arabia; 2Chemistry of Natural Compounds Department, National Research Centre, 12622 Giza, Egypt; Central Laboratory for Evaluation of Veterinary Biologics, 13181 Abbassia, Egypt

On our ongoing for investigating the bioactive compounds of A. hijazensis Lavarans & Collen. (Abd-Alla et al., 2009), one of 24 species of Aloe in Saudi Arabia (Collenette, 1999); the flowers and flower-peduncles were selected for the present study. Thirteen compounds were isolated from both flowers and flower-peduncles. The isolated compounds were classified into: five hydroxyquinones; ziganein, Ziganein 5-methyl ether, aloesaponarin I, chrysophanol, aloemodin, one dihydroxyocoumarin; feralolid, four flavonoids; homoplanigatin, isoorientin, luteolin 7-glu-
curonopyranoside, isovitexin, one phenolic acid; \( \beta \)-cumaric acid, the anthrone; aloin together with aloenin. Eleven compounds were attributed to the flowers and leaves to the seven-flower-peduncles. Homoplagantin and lutelin 7- glucuronopyranoside are reported here for the first time from Aloe spp. Evaluation of the antiviral activities of flowers, flower-peduncles, leaves, and roots of \( A. \) hijazensis against haemagglutinating viruses of avian paramyxovirus type-1, influenza virus type A, Newcastle disease virus, and group III adenoviruses; egg-drop syndrome virus in specific pathogen free chicken embryos were carried out. In general, the flowers and leaves showed the highest antiviral effect. This is the first report on the isolation of phytoconstituents from this plant parts and also the first time for its biological evaluation. Keywords: \( Aloe \) hijazensis, Phytotoxic Chemicals, Haemagglutinating Viruses Acknowledgement: Department of Botany, King Abdul-Aziz University, Jeddah, Saudi Arabia. Dr. Farg Abd-Allah Elghamdi. References: 1. Abd-Alia HI et al. (2009) Nat Prod Res 23: 1035 – 1049. 2. Collenette S (1999) Wild Flowers of Saudi Arabia. National Commission for Wild Life Conservation and Development. Riyadh.

The use of a new phyto drug Suttigen in gynaecology for treatment of inflammatory processes Rakhmatdinaeva S1, Aizan G1, Nijba B2, Gulbram B2
1Gazova Aisan Eurasian National University named after LN Gumilev, 5, Munaitpassov, 010008, Astana, Kazakhstan; 2Basharova Gulbram National Scientific Medical Center, 27, Kabanybaty, 010008, Astana, Kazakhstan

The aim of the research is an assessment according to the microbiological parameters of 3% suttigen ointment on the basis of Suttigen substance from a grass of \( Euphorbia \) soongarica Boiss., as vaginal swabs. In 185 female patients with inflammatory processes of the pelvic organs suttigen ointment was applied. The levomekol ointment (55 patients) and 10% the metiluratsil ointment (43 patients) was applied in the control groups. Along with a local therapy there was a general treatment with antibiotics, desensibilization and detoxification drugs. During the treatment, a quantitative microbiological examination of smears from the cervical canal, urethra and vagina was carried out in women with inflammatory conditions of the pelvic organs. The intake of material was within the time of patients admission and continued in the dynamics for 2 – 3 days. Before the treatment, semination wounds of \( Sphacelotheca \) aureus were 105 – 106 microbial bodies in genital habitats. On the 5th and the 6th days of the therapy with the use of this ointment was a completed resulting in a complete clearance of the genital habitat of \( Sphacelotheca \) aureus. The content of \( Sphacelotheca \) aureus in genital swabs in the control groups on the first day there were 105 – 106 microbial bodies, while on the third day 105 and on the fifth day – 103. In comparison with the ointments of levomekol and 10% metiluratsil, 3% suttigen ointment reduced the duration of a genital cleansing of bio-topes from microflora to more than 1.3 – 1.5 times and improved therapy.

Mathematical model for Glucose-Insulin interactions after administration of the \( Arctium \) lappa extract in diabetic rats Samiee F, Bahrami P
Biomedical Engineering, Science & Research Branch, Islamic Azad University, Tehran, Iran

Diabetes is a widespread chronic disease which is increasing at an alarming rate in the world. It can lead to a variety of vascular, neurological or metabolic complications. Maintaining blood glucose levels within the normal range by exogenous insulin administration or oral administration of plant extracts which increase plasma insulin levels can decrease these effects. Mathematical models have provided one means of understanding diabetes dynamics. In this study we used one of these models “minimal model” (1) based on our experimental data for estimation of plasma insulin from plasma glucose. We used a modified Michaelis Menten equations (2) in our model. Diabetes was induced by intraperitoneal injection of streptozotocin (80 mg/kg). After 24 hours of food deprivation, blood samples were collected from the orbital sinus before, and at 1, 2, 3 hours after oral administration of extracts. Blood glucose and insulin level were determined by glucose oxidase and standard radioimmunoassay methods, respectively. In diabetic rats, plant extract increased blood insulin levels (p < 0.05) and decreased blood glucose levels (p > 0.01). Results showed the above model can predict plasma insulin level from plasma glucose value. Keywords: diabetes, Arctium lappa, insulin-glucose References: (1) Bergman RN, Cobelli C (1980) Federation Proc 39: 110 – 115. (2) Lin J (2007) Robust modeling of the glucose-insulin system for tight glycemic control of critical care patients, Ph.D. Thesis, Department of Mechanical Engineering, University of Canterbury, New Zealand.

Bettulin is a pentacyclic triterpene alcohol belonging to the lupane series of compounds. It is extracted from the outer birch bark. The birch triterpenes have known antiallergic, antimicrobic, antitumor and hepatoprotective effects [1 – 3]. Although to our knowledge no reports about acute toxicity of bettulin were published. Acute toxicity of bettulin was studied on rats and mice. Betulin (98%) was provided by VIT and female outbred rats and mice were administered with betulin in single dose of 1000 – 16000 mg/kg intragastric and rats in single dose of 250 – 4000 mg/kg intraperitoneally. No significant effect of betulin administration on body weight of animals and no lethal effect were observed during 14 days after administration in rats and mice in all doses tested. LD50 was not reached in all experiments. Skin irritation, edema or infiltration at the injection site after intraperitoneal injection was not observed. Irritation of the gastrointestinal tract of rats and mice, and peritoneum in rats at the site of drugs injection has been not fixed. The form and location of all internal organs were without pathology both in rats and mice and there were no significant changes in mass coefficients of organs. In result of single intragastric administration or intraperitoneal injection of betulin in rats and mice no toxic effects were observed. Results of 14 days of observation of animals and data of necropsy evidenced about safety of this substance. Betulin is non-toxic and may be classified as substance of VI class toxicity [4]. Keywords: betulin, intragastric administration, intraperitoneal injection, mice, rats Acknowledgement: the study was done in frame of FORESTSPECS project, grant agreement 227239 References: 1. Alakurtti S et al. (2006) Eur J Pharm Sci 29: 1 – 13. 2. Kratsky PA (2006) Nat Prod Rep 23: 919 – 942. 3. Shikov AN et al. (2011) Phytomedicine 18: 141 – 146. 4. Puthong S et al. (2010) Phytotherapy Research 24: 1167 – 1173. PM46

Evaluation of acute toxicity of bettulin Makarova MN1, Shikov AN1, Avdeeva OI1, Pocharitskaya ON2, Makarenko IE2, Makarov VG1, Djachuk GI1
1St-Petersburg State Medical Academy named after I.I. Mechnikov, 47, Piskarevskiy pr., 195067, St-Petersburg, Russia; 2St.-Petersburg Institute of Pharmacy, 47/2, Piskarevskiy pr., 195067, St-Petersburg, Russia

Trigona laevis propolis: Chemical compositions and antiproliferative activity on cancer cell lines Chanchao C1, Umthong S1, Phawgraisirisarn P1, Puthong S3
1Department of Biology, Faculty of Science, Chulalongkorn University, Phya Thai Rd., Bangkok, 10330, Thailand; 2Department of Chemistry, Faculty of Science, Chulalongkorn University, Phya Thai Rd., Bangkok, 10330, Thailand; 3Institute of Biotechnology and Genetic Engineering, Chulalongkorn University, Phya Thai Rd., Bangkok, 10330, Thailand

Cancer is a leading cause of death worldwide and continue rising. Many cancer patients resisting to recent chemotherapeutic agents, so it is very important to search for new compounds with antitumor activity and develop to be anticancer drugs. Propolis of stingless bee (Trigona laevi-ceps) is focused in this research. It was extracted by 95% ethanol and partitioned through their polarities with 40% MeOH, CH 2Cl 2 and hexane. All parts were tested for antitumor activities against five tumor cell lines (Chago, KATO-III, SW620, BT474 and Hep-G2) by MTT assay. In addition, the cytotoxicity against two normal cell lines (Fibroblast and CH-liver) by MTT assay, the most effective fractions were 30DCM and 100DCM. Both were composed by several separated peaks by size exclusion chromatography, yielding totally 8 fractions (30DCM-F1, 30DCM-F2, 30DCM-F3, 30DCM-F4, 100DCM-F1, 100DCM-F2, 100DCM-F3, 100DCM-F4).
Ascorbic acid content, phenolic compounds and antioxidant capacity of Brazilian exotic fruits açai (Euterpe oleracea Mart.) and cupuaçu (Theobroma grandiflorum Schum.)

Olívia SC, Ramalho SA, Guadalcorte NC, Gomes ED, Miranda RM, Narcín IN
Laboratory of Flavor Analysis and Chromatography, Federal University of Sergipe, São Cristóvão, Sergipe, Brazil

It is well-known that diets high in fruits and vegetables may decrease the risk of chronic diseases, due to their low fat content and high levels of fiber and antioxidant substances, such as ascorbic acid and polyphenols. Current work describes the characterization of two Brazilian exotic fruits namely açai (Euterpe oleracea Mart.) and cupuaçu (Theobroma grandiflorum Schum.), for their: antioxidant capacity; ascorbic acid content; and total polyphenolic compounds. Antioxidant capacity was determined in pulps by 2,2-diphenyl-1-picrylhydrazyl (DPPH) method. Ascorbic acid was quantified by ultra-fast liquid chromatography using a Shimadzu™, UFLC-20A chromatograph with a reversed-phase octade-cylciane column XR-ODS™, and 0.025 M of a dihydrogen potassium phosphate solution as the mobile phase. Polyphenolic compounds were determined by the Folin-Ciocalteau method. Antioxidant capacity, expressed in terms of grams of pulp per 100 g of DPPH, was 1666.76 for the açai fruit, and 4366.71 for the cupuaçu fruit. Ascorbic acid was not detected in açai pulp, and its content was 7.04 mg per 100 g of pulp in cupuaçu pulp. Total phenolic compounds content, was 108.5 expressed in terms of gallic acid equivalent per 100 grams of pulp for the açai and 91.85 for the cupuaçu pulp. Results pointed out the nutritional and therapeutic potentialities of these exotic fruits, for their antioxidants properties. Keywords: food phenolics, data base, liquid chromatography, nutritional properties, Active oxygen species (ROS) have been recognized as playing an important role in the initiation and/or progression of various diseases such as atherosclerosis, inflammatory injury, cancer and cardiovascular disease. Many antioxidant compounds, naturally occurring from plant sources, have been identified as a free radical or active oxygen scavengers. Additionally, it has been determined that the antioxidant effect of plant products is mainly due to phenolic compounds, such as simple phenolic acids, flavonoids, isoflavonoids, hydrolyzable tannins and condensed tannins. The present study deals with the isolation and identification of the phenolic metabolites from *Acacia nilotica* (L.) Delile (Leguminosae) flowers and evaluation of its free radical scavenging activity. The aqueous alcoholic extract (MeOH; H₂O; 8: 2) of *Acacia nilotica* flowers was subjected to extensive repeated column chromatography on polyamide, cellulose and Sephadex LH-20 resulted in catechin, catechin-7-O-gallate, gallic acid, naringenin 7-O-β-glucopyranoside, quercetin 3-O-β-glucoside (2→1) glucopyranoside, quercetin 3-O-β-glucopyranoside, chalconaringenin 4′-O-β-glucopyranoside, naringenin and quercetin. The structure of the isolated compounds was elucidated on the basis of spectral analysis (UV, HRESI, 1/2D NMR). The radical scavenging activity of the extract was quantified spectrophotometrically, using DPPH radical. The total polyphenols showed excellent antioxidant potency when tested by radical scavenging methods. Keywords: *Acacia nilotica*, Phenolic compounds, antioxidant activity, DPPH

Salvia species belongs to the Lamiaceae are widely distributed in Turkey, 50% of the 89 *Salvia* species is endemic (1). Various parts of some *Salvia* species have been reported to have traditional uses (2,3). The common indications include GIT symptoms/disorders (colic, diarrhea, indigestion, and abdominal pain), respiratory tract symptoms/disorders (colds, sore throat, and cough), infections (tuberculosis, bacterial infections, influenza, and parasitic infections), pain (headache and arthralgia), and miscellaneous disorders (diabetes mellitus, liver diseases, barrenness, urticaria, and hemorrhage). In this study the analgesic activity of ethanol, butanol, chloroform and water extracts of *S. wiedemannii* Boiss. has been evaluated by using tail flick and acetic acid–induced writhing tests. The chloroform extract (500 mg/kg, i.p.) obtained from *S. wiedemannii* showed significant analgesic activity on tail flick assay, its efficacy was very close to morphine. The water, ethanol and butanol extracts showed analgesic activities similar to that observed with aspirin. Chloroform extract (500 mg/kg, i.p.) also inhibited number of writhings induced by acetic acid. Chloroform extract provided analgesic effects similar to morphine. Its effect was quick and durable. This in vivo study demonstrates that *S. wiedemannii* has strong analgesic effect in accordance with the public belief. Keywords: *Salvia wiedemannii*, analgesic activity, folk medicine References: 1. Davis PH (1982) flora of Turkey and the East Aegean Islands. Vol. 4, Edinburgh. 2. Honda G et al. (1996) Ethnopharmacol 53: 75 – 87. 3. Sezik E et al. (2001) Ethnopharmacol 75: 95 – 115.
Although a wide array of medicinal plants plays a role in the prevention and treatment of diabetes, there are few reports of the application of herbal medicines in amelioration of renal damage. The present study examined the effect of methanolic extract (25 and 50 mg/kg body weight) of Acacia nilotica (L.) Delile (Leguminosae) pods in streptozotocin-induced diabetic rats for 30 days, and its biochemical, histopathological and histochemical study in the kidney tissues. Diabetic rats exhibited loss of body weight, hyperglycemia, elevated serum urea and creatinine. Significant increase in lipid peroxidation (LPO), superoxide dismutase (SOD) and reduced glutathione (GSH) was observed in diabetic kidney. Histopathological examination revealed infiltration of the lymphocytes in the interstitial spaces, glomerular hypertrophy, basement membrane thickening and tubular necrosis with loss of their brush border in some of the proximal convoluted tubules in diabetic rats. Daily oral administration of Acacia nilotica extract reversed the adverse effect of diabetes in rats. Acacia nilotica extract lowered blood glucose levels, restored serum urea and creatinine and body weight loss. In addition, Acacia nilotica extract attenuated the adverse effect of diabetes on LPO, SOD and GSH activity. Treatment with Acacia nilotica was found to almost restore the normal histopathological architecture of kidney of streptozotocin-induced diabetic rats and ameliorate mitochondrial succinic dehydrogenase. However, glomerular size and damage area showed ameliorative effect after treatment with the extract. In conclusion, the antioxidant and antihyperglycemic properties of Acacia nilotica extract may offer a potential therapeutic source for the treatment of diabetes. Keywords: Acacia nilotica, streptozotocin, biochemical, histopathological, antioxidant activity

The three oral bacteria (members of the normal flora in the mouth) including Streptococcus mutans, Lactobacillus and Streptococcus sanguis play a major role in the production of dental caries so that the first two bacteria accelerate dental caries but the third reduce this process. The purpose of this study was to determine the antibacterial activity of hydro and methanolic extract from Salvia hyalanae DC. ex Benth. against the three oral bacteria and comparison with vancomycin antibiotic in vitro. At First, a sample of hydro and methanolic extract of the Salvia hyalanae was prepared and then its antibacterial activity against S. mutans (PTCC 1601), Lactobacillus (PTCC 1608) and S. sanguis (PTCC 1449) was evaluated by well diffusion (with concentration of 100 mg/ml) and agar serial dilution methods for determining of MIC (minimum inhibitory concentration) with dilution of 0.195 to 100 mg/ml. Also, we studied the antibacterial activity of vancomycin antibiotic on them by disk diffusion method. The results from the antibacterial tests Salvia hyalanae hydro and methanolic extract had not been affected against one of the bacteria. While S. mutans was sensitive to vancomycin and Lactobacillus and strep. Sanguis were resistant to it. This study demonstrated that hydro and methanolic extract of Salvia hyalanae may not be an effective antibacterial medication in the prevention of dental caries. Keywords: Salvia hyalanae, hydro and methanolic extract, antibacterial activity, vancomycin, oral bacterial

The results of Brueca javanica (Linn.) Merr (Simaroubaceae) are used as herbal remedies for treatment of human amebiasis, as well as for cancer in Chinese folklore. Here, we studied the anti-proliferative activity of the extract from fruit of B. javanica (BJE) and the fraction (BJEF5) against Hep G2 human hepatocarcinoma cells, and explored their mechanisms. From these studies, BJE showed growth inhibitory activity effect by the MTT assay in a dose-dependent manner with an IC50 value of 1.56 ± 1.02 µg/ml, and by HP20 resin chromatography, the fraction (BJEF5) of elute washing by 50% EtOH showed more potent effect in a dose-dependent manner with an IC50 Value of 0.44 ± 0.02 µg/ml. However, cell cytotoxic activity was not observed in the peripheral blood mononuclear cell (PBMC) treated with BJE or BJEF5 less than 30 µg/ml. Results from flow cytometry analysis also showed, BJE and BJEF5 induced cell cycle arrest in G1 phase as compared with the control groups, and the β-catenin transcription activity was inhibited in Hep G2 cells when treated with BJE and BJEF5, respectively. Furthermore, western blot analysis indicated that BJE and BJEF5 significantly reduced c-Myc, cyclin D1 protein levels leading to cell cycle arrest, and survivin protein level leading to apoptosis in a dose-dependent manner, respectively. Therefore, the BJE or BJEF5 deserves further exploration for its use as a potential agent in the therapy for hepatocellular carcinoma (HCC). Keywords: anti-proliferation, Brueca javanica, hepatocarcinoma Acknowledgement: The authors would like to thank the Ministry of Economic Affairs for the financial support of this research under contract No. MOEA 99-EC-17-A-02 – 04 – 0317.

The aim of this work was to evaluate the antimicrobial effects of wild garlic (Allium hirtifolium Boiss.) and peppermint (Mentha piperita L.) extracts and their combination on Staphylococcus aureus. In the present work the antimicrobial effects of the mentioned plant extracts were evaluated using disk diffusion method as a preliminary step and microdilution method. The mentioned extracts were introduced into TSB Broth at ten concentrations from 50% to 0.09%(v/v) in order to determine minimum inhibitory concentration (MIC) for Staphylococcus aureus using Bioscreen C device, which is based on optical density measurements. Results indicated that wild garlic and peppermint extracts showed MIC of 3.17%(v/v) and 12.5%(v/v) respectively for Staphylococcus aureus. The antimicrobial activity was enhanced in response to extract mixture than individual extracts of each species, as no growth was observed at the concentrations from 50 to 0.09%(v/v). In conclusion, edible plants can be a potential source for inhibitory substances for some pathogen. Both extracts studied in this research were effective on Staphylococcus aureus and the combination of them showed synergistic effect on the inhibition of the growth, so the potential of plant extracts when combined with each other can be used as a more effective barrier for preservation. Keywords: antimicrobial, extract, Staphylococcus aureus, Mint, Wild Garlic.
**PM58** Simultaneous determination of some phenolic compounds and antioxidant activity of *Inula viscosa* (L.) Alton

Gökbulut A1, Sarer E1, Satlmaz B2, Batooglu K2
1Department of Pharmacognosy, Faculty of Pharmacy, Ankara University, 06100, Tandoğan, Ankara, Turkey; 2Department of Biochemistry, Faculty of Pharmacy, İnönü University, 44280, Malatya, Turkey

*Inula* species are widespread in the world and used traditionally for ages by different cultures due to their various biological activities. The members of this genus contain terpenic compounds, especially sesquiterpene lactones, flavonoids, glycolipids and arachidonic acid derivatives (1,3). *I. viscosa*, suffrutescence and rank-smelling herb up to 1 - 2 m, is widespread in Mediterranean area (4). In this study, antioxidant activity of freeze dried water, methanol and ethyl acetate extracts of flower, leaf and radix of *I. viscosa* were evaluated via DPPH and ABTS methods. All the extracts showed antioxidant activity in different concentrations. Water extract of *I. viscosa* flower expressed strong antioxidant activity with lower IC50 values when compared with the other extracts. Ethyl acetate extracts of the investigated parts of the plant showed less antioxidant activity compared with the water and methanol extracts. It's obvious that phenolics are responsible for the antioxidant potential of the plants. For this reason, phenolic compounds such as chlorogenic acid, caffeic acid, rutin, myricetin, quercetin, luteolin and kaempferol were analyzed qualitatively and quantitatively in the flower, leaf and radix methanol extracts of *I. viscosa* by RP-HPLC. Chlorogenic and caffeic acids were found in all the investigated parts of the plant. Only myricetin was absent in the flower extract and chlorogenic acid was found in significant amount in radix extract. While myricetin was not determined in the plant, kaempferol was found only in the flower extract. Therefore, most of the investigated phenolics could be responsible for the potent antioxidant activity of *I. viscosa*. Keywords: *Inula*, Antioxidant activity, DPPH, ABTS, RP-HPLC References: 1. Zhao Y-M et al. (2006) Chem Biodivers 3: 371 – 384. 2. Danino O et al. (2009) Food Pharm Bull 29(3): 455 – 459. 4. Davis PH (1982) Flora of Turkey and The East Aegean Islands, Edinburgh University Press, Edinburgh.

**PM59** Metal chelating, radical-scavenging and anti-lipid peroxidative activities of various extracts from two endemic species belonging to the genus *Prangos* (Umbelliferae)

Oke Atunatas F1, Duman H1, Aslam B2
1Department of Biology, Faculty of Science, Gazi University, Ankara 06500, Turkey; 2Molecular Biology Research Center, Gazi University, Ankara 06500, Turkey

Medicinal applications have been reported for some *Prangos* species as emollient, carminative [1], antifungal [2], antioxidant [3], antibacterial, cytokine release inhibitor [4], and anti-HIV [5]. This study was undertaken to determine metal chelating, radical scavenging, and anti-lipid peroxidative properties of leaf extracts from two endemic species: *Prangos denticulata* Fisch. & Mey. and *Prangos platychloena* Boiss. et Tchihat. subsp engizkensis H. Duman et M.F. Watson. In addition, the amounts of total phenol compounds were determined. The methanol and the hot water extracts were more effective in all assays than the acetone extracts. *P. denticulata* hot water extract showed the highest metal chelating and radical scavenging ability (IC50 = 0.048 € 0.001 mg/ml). The strongest chelating effect was obtained from the *P. platychloena* subsp. engizkensis water extract (IC50 = 0.76 € 0.02 mg/ml). A significant relationship between the antioxidant activities and total phenolic contents were found (p < 0.05). Moreover, the hot water extracts showed a notable capacity to suppress lipid peroxidation. This study suggests that two endemic *Prangos* species can potentially be used as a readily accessible source of natural antioxidants. They can be exploited for its use against a number of disorders including cardiovascular diseases and cancer. Keywords: *Prangos denticulata*, *Prangos platychloena* subsp. engizkensis, endemic plant, radical scavenging, lipid peroxidation, metal chelating, antioxidant, total phenol References: 1. Zargari A (1988) Medicinal Plants. Tehran University Publications, Tehran. 2. Ozcan M (1999) Acta Alimentaria 28: 355. 3. Mavi A et al. (2004) Biol & Pharm Bull 27: 702. 4. Tada Y et al. (2002) Phytochemistry 59: 649. 5. Shikishima Y et al. (2001) Chem & Pharm Bull 49: 877.

**PM60** Antimicrobial activity and characterization of some phenolic compounds of *Inula peacockiana* (Aitch. & Hemsl.) Krovin

Gökbulut A1, Sarer E1, Sarer E1, Satlmaz B2, Batoglu K2
1Department of Pharmacognosy, Faculty of Pharmacy, Ankara University, 06100, Tandoğan, Ankara, Turkey; 2Department of Microbiology, Faculty of Pharmacy, İnönü University, 44280, Malatya, Turkey

The genus *Inula* (Asteraceae) has more than one hundred species and is found in Europe, Asia, Africa and mainly in the Mediterranean region. Some of the members of this genus are used as traditional herbal remedies throughout the world due to their anti-inflammatory, expectorant, diaphoretic, bactericidal, anti-inflammatory, antihepatic, antioxidant and antimalarial properties (1,2). *Inula peacockiana* (Aitch. & Hemsl.) Krovin is a perennial herb up to 2 m and naturally growing in Iranian-Turkey region (3). There are still many *Inula* species which were not studied or received a little attention, and one of these species appears as *I. peacockiana*. In this study, antimicrobial activity of the methanol extracts of flowers, leaves and radix of *I. peacockiana* were determined by agar dilution method against *S. aureus*, *E. faecalis*, E. coli, P. aeruginosa, C. albicans and *C. tropicalis*. All parts of the plant exhibited antimicrobial activity against all the investigated bacteria and yeasts. Flower extract was seemed to be more active against Gram positive bacteria and yeasts with lower MIC values. Some phenolic compounds such as chlorogenic acid, caffeic acid, rutin, myricetin, quercetin, luteolin and kaempferol were investigated qualitatively and quantitatively by RP-HPLC in the methanolic extracts of the plant parts. While chlorogenic and caffeic acids were found in all the investigated parts of *I. peacockiana*, quercetin was found in significant amount in the flower extract. All the investigated compounds were determined in the flower extract in various amounts. Consequently, some of the antimicrobial potential of the plant could be due to the presence of the investigated phenolics. Keywords: *Inula*, Antimicrobial activity, Agar dilution method, RP-HPLC References: 1. Zhao Y-M et al. (2006) Chem Biodivers 3: 371 – 384. 2. Bai N et al. (2005) Food Lip 12: 141 – 149. 3. Davis PH (1982) Flora of Turkey and The East Aegean Islands, Edinburgh University Press, Edinburgh.

**PM61** Anti-inflammatory effect of peat distillates in animal models

Makarova MN1, Shikov AN1, Pozharitskaya ON2, Makarenko IE3, Makarov VC4, Djachuk GI

1St.-Petersburg State Medical Academy named after I.I. Mechnikov, 47/5, Piskarevsky pr., 195067, St-Petersburg, Russia; 2St.-Petersburg Institute of Pharmacy, 47/5, Piskarevsky pr., 195067, St-Petersburg, Russia

Until now, the medical uses of peat derivatives have been very limited. One medicinal product made from peat is Torfot, a Soviet Union product used as a stimulator of regeneration processes, as well as non-specific immunomodulator. The aim of present study was to investigate anti-inflammatory effect of peat distillates. Two samples of peat (upper -PD 1 and deep -PD 2) were collected in October 2010 by Dr. N. Demidova (Northern Research Institute of Forestry, Arkhangelsk, Russia). The peat was distilled with steam. Female rats were injected intravenously with peat distillates in single dose of 0.3, 0.6 and 0.9 ml. Indomethacin was used as positive control. Edema was induced 1 h later by injection of 0.5% carrageenin solution in the plantar aponioure of the right hind paw. The edema volume was determined using the oncometric method at 3 h after the injection of carrageenin, and inhibition of edema rate was calculated [1]. Analgesic studies was carried out using the hot plate test [2]. Inhibition of edema rate after injection of PD 1 in dose 0.6 ml was 55.2% and PD 2 in dose 0.3 ml was 50.5%, while after administration of indomethacin it was 75.5%. Analgesic properties of distillates were observed in the hot-plate test at a dose of 0.9 ml. The latency time was increased in 2, 4, 6 and 14 times comparing to control group for indomethacin, PD 1 and PD 2 respectively. This is the first evaluation of anti-inflammatory effect of distillates of peat which exhibited significant anti-inflammatory and analgesic activity in rats. Keywords: upper peat, deep peat, indomethacin, edema, analgesic Acknowledgement: the study was done in frame of FORESTSPECS project, grant agreement 227239. References: 1. Shikov AN et al. (2010) Phytotherapy 17: 463 - 468 2. Shikov AN et al. (2008) Nat Med 52: 436 – 440.
Diuresis is important in the treatment of many diseases ranging from acute cases as renal failure to chronic cases as hypertension. Olive, *Olea europaea*, is a species of a small evergreen tree in the family Oleaceae, native to the coastal areas of the Mediterranean region. Olive leaves are used as anti-rheumatic, anti-inflammatory, antiseptic, antipyretic, vasodilator, diuretic, and hypoglycemic agents in traditional medicine. Recently, it has been shown that olive leaf extract (OLE) has calcium channel blocker property. The mechanism of the diuretic activity was studied through determination of saluretic, vasodilatory, hypotensive, diuretic and hypoglycemic agents in traditional medicine. Results showed that the petroleum ether extract and aqueous methanol extract of olive leaves possess efficient diuretic activity. Significant increase in creatinine urinary excretion and saluretic index was noticed on extract of olive leaves possess efficient diuretic activity. Significant in vitro anti-inflammatory activity of a *Passiflora alata* Curtis extract, was calculated, a Passiflora species endemic to Brazil. The extract was standardized in vitamin-2–O”–rhamnoside, the major component responsible for the biological activity. The content of vitamin-2–O”–rhamnoside in the dried extract is of 12%. The anti-inflammatory activity was evaluated by ELISA analysis for IL-6 and IL-8 in an in vitro model of human fibroblasts. The inflammatory stimuli were either UVB radiation or lipopolysaccharide. The results indicate the use of the presented extract as an anti-aging cosmetic ingredient. Keywords: inflammation, *Passiflora alata*, interleukins, IL-6, IL-8, aging, cosmetics References: 1. Giacomoni PU and Rein MA et al. (2008) Pharmaceutical Biology 46: 380 – 386. 4. Reyes-Chilpa R et al. (2002) Antimicrobial Agents and Chemotherapy 47: 1308 – 1314. 6. Palomino J C et al. (2002) Antimicrobial Agents and Chemotherapy 46: 106: 14711 – 14715. 2. Ito C (2002) J Nat Prod 65: 267 – 272. 3. Brenzan & Pharmacotherapy 62: 651 – 658. 4. Reyes-Chilpa R et al. (2002) Antimicrobial Agents and Chemotherapy 46: 2720 – 2722. 7. Papazis KI (1997) Immunol Methods 208: 151 – 158.

Despite the development of a number of effective treatments over the past half century, tuberculosis remains one of the most destructive bacterial infections in humans. The emergence of multidrug-resistant Mycobacterium tuberculosis leads to the research of new classes of antmycobacterial agents [1]. *Calliphora brasilienne* Cambess (Clusiaceae) is a tree popularly known as “guanandi” being a rich source of bioactive substances, including coumarins [2]. Previous studies of (-) mamea A/BB reported leshmanicidal (3) and anti-HIV (4) activities. This compound was identified by spectroscopic methods and comparison with literature data. A coumarin-type mammea was purified from a dichloromethane crude extract of *Calliphora brasilienne* leaves by chromatographic methods. In the current study, we evaluated the cytotoxicity and in vitro antymycobacterial activity of the (-) mamea A/BB. The compound was identified by spectroscopic methods and comparison with literature data. Antimicrobial activity was calculated against *Mycobacterium tuberculosis* H37Rv (ATCC 27294). The cytotoxicity assay was carried out by Sulforhodamine B colorimetric method [7]. The cytotoxicity for *M. tuberculosis* H37Rv macromolecules was compared using the selectivity index (SI). The coumarin (-) mamea A/BB showed significant activity against *M. tuberculosis* with MIC value of 31.2 μg/mL. The cytotoxicity against *M. tuberculosis* H37Rv macromolecules showed SI of 0.823. These results provide new perspectives on the development of novel drugs obtained from natural products with antmycobacterial activities. Keywords: *Calliphora brasilienne*, Mycobacterium tuberculosis, antmycobacterial Activity of (-) mamea A/BB from the Leaves of *Calliphora brasilienne* from *Mycobacterium tuberculosis*
One of the greatest challenges in endodontic treatment is the presence of bacteria as a biofilm, which confers stronger bacterial resistance to antimicrobial compounds [1]. In the present work, the in vitro antibiofilm activity of two natural pimarane-type diterpenes and one semi-synthetic derivative were investigated against nine bacteria responsible for dental root canal infections. The following anaerobic bacteria were evaluated in the present study: Porphyromonas gingivalis (clinical isolate), Prevotella nigrescens (ATCC), Prevotella intermedia (clinical isolate), Prevotella buccae (clinical isolate), Bacteroides fragilis (ATCC), Actinomyces naeslundii (ATCC), Peptostreptococcus micros (clinical isolate), and Aggregatibacter actinomycetemcomitans (ATCC). The diterpenes ent-pimar-8(14),15-dien-19-oic acid (1), its sodium salt (2), and ent-8(14),15-pimaradien-3-ol (3) (Figure 1) were used for determination of the minimum biofilm inhibition concentration (MBIC50) [2]. MBIC50 results varied between 6.25 and 25.0 µg/mL for the studied compounds. All the examined compounds displayed 50% or higher inhibition activity concerning biofilm formation. A maximum value of approximately 20-fold the MIC was attained for P. gingivalis (ATCC) [3] in the case of compound 1, and a minimum value of approximately onefold the MIC was achieved for most of the tested bacteria. The present results suggest that pimarane-type diterpenes are able to inhibit biofilm formation in vitro, and that their structure influence this anti-biofilm activity [3]. So this class of diterpenes should be considered in the search for new irrigating substances in the area of endodontic infections treatment. Studies of antibacterial activity linked to biofilm formation versus cytotoxicity of these compounds are being undertaken by our research group.

**Figure 1:** Chemical structure of diterpene type-pimarane

**Keywords:** Diterpene, Biofilm, Antibacterial activity, Anaerobe


#### Chemical analysis and biological activities of methanol extracts from Astragalus gombiformis Pomel (Fabaceae)

**Teytey H 1, Houta O 1, Lamari A 1, Neffati M 1, Douki W 1, Najjar M 1**

**1Biochemistry and Toxicology Laboratory, University Hospital of Monastir, Monastir 5000, Tunisia.**

**2Range Ecology Laboratory, Arid Land Institute, Mednine, Tunisia.**

**3Genetic Laboratory, Faculty of Medicine of Monastir, Monastir 5000, Tunisia.**

In the present study, wild Astragalus gombiformis Pomel extracts were tested for their biological activities and phenolic amounts. Antibacterial activity of this species against various bacteria was tested by the paper disk agar diffusion method and determination of the minimal inhibitor concentration. DPPH and ABTS assays were used to evaluate the antioxidant activity of methanol extracts. These extracts were also chemically investigated by spectrophotometric and HPLC analysis. For DPPH test, inhibitor concentrations 50% were 473.33 ± 64.29 and 626.66 ± 64.29 µg/mL, respectively, for aerial part and roots methanol extracts. Ascorbic acid, used as positive control, showed an inhibitor concentration 50% of 7.36 ± 0.70 µg/mL. ABTS test showed that roots and aerial part extracts contain respectively, 47.13 ± 0.05 and 79.81 ± 1.31 µmol of Trolox equivalents per g of dry plant material weight. Chemical investigation showed that total polyphenols and flavonoids were three folds higher in aerial part methanolic extracts. The antioxidant potential seems to be correlated to the phenolic content. Five of the tested extracts exhibited a diameter of inhibition zone equal or above 12 mm and with a minimal inhibitor concentration ranging between 233 and 1250 µg/mL. However, no insecticidal effect of aerial part extracts was shown against Culex pipiens. It appears that both roots and aerial part of A. gombiformis extracts possess antioxidant and antibacterial effects and should be more studied for identification of active compounds. Keywords: Astragalus gombiformis, Antibacterial, insecticidal, Antioxidant, phenolic amounts
extracts were tested, revealing five very active brown seaweeds: Cystoseira usnoidea (EC_{50} = 0.084 mg/mL), C. tamariscifolia (EC_{50} = 0.073 mg/mL), C. nodicaulis (EC_{50} = 0.041 mg/mL), Sphacelaria scoparium (EC_{50} = 0.265 mg/mL), and Fucus spiralis (EC_{50} = 0.110 mg/mL). After liquid-liquid partitioning, activity was found in the ethyl acetate fractions. These fractions were further analysed by HPLC-DAD, which showed compounds absorbing at 254, 280 and 350 nm. An attempt to identify these compounds by HPLC-NMR is currently in progress. Keywords: seaweeds, MAO-A inhibition, HPLC-NMR

Authors thank the Fundação Para a Ciência e a Tecnologia for the Post-Doc fellowship (SRH/BPD/62922/2009).

PM70

Diterpenes from Copaifera langsdorffii oleoresin against anaerobic oral pathogens

Veneziani RC¹, Souza AB², Martins CH², Heleno VC¹, Souza MG², Fartado NA³, Souta JP¹, Bastos JK¹, Cunha WR¹, Ambrósio SR¹
¹UNIFESP, Núcleo de Pesquisas em Ciências Exatas e Tecnológicas, Franca, Brazil; ²CFRPP-USP, Departamento de Ciências Farmacêuticas, Ribeirão Preto, Brazil

Anaerobic bacterial infections are the major cause of pulp and periodontal diseases [1] and Phorphyromonas gingivalis can be considered as the beginner of these pathological processes. Our research group has demonstrated that some diterpenes isolated from Copaifera langsdorffii Desf. oleoresin are able to inhibit the growth of various aerobic and anaerobic oral bacteria with very promising MIC values [2]. In view of these significant results against oral bacteria the investigation of the effect of these compounds on a panel of representative microorganisms responsible for root canal infections using the microdilution broth method [3] was done. The results indicate that (-)-copalic acid was the most active compound, displaying very promising MIC values against the main pathogens associated with these diseases (P. gingivalis). These results are supported by the other compounds also displayed some activity against the tested microorganisms. Keywords: Paphyromonas gingivalis, diterpenes, Copaifera langsdorffii Archer and Decker: Antimicrobial activity of Copaifera langsdorffii oleoresin against aerobic bacteria. Approved standard.

PM71

Observations with rapid micro-colony assay to screen antifungal activity of Origanum vulgare L., Zoster marina L. and Centaurea enisaefillis P.H. Davis extracts

Sevran M¹, Abaci Ö², Yaltos A¹, Boykan Erel S¹, Haliki Uzun R¹, Kanalci C¹, Hitcmedenofluo MG², Zeybek AU¹
¹Department of Pharmaceutical Botany, Faculty of Pharmacy, Ege University, Izmir, Turkey; ²Department of Biology and Basic Industrial Microbiology Section, Faculty of Sciences, Ege University, Izmir, Turkey

Currently, synthetic antifungal drugs are the main option to control fungal pathogens e.g. Candida albicans. C.P. Robin in humans. In turn, fungal pathogens generate resistance upon clinical treatments. Moreover, human pathogen Aspergillus fumigatus Fresenius isolates were suggested to generate resistance to azole antifungal drugs due to the examples of control plant fungal diseases in the field conditions. Thus, antifungal agents derived from natural products have vital importance for sustainable control of fungal infections. Plants accumulate plethora of antimicrobial compounds e.g. alkaloids, iridoids, flavonoids and lignans which could target the different sites e.g. cell wall formation and protein bio-synthesis in fungi. Alternative antifungal agent screening methods should be assayed for faster and sound detection. Previously, the micro-colony method i.e. the measurement of the early fungal development using microscopy was developed to screen dose response in the filamentous fungal species Fusarium efusum Winter. The micro-colony assay was tested to detect dose response in Candida albicans (ATCC 10231), A. fumigatus, Fusarium oxysporum Schlecht. emend. Snyder & Hansen on Origanum vulgare L. (Lamiaceae), Zoster marina L. (Zosteraceae) and Centaurea enisaefillis P.H. Davis (Asteraceae) extracts and reference fungicide flucanazole. The O. vulgare essential oil was toxic in all testing concentrations, but Z. marina methanol extracts were ineffective. C. enisaefillis methanol extract showed slight growth inhibition on C. albicans and A. fumigatus. This method provides a dose response in 24 hours. Additionally, method could be used to evaluate topical treat-

ments, pigmentation and filament morphology in fungi. Keywords: Antifungal drugs, natural products, Candida albicans, Aspergillus fumigatus, Fusarium oxysporum, dose response in fungi, rapid detection, Centaurea spp., Centaurea enisaefillis, Zoster marina L. Acknowledgement: Authors thank the bank Assoc. Prof. Dr. Sibel Konyalıoğlu for the utilization of the microscopy facility.

PM72

Can it be possible to use Caulerpa species for the treatment of some diseases?

Cengiz S¹, Cavus L², Yurdakok K³
¹Aksit University, Faculty of Science, Department of Chemistry, 07058, Ankara-Turkey; ²Dokuz Eylul University, Faculty of Science, Department of Chemistry, 35160, Izmir-Turkey

Many algae species have important secretions which are used for defensive purposes. These secretions generally have significant potential in terms of pharmaceutical industry. Among these secretions caulerpenyne (CYN) which is the main component of Caulerpa species is in an important position in medical investigations as a result of its determined properties such as cytotoxic, antiviral, antiinflammatory and apoptotic effects [2]. Fischel et al. [3] and Galgani et al. [4] focused on the used of CYN in many antifungal applications. In the present study, the inhibitory effects of CYN isolated from Caulerpa prolifera (Forsskål) J.V. Lamouroux (Caulerpaceae) on monounsaturated human acylglycerol lipase and lipoperoxidase were investigated. The results of the study presented that purified caulerpenyne inhibited soybean lipoperoxidase with an IC_{50} value of 5.14 mM. The results of the investigation conducted with monounsaturated lipoperoxidase lipase revealed that the inhibition of this enzyme were well in line with CYN concentration. The IC_{50} value of monounsaturated lipoperoxidase lipase inhibition with CYN was also determined as 98.4 μM. In conclusion, Caulerpa species can be a promising material for the treatment of monounsaturated lipoperoxidase lipase and lipoperoxidase-related diseases. Keywords: Caulerpa species, caulerpenyne, lipoperoxidase, monounsaturated lipoperoxidase, Inhibition Acknowledgement: We thank Prof. Dr. Georg Pohnert, Institute for Inorganic and Analytical Chemistry, Friedrich Schiller University of Jena for sharing his valuable knowledge about CYN purification with us. The authors are grateful to the Research Foundation of Dokuz Eylul University Project No: 2008. KF. FEN. 019 for financial support. Sevilay Cengiz thanks to The Scientific and Technological Research Council of Turkey (TÜBİTAK) for the scholarship. TÜBİTAK Project (109T512) is also acknowledged for financial support. References: 1. Barbier P et al. (2001) Life Sci 70: 415 – 429. 2. Cavas L et al. (2006) J Exp Mar Biol Ecol 339: 111 – 220. 3. NCCLS (2007) Methods for antimicrobial susceptibility testing of anaerobic bacteria, approved standard.


**PM74**

**Antibacterial capacity of Juglans sigillata green husks**

**Juglans sigillata** Dode., a fast growing deciduous tree species in the family Juglandaceae, is indigenous to mountain slopes and valleys of Tibet, Yunnan, Sichuan, and Guizhou provinces of southwest China [1, 2]. The green husk, cortex, kernel, nutshell, root and leaf of *J. sigillata* have a long history of being used in folk medicines to treat oxidative, inflammatory, rheumatic and nociceptive diseases, as well as to relief eczema, cancer, kidneys and stomach disorders. In the present study, antibacterial properties of *J. sigillata* green husk extracts were studied by hole-plate diffusion assay method described by Rios et al. [3] against Gram-negative bacteria, including *Salmonella enterica* and *Escherichia coli* and Gram-positive bacteria, such as *Bacillus subtilis* and *Staphylococcus aureus*. Aqueous EtOH (95% v/v) extracts from green husk were successively partitioned with a serious of polar solvents to get fractions soluble in n-hexane, CHCl₃, EtOAc, n-BuOH and H₂O. Results, expressed by diameter of inhibition zone, revealed that the 95% aqueous EtOH extract and all resulting soluble fractions from *J. sigillata* green husk revealed moderate or significant antibacterial effects against the four Gram bacteria, which indicated that *J. sigillata* green husk extracts have potential to destroy bacteria or suppress their growth or their ability to reproduce and could be used as excellent antibacterial agents. Keywords: antibacterial capacity, green husks, *Juglans sigillata*, Juglandaceae, hole-plate diffusion assay. Acknowledgement: This work was financially supported by Program for New Century Excellent Talents in University (NCET 2010). Foundation for the Development of Science and Technology Universities (NNSFC 20100016). National Natural Science Foundation of China (NSFC, No. 31000279) and Natural Science Foundation of Tianjin City (No. 09JCYBJC15800).

**PM76**

**Role of polyphenolic compounds on biological activity of collagenous materials**

Collagen, a unique connective tissue protein, is extensively used as biocompatible biomaterial in wound healing [1], cosmetics [2] and tissue engineering [3]. Due to its high sensitivity to enzymatic degradation, cross-linking of collagenous materials must be a compulsory step in their localization. Chemical cross-linking agents are able to form new covalent bonds in collagen structure, but are cytotoxic. The ability of natural plant polyphenolic compounds to stabilize collagen structure while preserving its cytocompatibility is now established [4]. This study aimed to investigate the interaction of small molecules from plants with collagen and their effect on its biological properties. Three mixtures of collagen with a polyphenolic extract of *Urtica dioica* L., a plant-derived flavonoid (quercetin) and a flavin from milk (riboflavin), respectively, were conditioned as porous materials by freeze-drying technique. A collagen-glutaraldehyde mixture was used as control. Free amino groups of the mixtures were spectrophotometrically assayed. An in vitro experimental model using bacterial collagenase was used to mimic the enzymatic attack on the collagenous materials implanted in vivo. The swelling capacity and in vitro cytocompatibility tested according to ISO 10993 - 5 on fibroblasts from NCTC cell line were evaluated. Results showed a good correlation between the free amino groups and the biodegradability of each mixture. The values of swelling capacity were at least 70-fold higher than the initial weight. Fibroblast viability and morphology showed a high cytocompatibility of the polyphenol-collagen mixtures. In conclusion, all these tests indicated an improved applicability of these mixtures for healing wounds: polyphenols, collagen, cross-linking, degradation, cytocompatibility, *Urtica dioica* Acknowledgement: This study was supported by Project PN II 62059. References: 1. Powell HM et al. (2006) Biomaterials 27: 5821 – 5827. 2. Helfrich YR et al. (2008) Dermatol Nurs 20: 177 – 183. 3. Graicunas G, Sienko S (2011) Cent Eur J Biol 6: doi: 10.2478/s11535-011-0012-1 4. Chuang TH et al. (2009) Tissue Eng Part A 15: 1809 – 1818. 5. Biagi M, Miraldi E, Figura N, Giachetti D (2009) Nat Prod Commun 4: 283-286. 6. Biagi M, Miraldi E, Figura N, Giachetti D (2009) Nat Prod Commun 4: 283-286.

References:

1. Altom JV et al. (1992) PM79
4. Centro de Estudos Farmacêuticos (CEF) and Centro de Neurociências e Biologia Celular (CNC), Universidade de Coimbra, Coimbra-Portugal; 5CEF, Universidade de Coimbra, Coimbra-Portugal and Departamento de Ambiente, Instituto Politécnico de Viseu, Viseu-Portugal; 6CEF, Universidade de Coimbra, Coimbra-Portugal; 7CNC, Universidade de Coimbra, Coimbra-Portugal and Faculdade de Farmácia, Universidade de Coimbra, Coimbra-Portugal; 8CEF, Universidade de Coimbra, Coimbra-Portugal and Faculdade de Farmácia, Universidade de Coimbra, Coimbra-Portugal

PM80

In East Asian countries such as Korea, China, and Japan, adzuki beans are consumed as ‘an’ or ‘anne’ (adzuki paste) or boiled and sweetened whole beans, and used in desserts, snacks, and confectionery items. Germination is a processing method by which the quality of this cereal can be improved for both digestibility and physiological function (2). The purpose of this study was to evaluate the antioxidant compounds and antioxidant activities of the methanolic extracts from adzuki bean (Vigna angularis (Wild.) Ohwi & Ohsawa) sprouts. To determine the antioxidant compounds in the methanolic extract and solvent fractions, the content of total polyphenol, flavonoid and tannin were measured by spectrophotometric methods. These were evaluated for antioxidative activities by DPPH and ABTS radical scavenging activities. The yield of methanolic extracts, hexane, chloroform, ethyl acetate, butanol, and water fractions of cockscomb were 25.20 – 32.35 and 2.70 – 7.32 mg/g, respectively. The total polyphenol, flavonoids, and anthocyanins contents on the ungerminated adzuki beans were 5.90 – 27.45, 8.17 – 27.23 and 14.69 – 16.13 mg/g sample, respectively. The DPPH and ABTS radical scavenging activities of the methanolic extracts on the cockscomb flowers were 5.24, 107.01 mg Trolox equivalent antioxidant capacity per g extract residue, respectively. The results of this study show that notable antioxidant activities in cockscomb flowers are considered to have significant health benefits. Keywords: Cockscomb (Celosia cristata L.), polyphenol, flavonoid, antioxidant activity References: 1. Wang Y et al. (2010) Fitoterapia 81: 1246. 2. Weng DB et al. (1995) Acta Nutrimenta Sinica 17: 59.

PM81

Inflammation is associated with several diseases and still exists an urgent need for the development of new and safer anti-inflammatory drugs. Since plant polyphenols are described to possess anti-inflammatory properties through modulation of pro-inflammatory signaling pathways, they could be used as source of a new anti-inflammatory drug. In a previous study using Cymbopogon citratus Stapf (Cy), an herb used in traditional medicine, we have demonstrated that polyphenolic compounds (PPs) from Cy have anti-inflammatory properties by inhibiting...
lipopolysaccharide (LPS)-triggered nitric oxide (NO) production and inducible nitric oxide synthase (iNOS) expression [1]. To further understand the underlying molecular mechanisms of PFs activity, the effect of Cy polyphenolic fractions was evaluated on the LPS-induced nuclear factor (NF)-κB pathway in the mouse macrophage cell line Raw 264.7. In Western blot assays, we observed that PFs inhibited the degradation and phosphorylation of inhibitory protein-xB (IκB). Next, the NF-xB transcriptional activity was assessed using cells transiently transfected with a NF-κB-dependent luciferase reporter plasmid. In addition to the interference with LPS-induced NF-xB activation observed in western blot, PFs inhibited the LPS-induced NF-xB transcriptional activity. In summary, these results demonstrate that PFs from Cymbopogon citratus inhibited the NF-xB pathway and therefore could be used as a natural anti-inflammatory agent. Keywords: Anti-inflammatory, Cymbopogon citratus, polyphenols, NF-κB Acknowledgement: Research supported by FCT PhD fellowship SFRH/BD/46281/2008, FCT project PDI/SAU-FCF/105429/2008 and FEDER/COMPETE (FCOMP-01 – 0124-FEDER-011096). References: 1. Francisco V et al. (2011) Ethnopharmacol 133: 818 – 827

PM82 Evaluation of anti-nitrosative activities of selected plant polyphenols

Awwad HM1, Mahmoud K2, Abd Alla HM1, El Toumy SA1
1Dept. Tanning Materials and Leather Technology, National Research Centre, 12622 Dokki, Cairo, Egypt; 2Dept. Pharmacognosy, National Research Centre, 12622 Dokki, Cairo, Egypt; 3Dept. Chemistry of Natural Compounds, National Research Centre, 12622 Dokki, Cairo, Egypt.

The involvement of free radicals as reactive oxygen (ROS) and reactive nitrogen species (RNS), specially their increased production, appears to be a common feature to most human diseases, including cardiovascular disease, neurodegeneration and cancer [1, 2]. The treatment with antioxidative substances and other strategies leading to the reduction of oxidative and nitrosative stress may represent a therapeutic intervention that could reduce the progression of the pathological process. As such, plant polyphenolics have been suggested to play particularly important role to fight against these diseases, by affording protection against free radical damage in cellular DNA, lipids and proteins [3 – 7]. Our goal was herein to investigate the scavenging capacity of some plant polyphenolic derivatives using different anti-nitrosative assays at different concentrations (from 0 to 300 μM). In addition, the anti-proliferative activity against different human tumor cell lines was estimated using the MTT and LDH assays. The ability of eight plant polyphenolic derivatives to react with the biologically relevant reactive nitrogen species, nitric oxide, peroxynitrite and nitrous acid were investigated indirectly by measurement of their ability to inhibit RNS-induced tyrosine nitration in vitro. All the investigated plant polyphenolic derivatives were found to be potent reactive nitrogen species scavengers and resulted in a significant inhibition of 3-nitrotyrosine (3-NT) formation in a dose-dependent manner. All the IC50 were being found at the μM level. These results indicate that these compounds may be utilized as promising sources of therapeutics. Keywords: reactive nitrogen species (RNS), nitric oxide, peroxynitrite and nitrous acid. Acknowledgement: This work was supported financially by the Science and Technology Development Fund (STDF), Egypt. Grant No 260. References: 1. Roberts RA et al. (2010) Toxicology 276: 85 – 94. 2. Lamas S et al. (1998) Trends in Pharmacological Sciences 19: 436 – 438. 3. Stephanie YH et al. (2006) Free Radical Biology & Medicine 40: 323 – 334. 4. Bolard SE et al. (2006) Biochemical and Biophysical Research Communications 350: 960 – 968. 5. Choi JS et al. (2002) Physotherapy Research16 (3): 232 – 235. 6. Oldreire C, Rice-Evance C (2001) Free Radical Research 35: 215 – 231. 7. Bartsch H et al. (1993) Basic Life Science 61: 27 – 44.

PM83 Adaptogens stimulate molecular chaperon Hsp70 expression in neuroglia cells

Amea A1, Kaar P1, Ponasson A1, Wikman G1
1Departments of Investigative Pathology, Scott & White Memorial Hospital and Clinic and The Texas AM Health Science Center College of Medicine, Temple, USA; 2Department of Research and Development, Swedish Herbal Institute, Askloster, Sweden

The seventy-kilo Dalton heat shock protein (Hsp70) plays an important role in the deterrence of protein damage during aging and their expression is required for longevity [1]. Recently, we demonstrated that ADAPT-232, a fixed combination of the extracts of three adaptogenic plants – Rhodiola rosea L., Schisandra chinensis K.Koch and Eleutherococcus cuniculosus Maxim., significantly increases the levels of circulating Hsp70 in the blood of rats [2]. Further, the long term treatment of aged rats with ADAPT-232 diminished or prevented a range of age-related disorders including malfunction of the central nervous system, loss of memory and loss of learning ability [3]. Similarly, ADAPT-232 improves cognitive function and mental performance in humans [4]. In this study, for the first time we demonstrate that ADAPT-232 stimulates the release of the heat shock factor protein (Hsp72) in isolated neuroglia cells via the upregulation of heat shock factor-1 (HSF-1). Taken together, our data suggests that the stimulation of HSP expression by adaptogens is associated with their anti-aging activity. Keywords: Adaptogens, Heat Shock Proteins, Neuroglia Cells, ADAPT-232, Hsp70 Acknowledgement: This work was supported in part by the Swedish Herbal Institute; Scott & White Memorial Hospital and Clinic; the Texas AM Health Science Center College of Medicine, the Central Texas Veterans Health Administration and an Endowment from the Cain Foundation. References: 1. Calderwood et al. (2009) Gerontology 55: 550 – 558. 2. Panossian et al. (2009) Phyto-medicine 16: 617 – 622. 3. Makarou et al. (2007) Abstract of International Congress Stress, Budapest, p. 242. 4. Aslanyan et al. (2010) Phyto-medicine 17: 499 – 494.

PM84 Antifungal activity of supercritical fluid extract obtained from Calophyllum brasiliense Cambess

Cymbalakis R1, Garcia VA2, Santos EM2, Filho LC3, Cabral VP3, Cortez DA4, Godoy JS5, Mendonça PS5, Svizdzinski TI
1Programa de Pós-graduação em Engenharia Química, Universidade Estadual de Maringá, Maringá, Paraná, Brazil; 2Programa de Pós-graduação em Agronomia, Universidade Estadual de Maringá, Maringá, Paraná, Brazil; 3Departamento de Engenharia Química, Universidade Estadual de Maringá, Maringá, Paraná, Brazil; 4Departamento de Farmácia Universidade Estadual de Maringá, Maringá, Paraná, Brazil; 5Departamento de Análises Clínicas e Biomedica, Universidade Estadual de Maringá, Maringá, Paraná, Brazil

Calophyllum brasiliense Cambess (Clusiaceae/Guttiferae) is a native Brazilian medicinal plant that is traditionally used in folk medicine for the treatment of several diseases, including infectious pathologies1. Leaves of C. brasiliense were extracted with supercritical fluid using CO2 as solvent (SF) at 40 and 60 °C and pressures of 105.2 and 244.1 bar. The extracts were tested against clinical isolates from patients' mouths, containing one of the following microorganisms: Candida tropicalis, Candida albicans or Candida glabrata, by determination of the minimal inhibitory concentration (MIC). The results indicated that both extracts exhibited antifungal activity against C. glabrata. The extract obtained by supercritical fluid at 60 °C and 244.1 bar showed better antifungal activity against C. glabrata, with MIC = 31.25 μg/ml for nine samples. This extract contained 30% of a mixture of mannose-α,B-type coumarins and the majority compound was identified as (-)-mammea A/BB by spectroscopy analyses. We conclude that SFE is an efficient method for obtaining bioactive compounds from plants, and that this method preserved the biological properties associated with antifungal activity. Keywords: Calophyllum brasiliense, coumarins, supercritical fluid, antifungal activity Acknowledgement: The authors are grateful to CNPq for providing a research grant and fellowships References: 1. Reyes-Chilpa R et al. (1997) Chem Ecol 23: 1901.

PM85 Phytochemical and biological characterization of Agrimonia eupatoria L.: an approach to structure-activity

Costa G1, Francisco V2, Liberal J1, Figuerinha A3, Cruz T1, Figueiredo T1, Lopes C1, Batista T2
1Faculdade de Farmácia, Universidade de Coimbra, Azeitão da Santa Comba, 3000 – 548 Coimbra, Portugal; 2Centro de Estudos Farmacêuticos, Universidade de Coimbra, Azeitão da Santa Comba, 3000 – 548 Coimbra, Portugal; 3Centro de Neurociências e Biologia Celular, Azeitão da Santa Comba 3004 – 517 Coimbra, Portugal; 4Departamento de Ambiente-UPV, Campus Politécnico de Repeses-3504 – 510 Viseu, Portugal

Plant polyphenols are well-known antioxidants, and recent studies have reported that they play an important role in prevention and treatment of
oxidative-related disorders, such as inflammatory diseases and cancer.
In this work, a phenol-rich fraction from Agrimonia eupatoria dry aerial parts (AePRF) was studied. Results revealed p-coumaric and ellagic acid derivatives, flavon and flavone glycosides, and monomers and oligomers of flavan-3-ols (proanthocyanidins). Some key features in molecular structure of flavonoids seem to be crucial to anti-inflammatory mechanisms: 4-oxo functional group and C2-C3 double bond at C-ring, 5- and 7-OH on A-ring and also OH functions on B-ring. Polymerization degree of proanthocyanidins plays a significant role in bioactivity, since dimers and higher oligomers are more effective than monomers, in inhibiting NO production. On the other hand, catechol moiety increases antioxidant activity, leading to presume that catechin-type proanthocyanidins are very active. Anti-inflammatory effect was evaluated in LPS-stimulated Raw 264.7 macrophage cell line by measuring the nitric oxide (NO) production through the Griess assay and AepRE cytopotoxicity was assessed by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay. Moreover, antioxidant capacity was determined by 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical. Total phenols and total flavonoids were also evaluated, and phenolic compounds were identified by high performance liquid chromatography, coupled to photodiode-array and electrospray ionization mass spectrometry detectors. Anti-inflammatory and antioxidant activities verified, as well as the phenolic profile established corroborate the traditional use of AePRF in inflammatory-related pathologies, since generous amount of the cited compounds were found in the phenol-rich fraction studied. Keywords: anti-inflammatory, antioxidant, phenolic, agrimonia, eupatoria.

Acknowledgement: This work was supported by FEDER/COMPETE (FCOMP–01–0124-FEDER-010196) and ICT, by the project PTD/SFU-FC/105492/2008 and the PhD fellowship SFRH/BD/46281/2008. A special thanks to LEMUC integrated in RNEM of Portugal for the HPLC/MS analyses.


PM86

Chilean medicinal plants as a source of norA efflux pump inhibitors against resistant Staphylococcus aureus strains

Holler JC1, Sloved HC2, Christenssen SB1, Mølgaard P1

1University of Copenhagen, Faculty of Pharmaceutical Sciences, Department of Medical Chemistry, Copenhagen, Denmark; 2Department of Microbiological Surveillance and Research, Statens Serum Institut, Copenhagen, Denmark

Staphylococcus aureus is a highly encountered pathogen in skin infections and multidrug resistance in this strain caused by native norA efflux pump inhibitors is a growing clinical problem. A path to solve this problem is the synergistic combination of an antibiotic with an efflux pump inhibitor (EPI) providing an effective drug. Ethnopharmacological knowledge on treatment of infected wounds may prove valuable in the search for anti-staphylococcal compounds. 24 plants traditionally used by the Huilliche people in southern Chile for wound healing therapy were used. Plant extracts were tested for norA efflux pump inhibitory activity in an assay based on fluorometric measurement of ethidium bromide transport by norA. Synergy studies were performed using the microdilution checkerboard method and MIC to rule out intrinsic activity. A total of 24 plant species were collected. Seven crude extracts was active (>50% inhibition) at 100 μg/ml, compared to reference drug reserpine at 20 μg/ml. None of the seven plants revealed antimicrobial activity in the concentration range tested. The two most potent efflux inhibitors were tested for dose-response activity and showed similar profile as reserpine, but had higher IC50 values of 11 and 14 μg/ml compared to 6 μg/ml of reserpine. Synergy studies of the two extracts G and M showed a 4-fold reduction in Moxifloxacin MIC at an extract concentration of 100 μg/ml. Extracts of Huilliche medicinal plants is likely to inhibit S. aureus norA facilitated EtBr efflux. Combination of extracts G and M with Moxifloxacin enhanced antibiotic action 4 fold. Acknowledgement: Glenn W. Kaatz of John D. Dingell VA Medical Center, Detroit for encouraging help on setting up the assay. Luca Guardabassi at LIFE-faculty University of Copenhagen for hosting our experiments.

PM87

Contribution of the components of STW 5 to its mode of action on inflamed rat small intestinal preparations

Hoser S1, Michael S2, Kelber O2, Weiser D3, Nieber K1

1University of Leipzig, Institute of Pharmacology; D-04013 Leipzig, Germany; 2Löwen-Apotheke-Waldheim; D-04736 Waldheim, Germany; 3Scientific Department; Steigerwald Arzneimittelwerk GmbH; D-64295 Darmstadt, Germany

STW 5 (Iberogast®) is successfully used in therapies of functional dyspepsia and irritable bowel syndrome. Given that clinical data suggest an inflammatory etiology of IBS, STW 5 and its components were examined on the production of the pro-inflammatory cytokine TNF-α and inflammation-induced cell death. The inflammation was induced by 2,4,6-trinitrobenzenesulfonic acid (TNBS, 10 μM, 30 min). The gene expression of TNF-α was determined in rat ileum preparation by realtime-RT-PCR. The release of TNF-α was measured in LPS-stimulated human monocytes using a commercially available ELISA. The cell death of THP-1 cells was determined using a commercially available LDH (lactate dehydrogenase)-assay. The TNBS-induced inflammation in ileal preparations was accompanied by increased TNF-α gene expression. STW 5 (500 μg/ml) inhibited the gene expression and reduced significantly the release of TNF-α by 87% in LPS (100 ng/ml)-stimulated human monocytes, while having no effect in untreated cells. In equivalent concentrations to STW 5, caraway, milk thistle, lemon balm and greater celandine had no effect on the LPS-induced increase in TNF-α release. Bitter candytuft, peppermint, chamomile, liqueur and anisica reduced the TNF-α release, though less pronounced as compared to STW 5. STW 5 (500 μg/ml) reduced TNBS (100 μg/ml)-induced cell death significantly by 51.2%. Apart from caraway all other components revealed a significantly decreased cell death in differentiated THP-1 cells after co-incubation with TNBS. Lemon balm had the strongest effect and caused a reduction to 2.64%. The results indicate that the herbal components contribute differently to the effect of STW 5. Keywords: Iberogast, Rat ileum, Inflammation, TNF alpha, THP-1 cells, LDH-test

PM88

Effects of STW 5 and STW 6 on rat ileal and colonic preparations: A comparative study

Voß U1, Michael S2, Kelber O2, Weiser D3, Nieber K1

1University of Leipzig, Institute of Pharmacy; D-04013 Leipzig, Germany; 2Löwen-Apotheke-Waldheim; D-04736 Waldheim, Germany; 3Scientific Department; Steigerwald Arzneimittelwerk GmbH; D-64295 Darmstadt, Germany

STW 5 (Iberogast®) is a fixed combination of nine plant extract with Iberis amara L. (STW 6) as its main component. It is successfully used for treatment of functional dyspepsia or irritable bowel syndrome (IBS). Because an inflammatory etiology of IBS the influence of STW 5 and STW 6 on tone and acetylcholine (ACh)-induced contractions of intact and inflamed intestinal preparations was examined. We used 1 – 1.5 cm long ileum and colon preparations of male Wistar rats to analyze region specific differences. Inflammation was induced by intraluminal instillation of 2,4,6-trinitrobenzenesulfonic acid (TNBS, 10 mM, 30 min). STW 5 (64 – 512 μg/ml) concentration dependently reduced the tone and decreased ACh-induced contractions of intact ileal and colonic preparations. STW 6 in equivalent concentrations (3 – 24.1 μg/ml) neither affected tone nor contractility. TNBS-induced inflammation was accompanied by a significantly reduced ACh contractions and morphological disturbances. Co-incubation of TNBS with STW 5 (512 μg/ml) or STW 6 (24.1 μg/ml) partially normalized the TNBS-induced attenuation of ACh-induced contractions and morphological damage in ileum preparations, whereas in inflamed colon segments only the co-incubation of TNBS with STW 6 in a high concentration (24.1 μg/ml) revealed similar effects. In conclusion, STW 5 influenced ACh contractions and tone in intact ileal and colonic preparations, whereas STW 6 does not contribute to these effects. In TNBS-inflamed ileum preparations STW 5 as well as STW 6 normalized morphological and contractile disturbances, while in colon preparations STW 6 but not STW 5 was effective. Our study confirms region specific effects of STW 5 and its main component STW 6. Keywords: Inflammation, Ileum, Colon, Gastrointestinal motility, Iberogast, Iberis amara
PM98

Agar-overlay assay; a useful and cost benefit method for detection of antibacterial peptides in plant seeds
Alisahmad A1, Rohghanian R2, Emtiazii C1, Chassempour A2
1Department of Biology, Faculty of Sciences, University of Isfahan, Isfahan, Iran; 2Department of Phytochemistry, Medicinal Plants and Drug Research Institute, Shahid Beheshti University, Tehran, Iran.

Multi-drug resistant bacteria are considered as a worldwide problem. Plant material have been an attractive candidate for overcoming human pathogens and amongst them plant defensins has been noticeably identified as a new class of promising antimicrobial substances. In this study, antimicrobial activity of some plant seeds components were assessed in two series of experiments. 8 different plant seeds were chosen according to data obtained from screening experiments which had been planned for accession of antimicrobial potential of different plant seeds methanol extract. Then an agar-overlay method, using fully separated proteins on SDS-PAGE gels, was used for initial determination of active putative proteins in total soluble proteins of seeds. 4 different gram positive and gram negative bacteria were subjected to the assay. For 2 of 8 selected plant seeds, there were clear and remarkable zones of inhibition in a region correspond to low molecular weight proteins in agar-overlay assays for all of tested gram positive bacteria but a smaller inhibition zone with several colonies for the gram negative bacterium. Clear and noticeable inhibitory zone in the case of our gram positive strains would be promising results and characterization of the effective peptides is now progressing in our laboratory. Even more important this approach may be used to establish different antibacterial activities of the peptides in a semi-quantitative manner which was not possible in routine screening test by using organic solvent extracted materials which are very complex matrix with unknown antagonistic or synergic effects on their components. Keywords: Plant defensins, Multi-drug resistant bacteria, Bioassay Acknowledgement: We acknowledge the support of Medicinal Plants and Drug Research Institute of Shahid Beheshti University. References: 1. Wisplinghoff H, Bischoff T, et al. (2004) Clin Infect Dis 39: 309 – 317. 2. Thevissen Ket et al. (2007) Drug discovery Today 12(21/22): 966 – 972. 3. Ko S-K, Ahn C-Bischoff T, et al. (2004) Clin Infect Dis 39: 309 – 317. 2. Thevissen Ket et al. (2007) Drug discovery Today 12(21/22): 966 – 972. 3. Ko S-K, Ahn C

PM99

Effect of methanolic extract of Harmal (Peganum harmala L.) on greenhouse whitefly Trialeurodes vaporariorum (Westwood) (Homoptera: Aleyrodidae)
Dehghani M1, Ahmadi K2, Zohadi H1, Asgharju M2
1Department of Plant Protection, Faculty of Agriculture, Shahid Bahoner University of Kerman, Kerman, Iran.; 2Department of Young Researchers Society, Shahid Bahoner University of Kerman, Kerman, Iran.; 3Department of Plant Protection, Faculty of Agriculture, Shahid Bahoner University of Kerman, Kerman, Iran.; 4Department of plant protection, Agricultural and Natural Resources Research Center of Kerman, Kerman, Iran.

The greenhouse whitefly, Trialeurodes vaporariorum is one of most serious pests of vegetables in the world [1]. The current insecticides mainly were caused resistance to insecticides and outbreaks of whiteflies [2]. The plant kingdom is by far the most efficient producer of chemical compounds, synthesizing many products that are used in defense against herbivores. Plants provide for pest control alternative to pesticides as a rich source of bioactive chemicals [3]. This experiment was studied to determine the effect of methanolic extract of harmal (Peganum harmala L.) on hatching rate and hatching time of the whitefly. The leaves of bean plants with eggs of the whitefly were placed in the round plastic Petri dishes (5 cm diameter) that filled with agar gel. The number of eggs on the leaf disc was counted and the eggs were treated with two concentrations of the harmal extract (40 & 80 mg/ml). In the concentration of 80 mg/ml, eggs were treated immediately after laying, while in 40 mg/ml, they were treated seven days after the expiration incubation period. In control treatments distilled water were applied. In concentration of 40 mg/ml, hatching rate and hatching time were 82.4% and 9.3 days, respectively. While the results of 80 mg/ml, hatching rate and hatching time in the other concentration (80 mg/ml) were 72.3% and 10.1 days, respectively. Hatching rate and hatching time in the both of concentrations were significantly different than control treatment. So, this extract could be affected on hatching rate and hatching time of this insect. Keywords: Trialeurodes vaporariorum, Peganum harmala, Hatching rate, hatching time References: 1. Lindquist R K (1972) J Econ Entomol 65: 1406 – 1408. 2. Andover D G (1990) Whiteflies; their bionomics, pest status and management. Intercept Ltd. UK. 3. Kim HG et al. (2005) Food Sci Biotechnol 14 (5): 685 – 688.

PM90

New pentacyclic diterpene polyesters isolated from Euphorbia falcata L. as resistance modulators in cancer cells
Martins A1, Sulyok E1, Vasas A1, Mohd r J1, Hofmann J1
1Institute of Pharmacognosy, Faculty of Pharmacy, University of Szeged, Szeged Hungary; 2Institute of Medical Microbiology and Immunobiology, Faculty of medicine, University of Szeged, Szeged Hungary

Resistance is a major cause of failure of chemotherapy and efflux is one of its major mechanisms that render the cancer cell resistant to more than one anti-cancer agent. The use of anti-cancer agents that restore the activity of already existing chemotherapeutics is a promising way to overcome resistance. Four new prenymsrianine-type (5 – 7) diterpene polyesters isolated from E. falcata L. were tested for their activity as efflux modulators. The compounds were identified as: (1) tetra- (2), penta- (3) and heptaesters (4), derivatives of a polyfunctional diterpene acylate, acylated with acetic, propanoic, isobutanoic, 2-methyl-butyric, n-hexanoic and benzoic acids. Compounds 2, 3, 4, 6 and 7 increased the retention of rhodamine123 in L5178 mouse T-cell lymphoma cells transfected with pHa

PS92

Potent anti-inflammatory compounds identified in Zingiber officinale Roscoe var. rubrum Thellandia: Mechanisms of action in psoriasis
Nordin NF, D’acquisto F, Gibbons S, Perret D
1Bone & Joint Research Unit, Barts and the London School of Medicine and Dentistry, London, United Kingdom; 2Biochemical Pharmacology, Barts and the London School of Medicine and Dentistry, London, United Kingdom; 3Translational Medicine and Therapeutics, Barts and the London School of Medicine and Dentistry, London, United Kingdom; 4Department of Pharmaceutical and Biological Chemistry, The School of Pharmacy, University of London, London, United Kingdom

Psoriasis is an autoimmune inflammatory skin disease associated with aberrant activation of T and B-lymphocytes. Increasing evidence indicates that T-helper 1 (Th 1) and Th17 lymphocyte subsets play key roles in the immunopathogenesis of the disease. In such a setting, activated Th 1/Th17 cells interact with keratinocytes leading to their proliferation and hyperplasia. Our studies are focused on developing new approaches for targeted therapy for psoriasis. Recent studies from our laboratories have identified therapeutic effects for compounds extracted from the ginger species Zingiber officinale Roscoe var. rubrum Thellandia, on pathogenic mechanisms in psoriasis. Initially, the therapeutic effects of chloroform extract (HB02) and selected fractions were assessed for their ability to suppress the production of pro-inflammatory mediators produced by macrophage. Four fractions, F5, F6, F7 and F10 with dual sup-
pressive effects on NO and PGF2α production were identified. The fractions had higher potency than L-NAME, a specific inhibitor of iNOS, and exhibited comparable effects to indomethacin in inhibiting of PGF2α. F6 had particularly potent inhibitory effects on inhibiting NO (IC50 = 6.7 ± 2.7 μg/ml) and suppressing iNOS gene transcription by 82.3 ± 3.73% at 20 μg/ml. Two compounds, 6-shogaol and a ferulate derivative were isolated from F6. Interestingly, the 2 compounds had additive effects in down-regulating iNOS and IL23 gene transcription. These compounds may therefore be key components responsible for the anti-inflammatory effects of HB02. Current experiments are focused on mechanisms of action and therapeutic efficacy of these compounds on suppressing psoriasis involving chemokine and cytokine production and keratinocytes proliferation using an in vitro human psoriatic skin model. Keywords: Psoriasis, Zinger officinalis Roscoe var. rubrum Theilae, 6-shogaol, ferulate derivative Acknowledgement: 1. Standards and Industrial Research Institute of Malaysia (SIRIM Berhad), Malaysia 2. Ministry of Science, Technology & Innovation (MOSTI), Malaysia

Effect of some medical plants on nymphal development and mortality of Brevicoryne brassicae (L.) (Homoptera: Aphiidae)

Nazarian A1, Almadni K2, Banadiami Y3
1Department of Plant Protection, Faculty of Agriculture, Shahid Bahonar University of Kerman, Kerman, Iran; 2Department of Plant Protection, Faculty of Agriculture, Shahid Bahonar University of Kerman, Kerman, Iran

The misuse and excessive use of synthetic insecticides may cause some undesirable effects not only to the agricultural ecosystem but also to human health due to insecticide residue in food. Therefore, several efforts have been created to reduce the use of synthetic pesticides particularly the use of synthetic insecticides. One of the efforts is the development of botanical insecticides as a novel and safer alternative strategy. Botanical insecticides, which contain plant extracts as active components, are safer as well as environmentally friendlier than synthetic insecticides [1]. Botanical pesticides are considered biodegradable [2] and their use in crop protection is a practically sustainable alternative. Ethanolic solution of three plant species, clove flower buds (Syzygium aromaticum L.), harnal seeds (Peganum harmala L.), Persian lilac fruits (Melia azedarach L.) were tested for their activities on duration of development and mortality of cabbage aphid [Brevicoryne brassicae (L.)] in the laboratory. The first instars (one day old) were sprayed with ethanolic solution (30 mg/ml) of different plant extracts, and nymphal duration as well as mortality were estimated. In the control treatment, the insects were sprayed with ethanol (95%). In harnal treatment, the duration of nymphal development was considerably longer than the control with a mean of 8.0 days. Total mortality (%) during the development of the aphid from N1 to adult emergence were 50.0%, 36.6%, 30.0% and 21.4% in clove, harnal, Persian lilac and control treatment, respectively. Keywords: Brevicoryne brassicae, plant extract, nymphal development, mortality

An approach to studying the mechanism of action of STW 5 in functional dyspepsia using the restraint stress model in rats

Khoyyal MT1, Abdel Aziz H2, Wadie W2, Zaki HH2, Kelber O2, Weiser D3
1Department of Pharmacology, Faculty of Pharmacy, Cairo University, Kasr-El-Aini Street, 11562 Cairo, Egypt; 2Departments of Pharmacology, Faculty of Pharmacy, Helios University, Egypt and Institute of Pharmaceutical Chemistry, Hittorfstr. 58, 48149 Münster, Germany; 3Scientific Department, Steigerwald Arzneimittelwerk GmbH, Havelerstr.5, 64295 Darmstadt, Germany

While the clinical efficacy of medicinal plants as therapeutic options in treating functional gastrointestinal diseases is widely accepted, the understanding of their mechanisms of action still remains uncertain. Two models for stress-induced functional dyspepsia were performed in order to choose the more adequate one for testing sensitivity changes of the fundus to various mediators. In one model, maternal separation (1) was performed on weanling rats starting from postnatal day 2 for 3 h each day for 3 weeks. Rats were then allowed to mature to an adult age. The other model was that of restraint stress (2,3). Adult animals were restrained for 90 min/day for 1 week. The animals were eventually sacrificed, the stomach fundus was isolated and its sensitivity in vitro to carbachol, potassium chloride, serotonin and adrenaline was tested. The sensitivity of the fundus strips from restrained rats towards these agents was more depressed than those from maternally separated ones. That model was therefore chosen to test the efficacy of STW 5 in restoring sensitivity to the agents mentioned. A group of animals received STW 5 orally once daily for 2 weeks before subjecting them to restraint stress. Treatment with STW 5 was effective in normalizing the depressed responses exhibited by animals subjected to restraint stress. Samples of blood were taken to assess levels of CRP and ghrelin. The findings throw further light on the mechanisms underlying the therapeutic usefulness of STW 5 in functional dyspepsia, especially when triggered by psychological stress. Keywords: Functional dyspepsia, STW 5, Stomach fundus, restraint-stress References: 1. Cheung CK et al. (2010) Gastroenterology 138: 576-582. 2. Zhang H et al. (2008) Phytomedicine 15: 602 – 611. 3. Zheng J et al. (2009) Am J Physiol Regul Integr Comp Physiol 296: R1358- R1365.

Reproduction and longevity of the cabbage aphid (Brevicoryne brassicae (L.)) after exposure to ethanolic extract of clove (Syzygium aromaticum L.)

Banadiami Y1, Almadni K2, Takalloozadeh H3
1Department of Plant Protection, Faculty of Agriculture, Shahid Bahonar University of Kerman, Kerman, Iran; 2Department of Plant Protection, Faculty of Agriculture, Shahid Bahonar University of Kerman, Kerman, Iran

Botanical pesticides are an important group of naturally occurring, often slow-acting crop protection that are usually safer to humans and the environment than conventional pesticides, and with minimal residual effects. Therefore the use of plant pesticides has been recommended ever more as a suitable alternative of plant protection with minimum negative risks [1, 2]. Especially botanical insecticides have long been a subject of research in an effort to develop alternatives to conventional insecticides. Therefore, this research was conducted to assess the effectiveness of ethanolic solution (30 mg/ml) of clove flower buds extract (Syzygium aromaticum L.) on reproduction and longevity of the cabbage aphid [Brevicoryne brassicae (L.)] in the laboratory. In this experiment, 50 newly 1st nymphal instars of the cabbage aphid were placed together into the round plastic Petri dishes on rape leaves and sprayed with the ethanolic solution. In control treatments only ethanol (95%) were applied. After feeding, the nymphs were transferred into the new Petri dishes with fresh leaves and reared until the adulthood. Afterwards, the adults were confined singly in other Petri dishes and reared until death. During the longevity experiments, reproduction of the adults was estimated during one week. The ethanolic plant extract caused a significant reduction of longevity of the cabbage aphid (8.7 days) when compared with the control treatments. Moreover, the plant extract had a significant deleterious effect on the mean total number of laid nymphs during the seven days with the mean of 5.8 nymphs. Keywords: Brevicoryne brassicae, plant extract, Syzygium aromaticum, Reproduction, longevity References: 1. Isman MB (2006) Ann Rev Entomol 51: 45 – 66. 2. Pavela R (2007) Pest Technol 1: 47 – 52.

Toxic effect of three medicinal plant extracts on Myzus persicae (Sulzer) (Hem.: Aphididae)

Salarie E1, Almadni K2, Zamani Dehyaghoobi R1, Najmizadeh H1, Takalloozadeh H2
1Department of Plant Protection, Faculty of Agriculture, Shahid Bahonar University of Kerman, Kerman, Iran; 2Department of Plant Protection, Faculty of Agriculture, Shahid Bahonar University of Kerman, Kerman, Iran

The peach potato aphid Myzus persicae (Sulzer) (Hem.: Aphididae) is one of the most noxious species [1]. It can infest plants of over 40 different families including many economically important ones world wide, and it is able to transmit over 100 plant viruses [2]. Therefore, in the present study the efficacy of aceton leaf extracts from three medicinal plants
were evaluated against 3–4-day-old individuals of the *M. persicae*. The plants were included *Eucalyptus globulus* Labill. (*Myrtaceae*), *Teucrium polium* L. (*Lamiaceae*) and *Ototogea persica* Boiss. (*Labiatae*). In order to obtain the crude extracts, the dried leaves were powdered and extracted with acetone. Experiments were carried out at 25 ± 1 °C temperature, relative humidity of 60 ± 10% and 16 hours of artificial light at an intensity of about 4000 lux. In control treatments only distilled water and DMSO (dimethyl sulfoxide) were applied. Topical treated aphids with three acetonic extract emulsion (in distilled water with DMSO) were placed on the broad bean leaf discs (4.5 cm diameter) in the round plastic Petri dishes (5.5 cm diameter), filled with a 0.5 cm-thick agar gel layer. The highest percentage of mortality (55.6%) was observed in the acetonic leaf extract of *O. persica* in the concentration of 80 μg/ml after 48 hours. While, it was less than 10% in the acetonic leaf extract of *E. globulus*. The acetonic leaf extract of *P. tremula* caused 14.5% mortality of *M. persicae*. It is concluded that *O. persica* is the most promising for future development and use as botanical pesticide. Keywords: *Myzus persicae*, medicinal plant, Toxic effect, Topical test References: 1. Blackman RL, Eastop VF (1984) Aphids on the World’s Crops. John Wiley and Sons. New York. 2. Clements KM et al. (2000) RevToxicol 3: 1 – 23.

### PM097

**Evaluation of antioxidant and antimicrobial activities of Ziziphus vulgaris (Rhamnaceae)**

**Ammoaghaie R**

**Biology Department, Shahrekord University, Shahrekord, Iran**

Ziziphus vulgaris Lam. is a deciduous shrub, native of the Mediterranean region and used to treat sore throats, alleviate stress and helps in the common colds. In present work 10 g of the dried plant material was soaked in 100 ml methanol and shaken for 24 h and clear filtrate was obtained. The fresh methanolic crude extracts were qualitatively screened for secondary metabolites. Results showed that: flavonoids, hydrolysable tannins, alkaloids, terpenes and saponins had reasonably high contents but anthraquinones and coumarines were low. In vitro antimicrobial assay and MIC determination growth inhibition activities of methanolic leaves extract of *Z. vulgaris* against gram-positive and gram-negative bacterial species using the conventional paper disc assay showed good inhibitory effects only against gram-positive with no antagonistic effects against gram-negative bacterial species tested. The MIC values of the crude methanolic *Z. vulgaris* extract on gram-positive was in range to 12.5 – 25.0 μg/ml, whereas extract exhibited very weak anti-microbial activity against gram-negative with very high values (1000 μg/ml) of MICs. Total phenolics assayed by the Folin-Ciocalteau method in plant extract also showed reasonably high contents of polyphenolics (300 mg/g extract). Results collectively suggest that *Z. vulgaris* is not only reliable natural sources of antimicrobials but also potential sources of phenolic compounds with antimicrobial activities. Further studies are necessary for intensive studies. Keywords: Ziziphus vulgaris, antimicrobials, phenolic antioxidants

### PM98

**Antioxidant activity and phenolic content of different extracts of Gentiana cruciata L.**

**Mihalovic V, Niciforovic N, Mladenovic M, Soljuic S, Stankovic M**

**Faculty of Science, University of Krugujevac, Radoja Donovnicova 12, 34000 Krugujevac, Serbia**

**Gentiana cruciata** L. is a perennial plant belonging to the genus Gentiana (fam. Gentianaceae) [1]. Gentiana species are widely used throughout the world as potential stomachic and hepatoprotective agents [2]. *G. cruciata* is used in the traditional medicine for loss of appetite, as a stomachic as well as component in preparations showing beneficial effects in gall and liver diseases [3]. The aim of this study was to evaluate the antioxidant and radical-scavenging activities of methanolic extract, chloroform, ethyl acetate and n-butanol fractions obtained from the methanolic extract of aerial part of *G. cruciata*. The total amount of phenols was calculated as the rutin equivalent (RU). The extracts were investigated for antioxidant capacity using two different assays: DPPH assay and inhibitory activity toward lipid peroxidation. The highest content of total phenols (109.8 mg GA/g) and flavonoids (110.9 mg RU/g) was determined in the n-butanol fraction. The most effective DPPH radical scavenger was n-butanol fraction (IC50= 114.7 μg/ml), while the methanol extract showed the highest inhibitory activity toward lipid peroxidation (IC50= 69.9 μg/ml). The results show a significant antioxidant activity of the investigated extracts compared to reference antioxidant compounds, such as butylated hydroxytoluene (BHT), ascorbic acid (AA), gallic acid (GA) and α-tocopherol. Keywords: Gentiana cruciata, antioxidant activity, phenolic content Acknowledgement: This work was supported by the Ministry of Science and Technological Development of the Republic of Serbia (project No. III 43004). References: 1. Struwe L, Albert V (2002) Gentianaceae-systematics and natural history, Cambridge University Press, Cambridge 2. Jiang R et al. (2005) Phytochemistry 66: 2674 – 2680. 3. Menkovski N et al. (2011) J Ethnopharmacol 133: 97 – 107.

### PM099

**Melissa officinalis**: an important dietary source of phenolic compounds with high antioxidant capacity

**Ammoaghaie R**

**Biology Department, Shahrekord University, Shahrekord, Iran**

Balm, Melissa officinalis L. a perennial herb native to southern climates of Europe and North America, and cultivated in Mediterranean and central Asian areas [1]. Oil of balm has been shown to have antiviral, antioxidant and antiapoptotic activity [2,3]. In this research, total phenolic content and related total antioxidant capacity of plant infusions was analyzed. Infusions were prepared in common way in which teas are prepared for human consumption. The total phenolics were measured by Folin-Ciocalteau assay. The total antioxidant capacity was estimated by Ferric Reducing/Antioxidant Power (FRAP) assay. Also, the phenol antioxidant coefficient (PAC) was calculated for plant infusion. The obtained results for Melissa infusions showed: high phenolic concentration, very high FRAP (> 20 mM/L) and PAC > 3. The fect of infusion time and temperature on the phenolic content, FRAP, and free radical scavenging ability was tested. Preparation of Balm infusions with hot (98 °C) and cold (20 °C) revealed that although antioxidants were liberated from leaves into the water at both of the temperatures studied, infusions prepared at higher temperature had more than 2-fold higher antioxidant capacity determined as FRAP. DPPH radical scavenging ability of Balm phenolics was similar to (+)-catechin but not as good as for quercetin. Compared to Trolox and vitamin C, Melissa phenolics were more efficient free ABTS radical scavengers. The results indicate that Melissa officinalis infusions could be an important dietary source of phenolic compounds with high antioxidant capacity comparable with red wines or beverages like tea. Keywords: Phenolic compound, antioxidant capacity; Infusions; Melissa officinalis; FRAP; DPPH; ABTS References: 1- Kennedy D, Little OW, Haskell CF, Scholey AB (2006) Phytother Res 20: 96 – 102 2-Weiman Z, Golhar A, Ziv I, Eshel A, Barak Y, Avidan M, Pedirajt 122:650 – 652 3- Wake G, Court J, Pictering A, Lewis R, Wilkins R, Perrey E (1999) J Ethnopharmacol 69: 105 – 114

### PM100

**In vitro and In vivo Antitumor Effects of Deoxylephonanthopin on Human Breast Cancer Cells**


**Agricultural Biotechnology Research Center, Academia Sinica, Taipei 115, Taiwan (R.O.C.)**

Breast cancer is one of the most common cancers in women, and a leading cause of death worldwide. It is often highly resistant to chemotheraphy, and there is no effective cure for patients in the advanced stages of the disease. In this study, we evaluated the effect of deoxylephonanthopin (DET), a phyto compound obtained from *Elephantopus scaber* L. (*Asteraceae*) for possible anti-tumor activities in the human breast cancer cell-line MDA-MB-231. Cell-apoptosis assay showed that DET treatment was able to effectively suppress the growth of test tumor cells in vitro. In addition, DET treatment significantly decreased expression level of transforming growth factor-beta (TGF-β), effectively inhibited cell growth by inducing G2-M phase cell cycle arrest and apoptosis, and reduced the clonogenicity in a concentration-dependent manner in MDA-MB-231 cells. DET also significantly inhibited the invasion and migration of test breast tumor cells. The effect of DET on suppression of NF-κB, via activation by TNF-α, was examined using electropherometric mobility shift analysis (EMSA). Decreased levels of expression of phosho-NF-κB and the downstream molecules of the NF-κB signaling path-
way, including survivin, Bcl-2, MMP-9 and VEGF, were observed in DET-treated MDA-MB-231 cells. In vivo, DET significantly inhibited tumor growth and the myeloid derived suppressor cell (MDSC) population in nude mice. Taken together, our findings suggest that DET may warrant a further systematic investigation for potential applications in the chemoprevention or control of breast cancers. Keywords: Deoxyelephantopin (DET), Elephantopus scaber, Asteraceae, anticancer

The anticancer potential of Artemisia afra
Spies L., Koekemoer TC, Sovemimo AK, Van De Venter M*
1Department of Biochemistry and Microbiology, Faculty of Science, Nelson Mandela Metropolitan University, Port Elizabeth, South Africa; 2Department of Pharmacognosy, Faculty of Pharmacy, College of Medicine, University of Lagos, Lagos, Nigeria

Artemisia afra Jacq. is one of the oldest, most well known and widely used traditional medicinal plants in South Africa. It is used to treat many different medical conditions, particularly respiratory and inflammatory ailments (Liu et al., 2009). There is no reported evidence of its use for the treatment of cancer but due to its reported cytotoxicity (Fouche et al., 2008; Mativandlela et al., 2008), we investigated the effect of A. afra extracts on 2 cancer cell lines. IC50 values of 18.21 μg/mL and 31.8 μg/mL of ethanol extracts were determined against U937 and HeLa cancer cells, respectively. An IC50 value of the aqueous extract was greater than 250 μg/mL. Dose response assays were also performed using confluent HeLa cells, yielding an IC50 value greater than 250 μg/mL. The effect of the cytotoxic ethanolic A. afra extract (20 μg/mL) on U937 and HeLa cells, progression through the cell cycle, apoptosis and mitochondrial membrane potential was investigated. Melphalan was used as a positive control. After 24 hours of treatment with melphalan using U937 cancer cells, an increase in sub G1 phase was evident. Treatment of cells with A. afra showed a delay in G2/M phase of the cell cycle. Apoptosis was confirmed using the TUNEL assay for DNA fragmentation, which was evident with the positive control and A. afra treatment at 24 and 48 hours. JC-1 staining showed a decrease in mitochondrial membrane potential at 24 hours. The results obtained suggest that A. afra potentially has medicinal anticancer properties. Keywords: Artemisia afra, apoptosis, cytotoxicity References: 1. Liu et al. (2009) S Afr J Bot 75: 185 – 195. 2. Fouche et al. (2008) J Ethnopharmacol 119: 455 – 461. 3. Mativandlela et al. (2008) Phytother Res 22: 841 – 845.

In vitro inhibition of 5-lipoxygenase by natural quinone compounds
Landa P*, Kutli Z*, Malik F, Kokoska L*, Widowitz U*
1Laboratory of Plant Biotechnology, Joint Laboratory of Institute of Experimental Botany AS CR, v.v.i. and Research Institute of Crop Production, v.v.i., Rozvozova 263, 165 02 Prague 6 – Lysolaje, Czech Republic; 2Department of Crop Sciences and Agroforestry, Institute of Tropics and Subtropics, Czech University of Life Sciences Prague, Kamýcka 129, 165 21 Prague 6 – Suchdol, Czech Republic; 3Department of Zoology and Fisheries, The Faculty of Agrobiology, Food and Natural Resources, Czech University of Life Sciences Prague, Kamýcka 129, 165 21 Prague 6 – Suchdol, Czech Republic; 4Department of Pharmaceutical Sciences, Karl-Franzens University Graz, Universitätsplatz 4/1, 8010 Graz, Austria

Dual inhibition of cyclooxygenase (COX) and lipoxygenase (LOX) pathways is promising approach in treatment of inflammatory diseases. Drugs able to block production of prostanoids together with leukotrienes should provide better anti-inflammatory properties and fewer side effects than non-steroidal anti-inflammatory drugs (NSAIDs) and selective COX-2 inhibitors [1,2]. Our previous studies revealed that some natural quinone compounds such as primin, alkannin, or shikonin are potent in vitro COX inhibitors [3]. In the current study, we tested 19 quinone compounds for 5-lipoxygenase (5-LOX) inhibition using in vitro assay according to [4] where neutrophile granulocytes with 5-LOX activity isolated from the human blood are incubated with arachidonic acid (substrate) and tested samples. After the incubation, the amount of leukotriene B4 (LTB4) is determined by commercial LTB4 EIA kit (Assay Designs). The most active quinone 5-LOX inhibitors were benzocoumarine primin and naphthoquinone shikonin which decreased LTB4 production by 93 and 87% at 50μM concentration, respectively. Reference inhibitor zileuton reduced LTB4 production by 91% at the same concentration. Based on these preliminary results obtained in 5-LOX assay together with data from previous studies targeted on COX inhibition the plant quinones such as primin and shikonin should be considered for further studies aimed on their potential of dual COX/LOX inhibition. Keywords: natural quinones, 5-lipoxygenase, enzyme inhibition, leucotriene B4, arachidonic acid pathway Acknowledgement: This study was supported by Czech Science Foundation project 525/09/P528. References: 1. Celotti F et al. (2001) Pharmacol Res 43: 420 – 436. 2. Leoni S et al. (2007) Curr Top Med Chem 7: 265 – 275. 3. Landa P (2009) Planta Med 75: 1059. 4. Adams M et al. (2004) Planta Med 70: 904 – 908.

Comparative assessment of antioxidant profile of Daucus carota L. ssp. sativus Hoffm. var. atrorubens Alfel. and a fermented local beverage, “Salgam”
Celep E1, Aydın A2, Kırımlıbekmez H1, Yeşilda E1
1Yeditepe University, Faculty of Pharmacy, Department of Pharmacognosy, 34755 Kayisdagı, Istanbul/Turkey; 2Yeditepe University, Faculty of Pharmacy, Department of Pharmaceutical Toxicology, 34755 Kayisdagı, Istanbul/Turkey

Salgam is a well-known traditional local beverage which is prepared by the lactic acid fermentation of black carrot [1]. It has been widely consumed in Turkish daily meal. This study aims to compare the antioxidant potentials of Salgam and its main ingredient, black carrot. Both salgam and freeze-dried black carrot juice showed a good activity at 125 mg/L concentration with values 91.76 ± 1.24 and 100.8 ± 1.72 mg AA/g dry extract, respectively. Ferric-reducing antioxidant power assay gave a result of 1012 ± 13.27 μM FeSO4/g dry extract for black carrot and 964 ± 7.21 μM FeSO4/g dry extract for salgam. These results indicate that although antioxidant potentials are close to each other, black carrot juice shows higher in vitro antioxidant activity when compared to that of salgam. Further in vivo studies are in progress. References: [1] Enern et al. (2008) Food Reviews International 24:352 – 359.

Bioactivity of in vitro glycoalkaloids from Solanum nigrum
Al Ashaal HA
National Research Centre, Dokki 12311, Giza, Egypt

Glycoalkaloids were produced from callus and regenerated plants of Solanum nigrum L. (Solanaceae) using different concentrations of auxins and cytokinins. The glycoalkaloids were separated by acid base precipitation, and determined by high performance liquid chromatography. The production of glycoalkaloids found to have antiparasitic activity against the selected carcinoma cell lines including liver, breast and lymphoplastic leukemia cell lines. Examination of the antiviral activity showed that glycoalkaloids had virucidal effect against the tested virus strains. In addition, callus glycoalkaloids were found to have antischistosomiasis and antifasciolas activities. Keywords: in vitro glycoalkaloids, Solanum nigrum, cytotoxicity, antiviral, antiparasitic

Determination of in vitro antioxidant potential of Cornus mas L. and its polyphenol content
Celep E1, Aydın A2, Kırımlıbekmez H1, Yeşilda E1
1Yeditepe University, Faculty of Pharmacy, Department of Pharmacognosy 34755 Kayisdagı Istanbul/Turkey; 2Yeditepe University, Faculty of Pharmacy, Department of Pharmaceutical Toxicology 34755 Kayisdagı Istanbul/Turkey

Cornus mas L. (Cornaceae) is one of the two species of the genus Cornus L. represented in the Turkish flora [1]. Its leaves have been widely used in Anatolian folk medicine against diarrhea and diabetes [2]. This study was designed to investigate the antioxidant potential of the 80% methanolic extract prepared from the leaves. The antioxidant properties were examined by using different in vitro systems. Ascorbic acid and BHT were used as reference substances. In the DPPH test IC50 value was
PM06
Mechanism of action of Fragraia vesca leaf extract on LPS treated macrophages

Liberal J1, Francisco V1, Amaral MT2, Marques C3, Lopes M1, Cruz MT1, Batista MT4
1Center for Pharmaceutical Studies, Faculty of Pharmacy, University of Coimbra, Coimbra, Portugal; 2Center for Neuroscience and Cell Biology, University of Coimbra, Coimbra, Portugal; 3Center for Pharmaceutical Studies, Faculty of Pharmacy, University of Coimbra, Coimbra, Portugal; 4Center of Ophthalmology and Vision Sciences, Institute of Biomedical Research in Light and Image, Faculty of Medicine, University of Coimbra, Coimbra, Portugal;

Fragaria vesca L., commonly known as Strawberry, has been used over the years by traditional medicine for the treatment of several diseases. However, scientific reports of its molecular action mechanism are lacking. Thus, this work aims to investigate the anti-inflammatory effects, of a Fragraia vesca leaf extract obtained by successive extractions with ethanol and 50% aqueous ethanol, on the macrophage cell line, Raw 264.7, stimulated with lipopolysaccharide (LPS). For this purpose nitric oxide (NO) production, scavenging activity and cytotoxicity of the extract were assessed. Furthermore, was evaluated the expression of proteins that are potential targets to prevent or treat chronic inflammation, namely iNOS, COX-2, phospho-IkB-α and Jnk-α. The results demonstrated that Fragraia vesca leaves extract was not cytotoxic and inhibit the production of NO triggered by LPS. Using (S)-Nitros-N-acetylpenicillamine as NO donor, the extract promoted a significant decrease of NO in the culture medium. Western Blot analysis showed that LPS triggered a significant increase on iNOS and COX-2, respectively, while phosphorylated IkB-α, strongly increased. However, an increase on the phosphorylation of Jnk-α occurred in cells co-treated with the plant extract and LPS, suggesting a potential reduction of proteasome degradation, since phospho-Jnk-α is a target for the ubiquitin-proteasome pathway. In conclusion, our data show that Fragraia vesca decreased the level of nitrites, mainly through direct NO scavenging activity of the extract. Keywords: Fragraia vesca, strawberry, anti-inflammatory properties, scavenger activity, proteasome inhibition


PM07
In vitro antiprotozoal activity of organic and aqueous extracts of several Turkish Lamiaceae species

Kirmazbekmez H1, Atay F1, Kaiser M2, Yesilada E1, Tasdemir D3
1Department of Pharmacognosy, Faculty of Pharmacy, University of Yeditepe, 34755 Sisli, Istanbul, Turkey; 2Department of Medical Parasitology, Swiss Tropical Institute, 4002 Basel, Switzerland; 3Centre for Pharmacognosy and Phytotherapy, School of Pharmacy, University of London, London WC1N 1AX, UK

The in vitro antiprotozoal activities of methanolic extracts and subextracts prepared from the aerial parts of five Lamiaceae plants (Salvia tomentosa Miller, S. sclarea L., S. dicroantha Stapf., Nepeta nuda L. subsp. nuda and Marrubium astracanicum Jacq. subsp. macdonn (Bormn.) P.H. Davis were evaluated against four parasitic protozoa, Trypanosoma brucei rhodesiensae, T. cruzi, Leishmania donovani and Plasmodium falciparum. The cytotoxic potentials of the extracts on L6 cells were also evaluated. Melarsoprol, benzimidazoles, miltefosine, chloroquine and podo- phylotoxin were used as reference drugs. The extracts showed antiprotozoal potential against three or four parasites. Hence, they were dispersed in water and partitioned against n-hexane and chloroform, respectively. From this, the traditional use was screened in the same test systems. The n-hexane extract of N. nuda was the most active against T. brucei rhodesiensae with IC50 value of 0.62 μg/mL, whilst the CHCl3 extracts of S. tomentosa and S. dicroantha showed significant activity against L. donovani (IC50 1.81 and 2.31 μg/mL, respectively). All organic extracts displayed moderate trypanocidal potential against T. cruzi with hexane extract of S. sclarea being the most active one (IC50 18.17 μg/mL). Again all organic extracts exhibited remarkable antimalarial activities and with IC50 values in the range of 2.54 – 3.78 μg/mL, the chloroform subextracts appeared to be slightly more potent than the hexane subextracts (IC50 values 3.37 – 4.64 μg/mL). The extracts displayed low or no cytotoxicity towards mammalian L6 cells. This is the first study reporting the antimalarial, leishmanicidal and trypanocidal effects of the genera Salvia, Nepeta and Marrubium that are native to Turkey. Keywords: Lamiaceae, Nepeta, Salvia, Marrubium, Antiprotozoal activity

PM08
Antinociceptive effect of chronic administration of green tea epigallocatechin gallate in a model of diabetic hyperalgesia in rat

Baluchnejadmojarad T1, Roghani M2
1Department of Physiology, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran; 2Department of Physiology, School of Medicine, Shahed University, Tehran, Iran

Considering antidiabetic and antiinflammatory potential of green tea epigallocatechin-gallate (EGCG), this study was designed to investigate the analgesic effect of two-month EGCG using formalin and tail-immersion tests in diabetic rats. Male rats were divided into control, EGCG -treated control, diabetic, EGCG -treated diabetic, and sodium salicylate-treated control and diabetic. EGCG was administered p.o. at doses of 20 and 40 mg/kg for seven weeks one week after diabetes induction. At the end of the study, pain threshold and nociception were evaluated using hot water tail immersion and formalin tests respectively. Diabetic rats exhibited a higher score of pain at both phases of the formalin test and EGCG-treated diabetic rats dose-dependently exhibited a lower nociceptive score at both phases of the test (p < 0.05). Regarding pain threshold, diabetes significantly reduced tail immersion latency (p > 0.05) and EGCG treatment did not produce a significant change in this respect. Although chronic treatment with EGCG does not affect pain threshold but significantly reduces nociception in an experimental model of hyperalgesia and this may be considered as an auxiliary treatment for diabetic hyperalgesia. Keywords: Epigallocatechin-3-gallate, Pain, Hyperalgesia, Diabetic rat
Hyperalgesia is considered as one of the marked signs of subchronic diabetes mellitus that could affect the life style of the patients. This study was designed to investigate the antinociceptive effect of chronic feeding of Allium schoenoprasum L. (AS) leaf in streptozotocin-diabetic rats using formalin and hot tail immersion tests. Rats were divided into control, AS leaf-treated control, diabetic, sodium salicylate (SS)-treated diabetic, and AS leaf-treated diabetic groups. The treatment groups received oral administration of AS leaf-mixed pooled feed (3%) for 8 weeks. Finally hyperalgesia were assessed using standard formalin and for hot tail immersion tests. AS leaf treatment of diabetic rats reduced pain score in chronic phase of formalin test from 2.4 ± 0.14 to 2.0 ± 0.12 (p < 0.05). Regarding hot tail immersion test, diabetic rats showed a significant reduction (5.9 s) in tail flick latency as compared to control ones (p < 0.05) and AS leaf treatment of diabetic rats did not significantly increase this latency relative to untreated diabetics. Taken together, 8-week administration of AS leaf could attenuate nociceptive score in chronic phase of formalin test in streptozotocin-induced experimental model of diabetes mellitus and has no effect on thermal pain and anti-inflammatory property of the plant is perhaps responsible for its analgesic effect. Keywords: Allium schoenoprasum, Diabetic hyperalgesia, Antinociceptive

The anti-inflammatory pharmacological profile of herbal extracts used in Tonsipret®-Kopfing B, Widowitz U, Blunder M, Haunschmidt F, Bauer R.

Institute of Pharmaceutical Sciences, Karl-Franzens-University Graz, Universitätsplatz 4, 8010 Graz, Austria; Bionorica SE, Kerschensteinerstr. 11 – 15, 92318 Neumarkt, Germany

Tonsipret® is a commercially available medicinal product for the treatment of sore throat and tonsillitis. The herbal extracts used in this preparation are hydro-alcoholic tinctures of dried, ripe fruits of Capsicum annuum L., re-epithelialization during SSFLO (1%, 5% or 10%), petrolatum jelly (negative control) or commercial emulsion of sunflower oil (positive control). At 14th days the animals were euthanized and the scar tissue was collected for histological and histomorphometric analysis to evaluate re-epithelialization, quantification of inflammatory cells, fibroblast cells, blood vessels and collagen density. All animals treated with SSFLO (1% or 5%) or commercial emulsion of sunflower oil (positive control) showed complete re-epithelialization, against only 33.33% showed by negative control group. In the morphometric evaluation a significant increase (p < 0.05) in the number of inflammatory cells in the group treated with 10% SSFLO was observed compared to the ESO control group. Among the remaining variables, there were no significant differences observed. The results clearly demonstrate that locally administered 1% and 5% SSFLO promote a significant re-epithelialization during the healing process, and might represent a novel therapeutic approach in cutaneous wounds. Keywords: Linum usitatissimum, re-epithelialization, healing, cutaneous wounds References: [1] Joshi K et al. (2006) PLEFA 74:17 – 21. [2] Otranto M et al. (2010) WRB 18: 629 – 636.

Histological analysis of rat cutaneous wounds treated with a semi-solid formulation of linseed (Linum usitatissimum L.) oil.

Blunder MD, Franco ED, Aquino CF, Oliveira AP, Rosas ST, Medeiros PL, Evencio LF, Goes AD

Department of Physiology, School of Medicine, Federal University of Pernambuco, Recife-PE, Brazil; Department of Pathology, Federal University of Pernambuco, Recife-PE, Brazil; Department of Histology and Embryology, Federal University of Pernambuco, Recife-PE, Brazil; Department of Antibiotics, Federal University of Pernambuco, Recife-PE, Brazil

The oil linseed of Linum usitatissimum L. (Linaceae) is popularly known as linen. Its chemical composition shows the presence polyunsaturated fatty acids, linolenic (56.6%) and linoleic acid (13.2%), and the monounsaturated fatty acid oleic (17.8%) which are important for the maintenance of normal dermal structure [1, 2]. The purpose of this study was to investigate the effects of a semi-solid formulation of linseed oil – SSFLO (1%, 5% or 10%) on re-epithelialization of excision wound model. Surgically standardized circular (± 78.5 mm²) wounds were made on the dorsum of Wistar rat. The animals were divided into five groups (n = 6) and treated for 14 days with SSFLO (1%, 5% or 10%), petrolatum jelly (negative control) or commercial emulsion of sunflower oil (positive control). At 14th days the animals were euthanized and the scar tissue was collected for histological and histomorphometric analysis to evaluate re-epithelialization, quantification of inflammatory cells, fibroblast cells, blood vessels and collagen density. All animals treated with SSFLO (1% or 5%) or commercial emulsion of sunflower oil (positive control) showed complete re-epithelialization, against only 33.33% showed by negative control group. In the morphometric evaluation a significant increase (p < 0.05) in the number of inflammatory cells in the group treated with 10% SSFLO was observed compared to the ESO control group. Among the remaining variables, there were no significant differences observed. The results clearly demonstrate that locally administered 1% and 5% SSFLO promote a significant re-epithelialization during the healing process, and might represent a novel therapeutic approach in cutaneous wounds. Keywords: Linum usitatissimum, re-epithelialization, healing, cutaneous wounds References: [1] Joshi K et al. (2006) PLEFA 74:17 – 21. [2] Otranto M et al. (2010) WRB 18: 629 – 636.

Tyrosinase inhibitory activity of selected medicinal plants.

Namjooyan F, Jahangiri A, Arkani E, Azemi M

Pharmacognosy Department, School of Pharmacy, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran; Medicinal Chemistry Department, School of Pharmacy, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran; Medicinal Plant Research Center, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran

Melanin is a pigment that is distributed widely in bacteria, fungi, plants and animals (1). Melanogenesis is initiated with oxidation of L-tyrosine by tyrosinase that is rate-limiting step in this process. Next reactions can proceed spontaneously (2). Tyrosinase is that a key enzyme in formation of melanin pigments, widely exists in animals and plants. Tyrosinase have a monophenolase activity as well as diphenolase activity that oxidizes o-diphenols to o-quinones (3). This study evaluated inhibitory effect of four plants including Phyllis alkekengi L., Alcea rosea L., Bunium persicum B. Fedtsch. and Marrubium vulgare L. on mushroom tyrosinase. In this study L-Dopa (Dihydroxyphenilalanin) is used as substrate, so diphenolase activity of mushroom tyrosinase was evaluated. Kojic acid was used as positive control. Extracts of Phyllis alkekengi, Alcea rosea, Bunium persicum (Total), Bunium persicum (defatted) and Marrubium vulgare showed IC50 values of 0.09, 0.38, 0.37, 0.38, 2.76 mg/ml respectively. IC50 values were defined as concentration of inhibitor that inhibited 50% of tyrosinase activity (4). Extract of Phyllis alkekengi showed greatest inhibitory effect on mushroom tyrosinase activity with IC50 value of 0.09 mg/ml. Kinetic and Inhibition parameters (Km, Vm, Ki) were calculated. In this study kinetic parameters (Km, Vm) are evaluated and Ki evaluated for P. alkekengi, A. rosea and B. persicum. Data has revealed that these three plants display a mixed- type inhibition. However Marrubium vulgare shows an uncompetitive inhibition (Table1). P. alkekengi that had the greatest tyrosinase inhibitor showed Ki value of 0.52 mg/ml comparing another study on total extract of Lavandula stoechas L. showed Ki value of 0.183 mg/ml (4). Finally calcu-
Influence of a semi-solid formulation of *Persea americana* oil fruit on the healing of cutaneous wounds in rats

Maia MD1, Oliveira AP1, Franco ED1, Aquino CF2, Melo RC1, Barbosa HE1, Paz SP2, Goes AD3

1Department of Physiology and Pharmacology, Federal University of Pernambuco, Recife-PE, Brazil; 2Department of Pathology, Federal University of Pernambuco, Recife-PE, Brazil; 3Department of Antibiotics, Federal University of Pernambuco, Recife-PE, Brazil.

*Persea americana* Mill. (Avocado) oil fruit presents polyunsaturated (oleic (ω-9) and linoleic (ω-6)) and monounsaturated fatty acids (linolenic (ω-3) [1,2]. Several studies have shown a role for ω-3, ω-6 and ω-9 in the process of tissue repair [3,4]. The purpose of this study was to investigate the effects of a semi-solid formulation of avocado oil (SSFAO) on cutaneous wound healing of rats. Wistar rats (200–250 g) were anesthetized with intraperitoneal injection of ketamine (75 mg/kg) plus xylazine (15 mg/kg) followed by shaving of the skin at wounding site and an circular area (78.5 mm²) of skin was surgically removed from dorsal region of the animals. After surgery, the animals were divided in groups (n=6) and treated with topical application of SSFAO (1%, 5%, 10% or 50%), avocado oil (AO), CuratecAge (positive control) and petroleo jelly (negative control) once daily for 14 days. Concerning to wound area (mm²) evolution, in the second day of treatment a statistically significant difference was observed between the AO group (118.88±14.37) compared to positive control (86.56±16.23), and in the fifth day the difference was observed between SSFAO 1% (59.52±9.74) compared to negative control (92.09±14.91). No difference was observed on the qualitative aspects (crust color and presence of fibrin, exudates, granulation and re-epithelialization) between SSFAO groups (1%, 5%, 10%, 50%) or AO when compared to controls. In conclusion, the top use of SSFAO (1%, 5%, 10% or 50%) or the AO appears to have no influence over the wound healing of rats. Keywords: *Persea americana*, wound healing References: [1] Salgado JM et al. (2008) CTA 28: 20–26. [2] Tango JS et al. (2004) RBF 26: 17–23. [3] Manhezi AC et al. (2008) RBE 61: 620–629. [4] Hatanaka E, Curi R (2007) RBF 88: 53–58. [5] Cardoso RB et al. (2004) WRR 12: 235–243.

### Table 1: effect of Extracts and kojic acid on the kinetic parameters of mushroom tyrosinase.

<table>
<thead>
<tr>
<th>Extract/acid</th>
<th>Km(M)</th>
<th>Vmax(U)</th>
</tr>
</thead>
<tbody>
<tr>
<td>B. radians</td>
<td>0.18</td>
<td>4.5</td>
</tr>
<tr>
<td>B. purpurascens</td>
<td>0.22</td>
<td>1.5</td>
</tr>
</tbody>
</table>

### Keywords:

---

**PM113**

Antioxidant capacity of *Bifora radians* Bieb. (Apiaceae = Umbelliferae) is represented by two species in Turkey, namely *Bifora testiculata* (L.) Sprengel and *B. radians* Bieb. [1] *B. radians* is an annual herb with typical odor in the fieldsides especially chalaky of Central Anatolia and known locally as “yabani kişinişotu, küçük kişinişotu or aşuţi” [1,2]. This species is used in traditional medicine as a stomachic and carminative in Turkey. Furthermore, the aerial parts of this plant has been used as an aromatic in foods especially *B. radians* is traditionally used for its diuretic effect comparable to furosemide (2). In the species described the presence of several flavonoid compounds is widely used for its diversity of actions among which is the hypoglycemic activity. Propyl gallate (IC50=0.09–0.18) is used as a positive control.

PM114

Evaluation of the hypoglycemic activity of extracts from *Boldoa purpurascens* Cav. González DM1, Hernández Y1, Borady B1, Vicet L1, Saucedo Y1, Pieters L2, Appers S2

1University of Martha Abreu Las Villas, Santa Clara, Cuba; 2University of Antwerpe, Antwerp, Belgium.

**Boldoa purpurascens** Cav. (1), a plant belonging to Nyctaginaceae family, is traditionally used for its diuretic effect comparable to furosemide (2). In the species described the presence of several flavonoid compounds was confirmed for its diversity of actions on glycemic control. The phytochemical analysis by 1H and 13C NMR spectroscopy allowed the presence of D-pinitol in the ethanol extract from the leaves of the plant (3). The aim of the investigation was checking the hypoglycemic effect of aqueous and alcoholic extracts obtained from *Boldoa purpurascens* at doses of 50, 100 and 200 mg/kg using insulin (U) as a positive control and NaCl 0.9% as negative control. Another experiment was performed similar but dried aqueous extract was used at doses of 50, 100 and 200 mg/kg: using metformin at dose of 50 mg/kg as a positive control and keeping the 0.9% NaCl as a negative control. The statistical analysis was carried out by the test of Kruskal Wallis with an interval of trust of 99%. The study concluded that the species possesses hypoglycemic activity at all doses tested in both extracts, being the reduction greater for the ethanol extract (40%), comparable to insulin. Keywords: *Boldoa purpurascens*, antidiabetic References: 1. Roig J T (1994) Dictionary of Cuban Common names, pp 225226. 2. Gonzalez D (2006) Doctoral Theses. 3. Bates S, Jones R, Bayley C (2000) Brit J Pharmacol 130:1944–1948.

**PM115**

The new source of biologically active substances – *Barbarea vulgaris* W. T. Alton

Marenich M, Rakhmadiyeva S, Albuldinov Y, Eurasian National University named L.N. Gumilyov, 5, Munatpasov str., Astana, 010008, Kazakhstan.

*Barbarea vulgaris* W. T. Alton (Brassicaceae) were collected in the Akmol region of the Republic of Kazakhstan in 2009. By means of spectrophotometric method the quantitative content of biologically active substances in the leaves, flowers and stems were determined: flavonoids (0.70%, 2.25%, and 0%) [5], carbohydrates (3.60%, 1.01%, and 0.36%) [3] and tannins (2.03%, 2.06%, and 0.08%); by method of titrimetry- organic acids (2.58%, 3.16%, and 0.58%) [2,4]. Determination of the mineral composition of ash from above-ground parts of plant was determined by mass spectrometry with inductively coupled plasma. There was found 31 elements in samples under analysis. As a result it was found that the plant is prone to the accumulation of such elements as iron (24.25×10–4%), silicon (8.99×10–4%), calcium (0.07%), potassium (0.14%), strontium (7.7×10–4%), magnesium (0.015%), sodium (0.014%), aluminum (9.94×10–4% – the given elements contained in the most concentration in the plant. A scheme was developed for the study of flowers. Water-alcohol extract was concentrated to a complete removal of ethanol. The resulting aqueous extract was separated from the sediment and then the liquor was exhaustively extracted with ethyl acetate. The separated sediment was processed with petroleum ether, benzene, ethanol, aqueous alcohol, water coherently [1]. Quercetin was identified in the ethyl acetate extract while rutin was found in the alcohol extract. References: 1. Fedoseeva L et al. (2005) Chem Plant Substances 3: 45–50. 2. State Pharmacopeia USSR (1990) 11:296–297. 3. Zaporozets M et al. (2003) Biochemical Phytobiochemicals 1:324–326. 4. Grinkevich N et al. (1983) Chem Anal Med Plants 1: 87–118. 5. Khaled A et al. (2004) Quantitative Content of Flavonoids 1: 356–358.
The genus *Michauxia* L’Hérit (Campanulaceae) is represented by five species in Turkey, namely *Michauxia campanuloides* L’Hérit ex Aiton, *M. laevigata* Vent., *M. tchatchevi* Fisch. et Mey. (E); *M. thyrsoides* Boiss. & Heldr. (E), and *M. nuda* and *loides* M. tchihatchewii Vent., Heldr. (E), and *M. campanuloides* Michauxia campanuloides; M. laeviga-
ta; M. tchatchevi; M. thyrsoidae; M. nuda; Antioxidant activity

A decrease of the intracellular concentration of doxorubicin by activation of ABC-transporters, mainly P-glycoprotein, leads to a reduction of its chemotherapeutical efficiency. To overcome multidrug resistance, digitonin, steroidal saponin, was selected to enhance cell permeability, increase intracellular accumulation, and anticancer effect of doxorubicin. We investigated the cytotoxicity and P-glycoprotein modulatory effect of digitonin in combination with doxorubicin in resistant leukemia and colon cells. MITT assay was applied to evaluate the cell viability and reversal effect of this combination. Rhodamine123 and calcein efflux assays were used for investigate P-glycoprotein function by flow cytometry. At the molecular level, RT-PCR confirmed the data obtained. Digitonin exhibits a significant effect on viability of Caco-2 and CEM/ADR5000 cells with IC50 values 15.17 μM and 16.02 μM, respectively. The co-incubation of doxorubicin with non-toxic concentration of digitonin (5 μM) resulted in an enhanced doxorubicin cytotoxicity in Caco-2 and CEM/ADR5000 cells by 1.9- and 1.2-fold, respectively. Digitonin increase Rhod123 and calcein accumulation in Caco-2 and CEM/ADR5000 cells in dose dependent manner. Moreover, 5 μM digitonin increases the accumulation of Rhod123 and calcein 1.3- and 1.1-fold of verapamil activity in Caco-2 cells. RT-PCR data indicate that 5 μM digitonin down-regulated P-gp/MDR1 mRNA to 80% of the control level. In conclusion, digitonin enhances the antitumor effect of doxorubicin and exhibits P-glycoprotein modulatory effect, so it considered as an efficient additive to the chemotherapeutic principle. Keywords: digitonin, anticancer, doxorubicin resistance

**PM119**

Cytogenetic analysis of genotoxicity of *Cynoglossum officinale* L. (Boraginaceae) extract from Bosnia and Herzegovina (W. Balkan)

Redzic A1, Redzic S2, Praxse N2
1Department of Biology and Human Genetics of Medical Faculty University, 90 Cekalusa St., 71 000 Sarajevo, Bosnia and Herzegovina; 2Department of Botany, Faculty of Science University of Sarajevo, 33 – 35 Zmaja od Bosne, 71 000 Sarajevo, Bosnia and Herzegovina

The *Cynoglossum officinale* L. have always been used in traditional med-
icine in Western Balkans [1]. The young shoots in some regions are used in nutrition [2]. A large number of medicinal plants of the family Bor-
aginaceae contains pyrrolizidine alkaloids and show genotoxic effects [3]. To expect a similar action of *Cynoglossum officinale*. For the analysis were taken samples of *Cynoglossum officinale* from different localities. The extracts were made of fresh aerial parts at concentrations of 0.5% and 1%. The genotoxicity was done by Allium-test. For each concentra-
tion were taken in 10 specimens. The effect of treatment, the extract was observed after 24 and 48 hours. After that, taken root and a fixed. It was examined in 10 preparations for each concentration and five for control.

then determined the mitotic index, the frequency of certain phases of mitosis, the frequency of chromosome aberrations and disturbances in the meiotic spindle. The analysis examined 10 x 1000 cells. It was found that extract of *Cynoglossum officinale* affect on mitotic activity and on other in-
vestigated parameters. Index mitosis after 48 hours is 3.5 (conc. 0.5%) and 2.7 (conc. 1%); control (4.8). The most cells were in prophase, at least in anaphase. The both of concentration of the extract cause of chromosome aberrations. There have been C-mitosis, anaphase abnormal-
ities, irregular metaphase and a few cells with two nuclei. More intensive effects express of 1% concentration. On the basis of research can be talking about cytostatic and genotoxic activity of the extracts of *Cynoglossum officinale*. Keywords: genotoxicology, cytogenetics, alka-

**PM120**

Modulation of P-glycoprotein, cytochrome P450, and glutathione-S-transferase by resveratrol in human cancer cells

El Readi MZ1, Eid SY2, Effert F1, Wink M1
1Institute of Pharmacy and Molecular Biotechnology, Heidelberg University, Im Neuenheimer Feld 364, 69120 Heidelberg, Germany; 2Institute of Pharmacy and Biochemistry, Johannes Gutenberg- University, Staudinger Weg 5, 55099 Mainz, Germany

Resistance of cancer cells to chemotherapy is controlled by a decrease of intracellular drug accumulation, increase of detoxification, and dimin-
ished propensity of cancer cells to undergo apoptosis. ABC-membrane transporters together with intracellular metabolic enzymes contribute to the complex and unresolved phenomenon of multidrug resistance (MDR). Resveratrol, a polyphenol of *Fallopia japonica* (MDR). Resveratrol, a polyphenol of *Fallopia japonica* (Houit.) Ronse Dccr., has antiinflammatory and antioxidant properties [1]. However, it is also interesting in the field of cancer therapy [2]. The mechanisms by which resveratrol might produce anticancer effects are not well under-
stood. In this study, resveratrol was shown to increase Rho123 and calcein accumulation in a concentration dependent manner (1–500 μM) in Caco-2 cells by 3–167% and 5–361% of verapamil. Moreover, the treatment of CEM/ADR5000 with 10–100 μM resveratrol significantly inhibited the Rhod123 and calcein efflux by 107–407%, and 164–460% as compared with verapamil (100X), respectively. The cyto-
toxicity of doxorubicin was enhanced by using 20 μM resveratrol; IC50 values were decreased from 4.15 to 1.23 μM, and from 33.67 to 1.81 μM, respectively. Furthermore, resveratrol significantly inhibited GST and cytochrome P450 enzyme activity in a dose dependent manner with IC50 values 33.30 μM and 11.49 μM, respectively. RT-PCR reveals a sig-
nificantly down-regulation of ABC-transporters and of smetabolic en-
zymes mRNA levels in Caco-2 cell lines in response to resveratrol treat-
ment. In conclusion, the inhibition of both ABC-transporters and of me-
**PM121**

**Variability of phenolic contents, antioxidant and antimicrobial activities of *Inula crithmoides* from Tunisia**

Kosari R, Jallali I, Medini F, Abdelly C

Laboratoire des Plantes Extremophiles, Centre de Biotechnologie à la Technopole de Bujér Cédria (CBBC), Hammamet, Tunisia

*Inula crithmoides* L. is a spontaneous halophyte thriving on waterlogged zones. This species is harnessing edible, medicinal, aromatic and economic potentialities. In fact, this plant is known for its richness on bioactive compounds, mainly on essential oils. In this study, we tried to carry out the richness of this species on phenolic compounds and to evaluate their biological activities. Different parts of the plant were collected from Kairouan (center of Tunisia) air dried, grounded to a fine powder then subjected to a selective extraction with petroleum ether, acetone 60% then ethyl acetate in order to have a phenolics enriched fraction. Dried extracts were dissolved in methanol to be used in the colorimetric quantification of phenolics and to estimate their antioxidant activities (DPPH, total antioxidant activity, reducing power and inhibition of the b-carotene bleaching tests) and antibacterial activity against four human pathogenic bacteria. Results revealed that *I. crithmoides* extracts contain interesting amounts of these phytochemicals, significantly variable within the different plant parts, with highest amounts recorded in flower extracts. Besides, the entire investigated antioxidant test showed that *I. crithmoides* extracts exhibited high antioxidant activities, especially flower extracts. The effect of *I. crithmoides* extracts on the degree of inactivation of selected food borne pathogenic bacteria was variable and depended on the strains in question and on the part of the plant. These finding suggest that *I. crithmoides* is an interesting source of phenolics having antioxidant and antibacterial potentialities allowing them to be used as preservative ingredients in the food, pharmaceutical, and cosmetic industry. Keywords: *Inula crithmoides*, phenolic compounds, biological activities

**PM122**

**Inhibitory activities of selected medicinal plants on mushroom tyrosinase**

Nemjeoygan F¹, Moosavi H², Esfandighir A, Azemi M²

¹Pharmacoogy Department, School of Pharmacy, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran; ²Medicinal Plant Research Center, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran; ³Medicinal Chemistry Department, School of Pharmacy, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran

Tyrosinase is a key enzyme in melanin synthesis from tyrosine. Using tyrosinase inhibitors has become increasingly important in medicinal and cosmetic products to prevent or treat pigmentation disorders (1). To evaluate inhibitory effects the extracts of *Urgeina maritima* (L) Baker [1], *Zhumeria majdae* Rech.f. & Wendelbo [2] and *Physalis divaricata* D.Don [3] on mushroom tyrosinase this study was designed. L-Dopa as used as substrate. Ethanolic extracts of *U. maritima* [bulb], Z. majdae [leaves] and P. divaricata [air organs] were used for their inhibitory effect in vitro on diphenolase activity of tyrosinase, using a spectrophotometric method. The extracts showed anti tyrosinase activity weaker than positive control (Kojic acid). The inhibitory activity of tested plants *Urgeina maritima*, *Zhumeria majdae* and *Physalis divaricata* against mushroom tyrosinase is expressed using IC₅₀ (concentration of inhibitor that inhibited 50% of tyrosinase activity) values of 2.79, 2.37, 3.34 mg/ml respectively. The kinetic study indicated that all extracts were uncompetitive inhibitors for tyrosinase. Keywords: *Urgeina maritima*, *Zhumeria majdae*, *Physalis divaricata*, mushroom tyrosinase, Inhibitor Acknowledgement: This research is a part of a project granted by Ahvaz Jundishapur University of Medical Sciences. References: Zhang X Hu et al. (2009) Biological and Pharmaceutical Bulletin 32(1): 86 – 90.

**PM123**

**Anti-inflammatory activity of ointments with dry extracts of rhizome and herb of *Aremia agrimonioides* (L.) DC (Rosaceae)**

Pilipovic S¹, Mulebegovic N², Mornjakov Z², Usunovic A¹, Elezovic A¹, Hadzidelic S¹

¹Agency for Medicinal Products and Medical Devices, Titova 9, 71000, Sarajevo, Bosnia and Herzegovina; ²Faculty of Medicine, University of Sarajevo, Cekalusa 90, 71000 Sarajevo, Bosnia and Herzegovina

*Aremia agrimonioides* (L.) DC (Rosaceae), also known as Bastard Agrimony, is a native plant species in the central Europe. The aqueous extracts of rhizome and herb were prepared by method of percolation with water. The liquid extracts were then evaporated in stream of nitrogen. The content of phenolics was determined by the method with Prussian Blue (1). The ointments with 1% dry extract of rhizome and 1% dry extract of herb were prepared in paraffin ointment. The anti-inflammatory effect of ointments was tested through the model of mouse ear model. Inflammation of both ears of albino mice (both sexes) was induced by applying 10μl of 3% solution of acetone-based croton oil (2). As control we used 1% hydrocortisone ointment. Ointments were applied once a day in the period of three days on the left ear, two hours after inducing inflammation. The right ear was not further treated. The appearance of the ears observed during three days was expressed in scores on 0 – 14 scale. The mean values recorded on the third day after the causing of ear inflammation were: for ears treated with ointment with extract of rhizome 6 ± 1, for ears treated with the extract of aerial part of plant 7 ± 1, for untreated ears 12 ± 2, and for ointment with 1% hydrocortisone 6 ± 1. Content of phenolic compounds in the extracts was 12.09% for the rhizome and 12.76% for the herb. The pharmacological response to both ointments was similar with the ointment with 1% hydrocortisone. Keywords: antiinflammatory, aremioidena, mouse ear References: 1. Price ML, Butler LG (1977) J Agric Food Chem 25:1268 – 1272. 2. Williamso EM, Okpako DT, Evans FJ (1996) Selection, Preparataion and Pharmacological Evaluation of Plant Material in: Pharmacological Methods in Phytotherapy Research; John Wiley Sons p.131 – 153.

**PM124**

**Change of total anthocyanins content and kernel lightness according ripening days after silking date in black waxy corn**

Lee J¹, Kim J¹, Son B¹, Baek S¹, Jung G², Kim S¹, Jung Y², Kim W³

¹National Institute of Crop Science, Suwon, Korea; ²Rural Development Administration, Suwon, Korea

This study was carried out to evaluate changes of total anthocyanins content and kernel lightness according ripening days after silking date in black waxy corn. Black waxy corns have pericarps colored black and black pericarps contain anthocyanins. Anthocyanins relate to antioxidant activities. Thirty black waxy corn inbred lines were planted at upland crop fields of National Institute of Crop Science in Korea, 2009. They were evaluated total anthocyanins content, respectively. Based on these results, they were classified by 3 groups. Three groups were mutually crossed. Their F₁ seeds were planted in upland crop fields of National Institute of Crop Science in Korea, 2010. They were classified 8 crossing groups by crossing combinations. These crossing groups were harvested at 19 days, 21 days, 23 days and 25 days after silking date, respectively. And their products were evaluated by total anthocyanins content and lightness. As increasing of harvest days, total anthocyanins content were increased, but lightness was not. The total anthocyanins content and lightness were analyzed correlation by SAS Enterprise Guide 4.2. They have negative correlation and coefficient of determinant (R²) was 0.8515. Keywords: black, waxy corn, anthocyanin, days after silking date References: 1. Lopez Martinez et al. (2009) Food Science and Technology 42: 1187 – 1192

**PM125**

**Quercetagelin, a component of premature *Citrus unshiu* (Swingle) Marcow., suppress the chemokines related with atopic dermatitis by regulating STAT1 signal**

Kang G, Han S, Kang H, Yoo E

Department of Pharmacology, School of Medicine, Jeju National University, Jeju, South Korea

Atopic dermatitis (AD) is an itchy and relapsing inflammatory skin disease. It was known that a predominant systemic Th2 dysbalance with...
increased IgE levels and eosinophilia is widely accepted in the pathogenesis of AD [1]. Thymus and activation-regulated chemokine (TARC/CCL17) and macrophage-derived chemokine (MDC/CCL22) are related with AD and are elevated in serum and lesional skin of AD patients [2, 3]. Citrus unshiu (CU) contains various flavonoids that have various bioactive effects [4]. In present study, we investigated the effect of a component of premature CU, quercetin, on the production of TARC and MDC in HaCaT human keratinocytes. As results, quercetin significantly inhibited the phosphorylation of STAT1, key transcription factor initiating IFN-γ signaling pathway, in a time- and dose-dependent manner. These results suggest that quercetin, a component of premature CU, may have an anti-atopic activity by inhibiting the inflammatory chemokines (TARC and MDC) via the STAT1 pathway. Keywords: quercetin, Citrus unshiu, Atopic dermatitis, TARC/CCL17, MDC/CCL22, Jak-STAT pathway References: 1. Bieber T (2010) Ann Dermatol 22 (2): 125 – 137. 2. Hijnen D et al. (2004) J Allergy Clin Immunol 114 (4): 296 – 301. 4. Kim YD et al. (2009) Korean J Nutr 42(3): 278 – 290.

Solvent extracts of Carpinus tschonoskii suppress the expression of atopic inflammatory cytokines and chemokines in RAW264.7 macrophages and HaCaT keratinocytes Han S1, Kang G2, Park D3, Kang H4, Yoo B5, Yoo E7 1Department of Pharmacology, College of Medicine, Jeju National University, Jeju, South Korea; 2Department of Histology, College of Medicine, Jeju National University, Jeju, South Korea; 3Cosmetic R&D center, COSMAX Inc. Hwa Sun, Gyeonggi, South Korea. Atopic dermatitis (AD) is a common, chronic relapsing, inflammatory skin disease characterized by pruritus and inflammation and accompanied by cutaneous physiological dysfunction through chemokine-mediated infiltration of numerous mononuclear cells in lesional skin [1]. TARC (thymus and activation-regulated chemokine/CCL17) and MDC (macrophage-derived chemokine/CCL22) that bind to the chemokine receptor CCR4 which is highly expressed on T-helper 2 cells lead to preferential influx of Th2-type lymphocytes to the lesional skin in AD [2]. Furthermore, cytokines are other triggers of AD and the expression of inflammatory cytokines (TNF-α, IL-1β, and IL-6) increase in lesional skin macrophages of AD patients [3]. In present study, we investigated the anti-inflammatory effects of Carpinus tschonoskii Maxim. in RAW264.7 murine macrophage and HaCaT human keratinocytes. As results, the CHCl3 sub-fractions (C-4, -5, and -6 fr.) dose-dependently inhibited the TNF-α and IL-1β production of TNF-α and IL-1β, and RAW264.7 murine macrophage. Also, they inhibited the mRNA expression and protein level of TARC and MDC via suppressing the phosphorylation of STAT1 protein in IFN-γ-stimulated HaCaT human keratinocytes. These results suggest that C. tschonoskii may be an effective source for improving the symptoms of AD by inhibiting the inflammatory cytokines and chemokines. Keywords: Carpinus tschonoskii, Atopic dermatitis, TARC/CCL17, MDC/CCL22, RAW264.7 macrophages, HaCaT keratinocytes References: 1. Bieber T (2008) N Engl J Med 358:1483 – 94. 2. Sekiya T et al. (2000) J Immuno 165:2205 – 2213. 3. Grossman RM et al. (1989) Proc Natl Acad Sci USA 86: 6367 – 71.

Moringa oleifera Lam. (Family: Moringaceae) is commonly known as drumstick tree or horseradish tree. The leaves are highly nutritious, being a significant source of beta carotene, Vitamin C, protein and antioxidants [1]. In the present study, protective effect of hydroalcoholic extract of the leaves of M. oleifera on experimental reflex eosinophilagis in rats was investigated. Rats received M. oleifera extract (200, 400 mg/kg), omeprazole (30 mg/kg) given at 1 h prior to surgery [2]. M. oleifera extract at doses 200, 400 mg/kg significantly inhibited the eosinophil index (P < 0.001) as compare to control. Further, acid and pepticin put out of gastric contents were significantly decreased in treated groups. M. oleifera extract (400 mg/kg) significantly inhibited the lipid peroxidation (from 0.58 ± 0.03 to 0.38 ± 0.02 nmol of malondialdehyde (MDA)/mg protein) (P < 0.001) and increased in levels of catalase to 25.4 ± 2.8 units of catalase activity/mg protein and superoxide dismutase (SOD) to 712.5 ± 5.8 units/mg protein (P < 0.001). M. oleifera extract (200 mg/kg) and omeprazole also showed significant inhibition in lipid peroxidation (P < 0.05) and enhanced the activities of catalase (P < 0.01) and SOD activity. Further, it altered the elevated levels of salicylic acid and heoxene contents in oesophageal tissue. Indeed, M. oleifera significantly decreased the elevated plasma histamine content (P < 0.05). The results suggested that antioxidants potential of M. oleifera could attenuate the severity of reflex eosinophagis and prevent the oesophageal mucosal damage. Keywords: Moringa oleifera, Reflux Eosinophilagis, Antioxidant References: 1. Verma A R (2009) Food Chem Toxicol 47: 2196 – 2201. 2. Rao CV, Vijnalakumar M (2008) Eur J Pharmacol 589: 233 – 238.

Solvent extracts of Carpinus tschonoskii suppress the expression of atopic inflammatory cytokines and chemokines in RAW264.7 macrophages and HaCaT keratinocytes Han S1, Kang G2, Park D3, Kang H4, Yoo B5, Yoo E7 1Department of Pharmacology, College of Medicine, Jeju National University, Jeju, South Korea; 2Department of Histology, College of Medicine, Jeju National University, Jeju, South Korea; 3Cosmetic R&D center, COSMAX Inc. Hwa Sun, Gyeonggi, South Korea. Atopic dermatitis (AD) is a common, chronic relapsing, inflammatory skin disease characterized by pruritus and inflammation and accompanied by cutaneous physiological dysfunction through chemokine-mediated infiltration of numerous mononuclear cells in lesional skin [1]. TARC (thymus and activation-regulated chemokine/CCL17) and MDC (macrophage-derived chemokine/CCL22) that bind to the chemokine receptor CCR4 which is highly expressed on T-helper 2 cells lead to preferential influx of Th2-type lymphocytes to the lesional skin in AD [2]. Furthermore, cytokines are other triggers of AD and the expression of inflammatory cytokines (TNF-α, IL-1β, and IL-6) increase in lesional skin macrophages of AD patients [3]. In present study, we investigated the anti-inflammatory effects of Carpinus tschonoskii Maxim. in RAW264.7 murine macrophage and HaCaT human keratinocytes. As results, the CHCl3 sub-fractions (C-4, -5, and -6 fr.) dose-dependently inhibited the TNF-α and IL-1β production of TNF-α and IL-1β, and RAW264.7 murine macrophage. Also, they inhibited the mRNA expression and protein level of TARC and MDC via suppressing the phosphorylation of STAT1 protein in IFN-γ-stimulated HaCaT human keratinocytes. These results suggest that C. tschonoskii may be an effective source for improving the symptoms of AD by inhibiting the inflammatory cytokines and chemokines. Keywords: Carpinus tschonoskii, Atopic dermatitis, TARC/CCL17, MDC/CCL22, RAW264.7 macrophages, HaCaT keratinocytes References: 1. Bieber T (2008) N Engl J Med 358:1483 – 94. 2. Sekiya T et al. (2000) J Immuno 165:2205 – 2213. 3. Grossman RM et al. (1989) Proc Natl Acad Sci USA 86: 6367 – 71.

Effect of Moringa oleifera extract on experimental reflex eosinophilagis in rats Vijayakumar M, Eswaran B, Rao CV, Rawat AS 1Department of Pharmacology, College of Medicine, Jeju National University, Jeju, South Korea; 2Department of Histology, College of Medicine, Jeju National University, Jeju, South Korea; 3Cosmetic R&D center, COSMAX Inc. Hwa Sun, Gyeonggi, South Korea. Atopic dermatitis (AD) is a common, chronic relapsing, inflammatory skin disease characterized by pruritus and inflammation and accompanied by cutaneous physiological dysfunction through chemokine-mediated infiltration of numerous mononuclear cells in lesional skin [1]. TARC (thymus and activation-regulated chemokine/CCL17) and MDC (macrophage-derived chemokine/CCL22) that bind to the chemokine receptor CCR4 which is highly expressed on T-helper 2 cells lead to preferential influx of Th2-type lymphocytes to the lesional skin in AD [2]. Furthermore, cytokines are other triggers of AD and the expression 0.001) as compare to control. Further, acid and pepticin put out of gastric contents were significantly decreased in treated groups. M. oleifera extract (400 mg/kg) significantly inhibited the lipid peroxidation (from 0.58 ± 0.03 to 0.38 ± 0.02 nmol of malondialdehyde (MDA)/mg protein) (P < 0.001) and increased in levels of catalase to 25.4 ± 2.8 units of catalase activity/mg protein and superoxide dismutase (SOD) to 712.5 ± 5.8 units/mg protein (P < 0.001). M. oleifera extract (200 mg/kg) and omeprazole also showed significant inhibition in lipid peroxidation (P < 0.05) and enhanced the activities of catalase (P < 0.01) and SOD activity. Further, it altered the elevated levels of salicylic acid and heoxene contents in oesophageal tissue. Indeed, M. oleifera significantly decreased the elevated plasma histamine content (P < 0.05). The results suggested that antioxidants potential of M. oleifera could attenuate the severity of reflex eosinophagis and prevent the oesophageal mucosal damage. Keywords: Moringa oleifera, Reflux Eosinophilagis, Antioxidant References: 1. Verma A R (2009) Food Chem Toxicol 47: 2196 – 2201. 2. Rao CV, Vijnalakumar M (2008) Eur J Pharmacol 589: 233 – 238.
which is characterized by the absence of alkaloids [3]. This extract was tested for estrogenic activity in a panel of suitable test models. Besides a significant competitive binding to estrogen receptors alpha (ER alpha) and ER beta, induction of alkaline phosphatase in Ishikawa endometrial adenocarcinoma cell was observed. Unfortunately, the extract did not display any estrogen receptor selectivity and promoted uterine growth in ovariectomized rats. Hence, it was considered inappropriate for the treatment of climacteric complaints and precluded from further product development. Keywords: Sophora flavescens, antitumorogenic activity


PM130

Antibacterial activity of plant extracts highly depends on extraction solvent
Sperl C, Mader E, Henkli S, Teichmann K, Schatzmayr G Biomin Research Center, Tulln, Austria

As an alternative to antibiotic growth promoters in animal nutrition, that have been banned in the EU in 2006, the demand for plant derived substances (phytogenic) is emerging to counteract bacterial infections in swine and poultry. In contrast to antibiotics, phytotherapeutics are expected to refrain from causing transmissible bacterial resistances and leaving critical residues in animal tissue. Looking for potential phytotherapeutics, five different plant raw materials (Berberis aristata DC. root, Sophora flavescens Aiton root, Holarrhena antidysenterica (L.) Wall. bark, Bridelia ferruginea Benth. bark, and leaves) were selected. Dry extracts were produced from each material using different extraction solvents (ethanol abs., water and 50/50 (v/v) ethanol/water). The antibacterial activity of the extracts on two pathogenic bacteria, Salmonella typhimurium and Clostridium perfringens Type C, was examined with a turbidimetric microdilution method. The bacterial cultures with defined microbial count were incubated in optical density of the bacterial culture led to a quantitative result, which is characterized by the absence of alkaloids [3]. This extract was tested for estrogenic activity in a panel of suitable test models. Besides a significant competitive binding to estrogen receptors alpha (ER alpha) and ER beta, induction of alkaline phosphatase in Ishikawa endometrial adenocarcinoma cell was observed. Unfortunately, the extract did not display any estrogen receptor selectivity and promoted uterine growth in ovariectomized rats. Hence, it was considered inappropriate for the treatment of climacteric complaints and precluded from further product development. Keywords: Sophora flavescens, antitumorogenic activity


PM131

Phytochemistry and biological activities of the ethanolic extract of Onosma aucherianum Mašković P, Nisic N, Soljačić S, Manojlović N, Ćvijović M, Mladenović J, Academic Djojkov G, Radijoković M Department of Chemistry and Chemical Engineering, Faculty of Agronomy, University of Kragujevac, Cara Dušana 34, 32 000 Čačak, Serbia; 2Faculty of Science, University of Kragujevac, Radoja Domanovica 12, 34 000 Kragujevac, Serbia; 3Department of Pharmacy, Medical Faculty, University of Kragujevac, 34 000 Kragujevac, Serbia; 4Department of Pharmaceutical Engineering, Faculty of Technology, University of Novi Sad, Novi Sad, Serbia

This study was aimed at evaluating the antioxidant activity and efficacy of the ethanolic extract of the endemic plant species Onosma aucherianum DC. in inhibiting the development of selected fungi and bacteria. The highest susceptibility to the ethanolic extract of O. aucherianum among the bacteria tested was exhibited by B. subtilis and S. aureus (MIC = 15.62 µg/ml). Among the fungi, A. niger (MIC = 15.62 µg/ml) showed the highest susceptibility. Total phenolic, flavonoid, condensed tannin and galloloidin contents were 90.26 ± 0.00 mg GA/g, 35.24 ± 0.55 mg RU/g, 74.65 ± 0.75 mg GA/g and 31.74 ± 1.05 mg GA/g, respectively. Total antioxidant capacity was 78.45 ± 0.98 µg AA/g. IC50 values were determined for each measurement: 21.45 ± 1.55 µg/ml for DPPH free radical scavenging activity, 36.46 ± 1.68 µg/ml for inhibitory activity against superoxide dismutase, 99.11 ± 0.2 µg/ml for hydroxy radical scavenging activity and 45.91 ± 0.88 µg/ml for chelating ability. The rosamary acid was found to be the dominant phenolic compound of the extract. Keywords: antimicrobial activity, antioxidant activity, Onosma aucherianum, HPLC analysis, phenolic compounds Acknowledgement:

PM132

Topical anti-inflammatory activity of Plantago lanceolata L. leaves: the relevance of triterpenic acids
Sosa S1, Faudale M1, Zacchiga M1, Cateli P1, Del Favero G2, Tubaro A1, Della Loggia R3

1Dipartimento di Ingegneria Industriale e dell’Informazione, Università di Trieste, Via A. Valerio 6, 34127 Trieste, Italy; 2Dipartimento di Scienze Chimiche e Farmaceutiche, Università di Trieste, Pile Europa 1, 34127 Trieste, Italy

The leaves of Plantago lanceolata L. (Plantaginaceae) are used in traditional medicine for the topical treatment of skin inflammatory affections [1]. Although P. lanceolata leaf extracts and some of their constituents have been shown to inhibit in vitro enzymes involved in inflammation [1, 2], the in vivo topical anti-inflammatory properties of the leaves have not been investigated. Therefore, P. lanceolata leaves have been studied for their topical anti-inflammatory activity by the Croton oil-induced ear dermatitis assay in mice [3]. P. lanceolata leaves were sequentially extracted with n-hexane, chloroform and methanol and the relevant extracts were evaluated for their ability to inhibit the mouse ear edema induced by Croton oil. Each extract (300 µg/cm²) provoked a significant edema reduction, the chloroform one being the most active. Its potency was only two fold lower than that of the reference non steroidal anti-inflammatory drug indomethacin: their ID50 (dose inducing 50% edema inhibition) values were 186.4 and 97.5 µg/ml, respectively. By column chromatography, the chloroform extract was separated in five fractions (A-E), concentrating its activity fraction C, which was constituted mainly by ursolic acid (44%) and oleanolic acid (27%). These compounds induced a dose-dependent edema inhibition, and ursolic acid (ID50 = 56 µg/ml) was more active than oleanolic acid (ID50 = 132 µg/ml) and indomethacin. The two triterpenes, which give a significant contribution to the anti-inflammatory activity of the parent extract, can be proposed as parameters in the quality control of P. lanceolata leaf preparations for the topical use against skin inflammations. References: 1. Beara IN et al. (2010) J Pharm Biomed Anal 52: 701 – 706. 2. Vigo E et al. (2005) Pharm Pharmacol 57: 383 – 391. 3. Tubaro A et al. (1985) Agents Actions 17: 347 – 349.

PM133

Cyathula prostrata inhibits in vitro cancer cell growth via multiple targets
Van De Venter M1, Schnabelger GE1, Baatjies L1, Koekemoer TC2, Sowemimo A2

1Department of Biochemistry and Microbiology, PO Box 77000, Nelson Mandela Metropolitan University, Port Elizabeth 6030, South Africa; 2Department of Pharmacognosy, Faculty of Pharmacy, University of Lagos, Lagos, Nigeria

The in vitro anticancer activity of an 80% ethanol extract of Cyathula prostrata (L.) Blume, an annual branching shrub used by traditional healers in Nigeria to treat cancer was investigated. IC50 values were 100.8 µg/ml and 64.4 µg/ml for HeLa (cervical cancer) and U937 (mye-lo-monocytic) cell lines, respectively. Further experiments were performed using 125 µl C. prostrata leaf extract and 50 µM cisplatin as positive control. More than 80% of the cells were arrested in the G1 phase after 48 hours of C. prostrata treatment. The annexin V-FITC/PI assay revealed an increase in percentage apoptotic cells from 4.9% to 53.1% at 24h. Cell cycle arrest was not accompanied by increased levels of the cyclin-CDK inhibitor p21. Increase in caspase-8 activation was observed in response to treatment with the extract with no cyt-c release from the mitochondria. The lack of cyt-c release was due to no change in mitochondrial membrane potential, which was investigated with the aid of fluorescent mitochondrial dyes and flow cytometric techniques. The results therefore show that C. prostrata extract induces apoptosis via the extrinsic pathway and this activation is independent of the mitochondrial dysfunction. Levels of p53 expression, the catalytic subunit of telomerase, were also shown to decrease upon C. prostrata treatment. The findings from this study suggest that the extract acts through multiple targets, by inducing: cell cycle arrest in the G1 phase through an unknown mechanism; apoptosis through an extrinsic death receptor pathway and replicative senescence through inhibition of telomerase. Keywords: Cyathula prostrata apoptosis, caspase 8, telomerase, cell cycle arrest Acknowledgement:

The antioxidant activities of the methanolic extracts of *Ephedra sarcocarpa* Aitch. & Hems. growing in Iran was evaluated using ferric reducing antioxidant power (FRAP) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assays. FRAP values 2.1 mmol eq quercetin/g extracts, and DPPH assay 4.6 mg/mL. This plant showed the high antioxidant activities. This plant showed the high antioxidant activities. This plant showed the high antioxidant activities. FRAP and DPPH assay showed good correlations with the total phenolic contents [3] of the plants, measured by the Folin–Ciocalteau assay. <sup>1,2,3</sup> The results obtained indicate that *E. sarcocarpa* may become important in the obtaining of a noticeable source of compounds with high protective potential and antioxidant activity. Keywords: Antioxidant(s), FRAP, DPPH. Total phenol, Antimicrobial, Ephedra sarcocarpa References: 1. Benzie I F, Strain J (1996) Journal of Analytical Biochemistry 239: 70 – 76. 2. Hwang BY et al. (2001) Journal of Natural Products 64: 82 – 4. 3. Singleton VL, Rossi JA (1965) American Journal of Enology and Viticulture 16: 144 – 158. 4. Bauer AW, Kirby WMM, Sherries JC, Turck M (1966) American Journal of Clinical Pathology 45: 493 – 496. Is the inhibition of STAT3 phosphorylation in vascular smooth muscle cells by indirubin-3'-monoxime redox-dependent? Blazevic T, Schwaibaer A, Schreiner CE, Heiss EH, Aratamov AG, Dirsch VM Department of Pharmacognosy, University of Vienna, Althanstraße 14, A-1090 Vienna, Austria Indirubin is a natural product found in the traditional Chinese antileukemic recipe, Daogu Longhu Wang. Its reported pro-antiproliferative activity makes it a promising candidate in the treatment of cardiovascular diseases (CVDs). We showed recently that the derivative indirubin-3'-monoxime (I3MO) inhibits the proliferation of vascular smooth muscle cells (VSMC) by inhibition of STAT3 phosphorylation. The importance of reactive oxygen species (ROS) in STAT3 activation has been reported and oxidative stress has been implicated in many CVDs. Here, we examine the role of ROS as a putative target of I3MO acting upstream of STAT3. Employing the fluorescence probes 2',7'-dichlorodihydrofluorescein and Amplex Red, I3MO was shown to inhibit PDGF-induced STAT3 phosphorylation. Selective downregulation of Nox1 and Nox4 isoforms, using the inhibitory peptide, gp91tat and the siRNA approach, respectively, did not prevent I3MO-mediated STAT3 inhibition, which was linked to its anti-proliferative effect on VSMCs, shown to be redox-dependent. Despite being considered the major ROS-source in VSMCs, Nox isoforms, Nox1 and Nox4, are not targets of I3MO responsible for the effect on STAT3 phosphorylation. Keywords: indirubin, vascular smooth muscle cells, proliferation, atherosclerosis, STAT3, PDGF, reactive oxygen species, NAD(P)H oxidase. Acknowledgement: This work was supported by the Austrian Science Foundation (FWF) (P23317 to E.H.H., NFN S 107-BO3 to V.M.D. and P 18082 to V.M.D.) References: 1. Meijer L et al. (1999) Nature Cell Biol 1: 60 – 7. 2. Schwaibaer A et al. (2010) ATVB 30: 2475 – 81. 3. McCormick J et al. (2006) FASEB J 20: 2115 – 17. 4. Bronas, UG, Dengel DR (2010) Am J Lifestyle Med 4: 521 – 534. Antioxidant, antimicrobial, anti-inflammatory and anticancer activities of *Carthamus tinctorius* flowers Boureux NK, Quesslat S, Falleh H, Harboou F, Kouati R, Lagraoui B, Lachaal M<sup>1</sup> Unité de Physiologie et de Biochimie de la Tolérance au Sel des Plantes, FST, Campus Universitaire, 2022 Tunis El Manar, Tunisie; <sup>2</sup>Laboratoire des Plantes Extrêmesphiles, Centre de Biotechnologie à la Technopole de Borj Cédria (CBRC), BP 3501, 2600, Hammam-Lif, Tunisie; <sup>3</sup>Laboratoire LASEVE à l’Université du Québec à Chicoutimi, Québec, Canada. Carthamus tinctorius L. (Asteraceae) is an aromatic and folkloric medicinal plant thanks to its multiple virtues. However, few scientific studies investigated its biological activities. For that, this study aimed to investigate antioxidant, antibacterial, anti-inflammatory and anticancer activities of methanolic flower extracts of *Carthamus tinctorius* in order to validate some of its ethnopharmacological claims. Antioxidant activity was assessed via the ABTS radical scavenging and β-carotene inhibition tests, the antibacterial capacity were tested against human pathogen strains. Whereas, anti-inflammatory activity were estimated using inhibitory NO release in LPS-stimulated Raw 264.7 macrophages, in comparison with N(G)-nitro-L-arginine methyl ester (L-NAME), which was used as a positive control. In addition, anticancer activity was evaluated against Human lung carcinoma (A-549) and Human colorectal adenocarcinoma (DLD-1) cell lines. Main results showed that the flowers exhibit interesting biological activities. Indeed, flower extract displayed an inhibition percentage against ABTS equal to 30% and over 40% for the β-carotene inhibition assay. Antimicrobial activities were important especially against *M. luteus* strains (100% inhibition). Concerning anti-inflammatory activity, methanolic extract was able to inhibit NO release by 80% at 160 mg/mL. Furthermore, *C. tinctorius* extract showed an anticancer activity against tumor cell lines DLD-1 with an IC50 value of 17.9 μg/mL. These findings demonstrate the interesting potentiality of *Carthamus tinctorius* flowers as valuable source of antioxidant compounds which exhibit novel biological activities as antibacterial, anti-inflammatory and anticancer capacities. Keywords: *Carthamus tinctorius*, antioxidant capacity, antibacterial activity, anti-inflammatory activity, anticancer activity. Evaluation of Hydroalcoholic extract of *Astragalus fasciculifolius* Boiss. on Immunological factors IFN-γ, IL-4 in early sensitized mice induced by Ovalbumin Azemi M<sup>1</sup>, Ghaforian Boroujerdina M<sup>2</sup>, Namjooyan P<sup>2</sup>, Saintian H<sup>1</sup>, Yousef Naanaei S<sup>3</sup>, Hemmati A<sup>1</sup> <sup>1</sup>Medical Plant Research Center, Pharmacognosy Department, School of Pharmacy, Ahvaz Jundi Shapur University of Medical Sciences, Ahvaz, Iran; <sup>2</sup>Immunology Department, School of Medicine, Ahvaz Jundi Shapur University of Medical Sciences, Ahvaz, Iran; <sup>3</sup>Pharmacology-Toxicology department, School of Pharmacy, Ahvaz Jundi Shapur University of Medical Sciences, Ahvaz, Iran The genus Astragalus is a very large group of more than 2,000 species and about 800 species in Iran. Currently, much of the pharmacological research on Astragalus is focused on its immune-stimulating polysaccharides and other active ingredients useful in treating immune deficiency conditions. Astragalus has demonstrated a wide range of potential therapeutic applications in immunodeficiency syndromes, as an adjunct cancer therapy, and for its adaptogenic effect on the heart and kidneys. Astragalus can modulate the balance of Th1/Th2 cytokines; it decreases IL-4 and increases IFN-γ. Since, allergy conversely disturbs the balance of Th1/Th2, increases IL-4 and decreases IFN-γ, we decided to use Astragalus fasciculifolius Boiss. to improve the balance. Hydroalcoholic extract of *Astragalus fasciculifolius* assessed by phytochemical tests to recognize the main active constituents. Mice were sensitized with subcutaneous injection of 100 μg of ovalbumin, 1 mg aluminum hydroxide, days 1 and 7. Efficiency of sensitization was assessed by blood IgE levels. then 14
We have also found a good correlation ($R^2 = 0.684$) between DPPH radi- cal scavenging activity and $\alpha$-glucosidase inhibition. Our results support the findings that antioxidants (specifically, radical-scavengers) play an important role in the control and management of diabetes [2]. Keywords: Rubiaceae, Rubioidae, anti-diabetic, anti-inflammatory, $\alpha$-glyco- sidase, NO inhibition Acknowledgement: Research and Management Institute (RMI), University of Vienna, Austria; Ministry of Higher Educa- tion (MOHE) for research grant 600-RMI/STF/RGS 5/3(FST35/2009) and Dr Shamshul Khamis from Universiti Putra Malaysia for identification of plants. References: 1. Ahmad R et al. (2010) African Journal of Biotechnol- ogy 9: 7948 – 7954. 2. Rahimi R, Nikfar S, Larjani B & Abdollahi M (2005) Biomedicine and Pharmacotherapy 59: 365 – 373.

**PM138**

Betulonic acid enhances glucose uptake in 3T3L1 adipocytes after long term treatment

Kramer MP, Baumgarner RR, Atanasov AG, Dirsch VM, Heiss EH

Department of Pharmacognosy, University of Vienna, Althanstrasse 14, A-1090 Vienna, Austria

The metabolic syndrome including hyperglycaemia and insulin resist- ance is on the rise worldwide and consequently also cardiovascular diseases and Diabetes Mellitus Type 2. Currently used drugs for these indications are effective, but possess side effects when used chronically. Nature could provide a variety of compounds with undetermined poten- tial to treat and prevent these disorders. In this study, we tested betu- linic acid (BA), a naturally occurring pentacyclic triterpenoid, in two diabetes-related assays, namely inhibition of the Protein Tyrosine Phos- phatase 1B (PTP1B) in vitro and 2-deoxy-D-glucose (3 H-DG) uptake in 3T3L1 adipocytes. BA inhibited PTP1B activity and did not influence 3 H-DG uptake. However, in differentiated 3T3L1 adipocytes, BA (10 $\mu$M) elicited a 1.8-fold increase of the basal glucose (3 H-DG) up- take rate after 48 hours of treatment. The observed increase in glucose uptake was further enhanced by insulin stimulation and not accompa- nied by a decrease in cell viability as evident by unaltered cell morphol- ogy under the microscope and lack of procaspase 3 cleavage shown by immunoblots. Interestingly, incubation of RAW264.7 macrophages and immortalized human umbilical vein endothelial cells (HUVECs) with 10 $\mu$M BA also increased their basal glucose uptake rate approximately 1.4-fold and 1.7-fold, respectively. Given the vast number of so far re- ported anti-fungal, anti-viral, anti-bacterial and anti-cancer properties of BA [1], our data indicate that BA may be successfully repurposed also for metabolic disorders (hyperglycaemia), and warrant further investiga- tions concerning the underlying mode of action. Keywords: adipocytes, hyperglycemia, triterpenoids, metabolic syndrome References: [1] Mula- lauer FB et al. (2010) Anticancer Drugs 21(3):215 – 227

**PM140**

Anti-sickling properties of Nigerian plants

Elsuwan CA1, Oluagba TA2

1Drug Research and Production Unit, Faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife, Nigeria; 2Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife, Nigeria

Sickle cell disorder is a public health problem in many countries parti- cularly in Africa. It is one of the most prevalent hematologic genetic disorders which results from a single point mutation of $\beta$Glu in Hb to $\beta$Val in Hbs (1). No drug could effectively cure the disorder but a potentially useful drug if available should effectively provide relief by alleviation of its symptoms. Nonetheless, there are few anti-sickling agents to date available for clinical use (2). In view of its genetic origin, advocacy remains the only option for the prevention of the disorder. However, with over 1 million individuals worldwide with sickle cell disorder, the search for ideal anti-sickling drugs is a major priority. The study reports the anti-sickling properties of eight Nigerian plant species with inhibitory and reversal properties. Extracts of the Cola species tested, in particular, showed the same order of activity as p-hydroxybenzoic acid, the positive control. Keywords: Sickle cell disorder, plants, Cola species, anti-sickling properties References: [1] Quartta B et al. (2009) Phytomedicine 16: 125 – 129 2. Martin KS et al (2004) Med Chem 47: 4665 – 4676

**PM141**

Evaluation of anti-diabetic and anti-inflammatory properties of Malaysian Rubiaceae and correlation to their antioxidant potential

Ahmad R1, Mahbob EN2, Luijs NH1, Shaari K2, Ahmad S2

Faculty of Applied Sciences, University Technology MARA, Shah Alam 40450, Selangor, Malaysia; Institute of Bioscience, University Putra Malaysia, Serdang 43400, Selangor, Malaysia

We have previously reported the antioxidant activities of methanolic extracts of 22 species of Rubiaceae plants (family Rubiaceae) [1]. In this paper, we now report the antihyperglycemic and anti-inflammatory properties of the 22 species. The assays employed were $\alpha$-glucosidase inhibition assay for antidiabetic potential and Griess assay for the mea- surement of nitric oxide (NO) inhibition in lipopolysaccharide (LPS) and interferon-$\gamma$ (IFN-$\gamma$)-treated RAW 264.7 cells. In the $\alpha$-glucosidase inhibi- tory assay, extracts of Hydronymphium formicarum Jack, Psychotria grif- fithii Hook.f. and Urophyllynum griffithianum Hook.f. were shown to be effective inhibitors against $\alpha$-glucosidase. The results indicated that H. formicarum and P. griffithii showed high percent inhibition in the $\alpha$- glucosidase inhibitory assay with percent inhibition of 89.8% and 87.7%, respectively. U. griffithianum showed moderate activity with per- cent inhibition of 68.4% while other species showed no activity. In the anti-inflammatory assay, Hedyotis philippinensis (Wild. ex Sprague) Merr. ex C.B. Rob. (leaves and stems), Spermacoce exilis (Wild. ex Spreng.) Merr. et C.D. Ainsworth and Damarae seenii (L.O.Williams) C.D.Ainsworth showed potent inhibitory activity on NO production in LPS and interferon-$\gamma$ (IFN-$\gamma$)-treated RAW 264.7 cells. We have also found a good correlation (R$^2$ = 0.684) between DPPH radi- cal-scavenging activity and $\alpha$-glucosidase inhibition. Our results support the findings that antioxidants (specifically, radical-scavengers) play an important role in the control and management of diabetes [2]. Keywords: Rubiaceae, Rubioidae, anti-diabetic, anti-inflammatory, $\alpha$-glucosidase, NO inhibition Acknowledgement: Research and Management Institute (RMI), University of Vienna, Austria; Ministry of Higher Educa- tion (MOHE) for research grant 600-RMI/STF/RGS 5/3(FST35/2009) and Dr Shamshul Khamis from Universiti Putra Malaysia for identification of plants. References: 1. Ahmad R et al. (2010) African Journal of Biotechnol- ogy 9: 7948 – 7954. 2. Rahimi R, Nikfar S, Larjani B & Abdollahi M (2005) Biomedicine and Pharmacotherapy 59: 365 – 373.

The arachidonic acid metabolism is the main target for non-steroidal anti-inflammatory drugs (NSAIDs). Two cyclooxygenases (constitutive COX-1 and inducible COX-2) and lipooxygenase (5-LOX) enzymes are responsible for transformation of arachidonic acid into the potent bio- logically active lipid mediators that are intimately involved in inflam- mation [1]. The newly developed COX-2 selective inhibitors seem to possess lower risk of unwanted side-effects than traditional NSAIDs. In our previous study, we identified potential COX-2 inhibiting plant ma- terial in Ranunculaceae family [2]. It is now being perceived, that dual blocking of both COXs and 5-LOX is promising approach to treatment of inflammatory diseases [3]. Thus we decided to evaluate the in vitro inhibitory activity against both COXs and 5-LOX of ethanolic extracts prepared mainly from roots of more than 30 plant species belonging to plant family Ranunculaceae using method previously described by Re- ininger and Bauer [4] and Adams et al. [5], respectively. The amounts of prostaglandin E2 (for COX) and leukotriene B4 (for 5-LOX) were deter- mined by commercial ELA kits (Assay Designs). The highest prevention against production of COXs and 5-LOX derived eicosanoids possessed extract from roots of Helleborus purpurascens Waldst. & Kit., where COX-1/COX-2/5-LOX scavenging rate 1.5/1/1.2 was recorded. The con- sequent bioactivity guided fractionation showed, that isomers of linoleic acid seem to be responsible for blocking of even COXs or 5-LOX. Cimic- fuge racemosa (L.) Nutt. and Trollius altissimus Crantz had been deter- mined as other promising plant materials, suggesting these species po- tential for further research for new anti-inflammatory substances. Keywords: cyclooxygenases, lipooxygenase, anti-inflammatory, Ranuncula- ceae, in vitro Acknowledgement: This research was supported by Czech Science Foundation (Project No. 525/08/1179). References: 1. Clara J.
Sutherlandia frutescens targets adipose tissue mitochondrial metabolism
Koekemoer T, Mackenzie J, Deauty G, Roux S, Van De Venter M
Nelson Mandela Metropolitan University, Port Elisabeth, South Africa

Sutherlandia frutescens (L.) R.Br. ex W.T.Aiton is an indigenous South African medicinal plant traditionally used to treat a number of ailments including diabetes. While previous in vivo studies have confirmed its anti-diabetic properties, the precise molecular mechanism of action has not been elucidated. In the present study we have established that S. frutescens treatment specifically attenuates a number of adipose tissue related parameters, including circulatory and adipose tissue free fatty acid and triglyceride levels. The lack of any significant changes in adipose tissue nitrotyrosine and plasma MCP-1, both classical markers for adipose inflammation, indicates that these effects are not attributable to anti-inflammatory properties. In 3T3-L1 preadipocytes, treatment led to a significant increase in the rate of glucose consumption despite the complete absence of triglyceride accumulation. This increased glucose consumption is reflected by a corresponding dose dependent increase in lactate production, suggesting an increased glycolytic flux in treated cells. Taken together in vivo and in vitro findings are consistent with a hypothesis in which S. frutescens induces mitochondrial uncoupling in adipose tissue, resulting in a reduced efficacy of oxidative phosphorylation and a consequent up-regulation of glycolysis. In this manner the carbon flux is redirected away from lipid synthesis resulting in both decreased free fatty acid production and triglyceride accumulation in adipose tissue. Increased markers for mitochondrial function, elevated levels of phosphorylated AKT and the effects on PI3K regulated glucose uptake in 3T3-L1 treated cells provide further support that S. frutescens counteracts adipocyte dysfunction associated with the development of diabetes. Keywords: Sutherlandia frutescens, adipose tissue, lipid metabolism, mitochondria.

Effects of the extracts from Turkish medicinal plants on NF-κB activation on LPS-induced RAW 264.7 macrophages
Atay I, Iler AZ, Tekci D, Kirmizibekmez H, Yesilada E
1Department of Pharmacogony, Faculty of Pharmacy, University of Yeditepe, 34755 Kayisidagi, Istanbul, Turkey; 2Department of Genetics and Bioengineering, Faculty of Engineering and Architecture, University of Yeditepe, 34755 Kayisidagi, Istanbul, Turkey

NF-κB is a transcription factor mediating the expression of several genes involved in inflammation and its inhibition might be a valuable strategy to develop effective anti-inflammatory agents [1]. Sambucus ebulus L., S. nigra L. (Caprifoliaceae) and Cistus laureifolius L. (Cistaceae) which are used in Turkish folk medicine for treatment of rheumatism and related inflammatory problems, were evaluated for NF-κB inhibitory activity [2,3]. Each plant was extracted with ethanol or methanol and then fractionated by successive solvent extractions to obtain subextracts; i.e. hexane, chloroform, ethyl acetate, and methanol. Effects of the extracts on the viability of RAW 264.7 macrophages were determined by using WST-1 cell viability assay. Effects on NF-κB activation in lipopolysaccharide (LPS) induced RAW 264.7 macrophages were studied by using Electromobility Shift Assay (EMSA). RAW 264.7 cells were preincubated for two hours with indicated non-toxic concentrations of extracts and then stimulated with LPS (1 μg/ml). Cells were harvested, nuclear proteins were extracted and assayed for NF-κB-DNA binding affinity by EMSA. Results were quantified by densitometric analysis using Image J program. S. ebulus hexane subextract (50 μg/ml) exhibited the highest activity which led to 51.3% decrease of NF-κB activation followed by ethyl acetate (100 μg/ml) and chloroform (100 μg/ml) subextracts which showed 32.6%, 28.1% inhibition respectively, while the remaining water subextract (100 μg/ml) showed the lowest activity (9.3%). Only hexane extract of S. nigra exhibited an inhibition (25.3%) of NF-κB activation at 50 μg/ml. The hexane, chloroform and remaining water subextracts of C. laureifolius showed 10.7%, 11.9% and 18.8% inhibition at 100 μg/ml, respectively. Keywords: NF-κB, RAW 264.7, Sambucus, Cistus, anti-inflammatory
Acknowledgement: This study is supported by Turkish Scientific and Technological
PM146

Wound Healing Effects of New Cream Formulations
Ağdıç D1, Üzuner YV1, Kilic E2
1Yeditepe University, Faculty of Pharmacy, Department of Pharmaceutical Technology, 34755 Kayıdağları Istanbul/Turkey; 2Yeditepe University, School of Medicine, Department of Physiology, 34755 Kayıdağları Istanbul/Turkey

Some of the many potentially beneficial ingredients that are traditionally used in wound healing, are obtained from some plants but their effectiveness has not been scientifically evaluated yet. In this study, new cream formulations (Levant storax and Complex creams) with the same cream base were developed. The composition of the cream base was as follows: (1) Butyroperspermum parkii extract, (2) squalane, (3) cetyl alcohol, squalene, (4) cetyl stearal alcohol, (5) caprylic/capric triglycerides, (6) petrolatum, (7) glycerine, (8) tetrasodium EDTA, (9) methylparaben, ethylparaben, propylparaben, butylparaben and (10) deionized water. In addition to the ingredients used in the cream base, Levant storax cream also contained balsam of Peru, Kotschy, squalane, cetyl alcohol, sorbitan olivate, and, in particular, PGF2α. We comparatively assessed the wound healing potential of the two formulations against a reference cream, Madecassol and the cream base by using in-vivo excisional wound model on rats. All wounds were photographed in the presence of a standard ruler by Dlite Analog Microscope in the first and last day of the study. The wound areas were computed by using the Image J software and the wound contraction rates were calculated as a percentage of the reduction in wounded area and analyzed for statistical significance. According to the results, Levant storax cream was the best formula with the highest contraction rates, the Complex cream was as effective as the reference cream and better than the cream base. Keywords: Wound, Excision, Liquidambar orientalis, Wound healing References: 1. Bruneton J (1999) Pharmacognosy, Phytochemistry, Medicinal Plants. 2nd edition, Lavoisier Tec&Doc, Paris, 520 2. Hafizoglu H, Reunanen M, Istek A (1996) Holzforschung 50: 116 – 117

PM147

Hair-growth promoting effect of bimatoprost
Kang J1, Kim S2, Kim E1, Park D2, Koh Y1, Yoo E1, Kang H1
1Department of Pharmacology, School of Medicine, Institute of Medical Science, Jeju National University, Jeju, South Korea; 2Department of Histology, School of Medicine, Institute of Medical Science, Jeju National University, Jeju, South Korea; 3Department of Microbiology, School of Medicine, Institute of Medical Science, Jeju National University, Jeju, South Korea

Importance of prostaglandin pathway in hair growth has been reported and, in particular, PGF2α was reported to promote hair growth. We thus examined the efficacy of several PGF2α analogues such as latanoprost, bimatoprost, unoprostone and travoprost, on the proliferation of dermal papilla cells (DPC), regulator of hair cycle and length of hair follicles, using immortalized DPC from rat vibrissa follicles. Among these compounds, bimatoprost showed outstanding effectiveness on the proliferation of DPC. When rat vibrissa follicles were treated with bimatoprost, hair-fiber length of vibrissa follicles significantly increased. When we examined the effect of bimatoprost on the regulation of cell cycle, bimatoprost was found to decrease the Sub-G1 population and to increase the expression of cell cycle regulated proteins such as CDK2 and Cyclin E in DPC. Bimatoprost also increased the expression of β-catenin as well as the expression of Cox-2, target gene of β-catenin. Taken together, our results suggest that bimatoprost increased the hair growth by progression of cell cycle through upregulation of CDK2, Cyclin E and β-catenin. Keywords: bimatoprost, hair growth, dermal papilla cells, cell cycle, vibrissa follicle

PM148

Anticancer effect of a cembrendolide diterpene LS-1 in colon cancer cells through activation of oxidative stress
Kim E1, Hong J2, Kang J3, Park D2, Koh Y1, Yoo E1, Kang H1
1Department of Pharmacology, Jeju National University, Jeju, South Korea; 2Department of Histology, Jeju National University, Jeju, South Korea; 3Department of Microbiology, Jeju National University, Jeju, South Korea

We observed that LS-1, cembrendolide diterpene, inhibited growth and induced apoptosis in colon cancer cells via a ROS dependent mechanism. Treatment of HT-29 cells with LS-1 resulted in ROS generation, which was accompanied by disruption of mitochondrial membrane potential, cytochrome c release, and activation of Bid, caspase-3, -8, and -9, and cleavage of PARP along with the suppressive expression of Bcl-2. All these effects were significantly blocked on pretreatment with the ROS inhibitor N-acetylcystein (NAC), indicating the involvement of increased ROS in the proapoptotic activity of LS-1. Moreover, we showed that LS-1 induced the phosphorylation of JNK and dephosphorylation of p38, ERK, Akt, Src and STAT3, which were effectively attenuated by NAC. In addition, the expression of antioxidant catalase was abrogated by treatment using LS-1 with or without NAC. These findings reveal the novel anticancer efficacy of LS-1 mediated by the induction of apoptosis via ROS generation in human colon cancer cells. Keywords: LS-1; cembrendolide diterpene; HT-29; Apoptosis; ROS

Acknowledgement: This work was supported by the National Research Foundation of Korea Grant funded by the Korean Government (NRF-2009 – 315 – 2-E00072) and Jeju National University Hospital Research Fund (2010).

PM149

Cytotoxic properties of five Centaurea L. species from Anatolia
Baykan Erel S1, Demir S2, Aydin Kose F2, Ballar P3, Karaalp U1
1Department of Pharmaceutical Botany, faculty of Pharmacy, Ege University, 35100, Bornova, Izmir, Turkey; 2Department of Biochemistry, Faculty of Pharmacy, Ege University, 35100, Bornova-Izmir, Turkey

The genus Centaurea L. (Asteraceae) comprises about 192 taxa in the flora of Turkey distributed throughout the Anatolian peninsula, with 61% being endemic (1 – 3). Many species of the genus have long been used traditionally to treat various ailments e.g. cough, hemoroid, peptic ulser and abscess (4). Pharmacological studies on some Centaurea species have reported antiinflammatory, antimicrobial, antipyretic, cytotoxic and immunological activities (5). In this study, methanolic extracts of five Centaurea L. species (C. iberica Boiss., C. athoa DC, C. hitchclisia DC, C. ibirica Trev. and C. polyclada DC) were investigated for their cytotoxic activities against three human cancer cell lines; MCF7 (human breast cancer), A549 (human lung cancer), U2OS (human osteocarcoma) and one non-cancer cell line, 293HEK (human embryonic kidney) by cell proliferation assay using WST-1 reagent. C. polyclada extract was the most active one against MCF7 (IC50: 61 μg/ml), U2OS (IC50: 63 μg/ml) and 293HEK (IC50: 72 μg/ml) cell lines. C. iberica also showed significant effect on 293HEK (IC50: 85 μg/ml) and MCF7 (IC50: 90 μg/ml). This is the first cytotoxic activity report for the Centaurea species mentioned above. Keywords: Centaurea, Asteraceae, cytotoxicity References: 1. Bona B et al. (2008) IJUS J Biology 67(1):55 – 63 2. Wagenitz G (1975) In: Flora of Turkey and the East Aegean Islands, Davis P.H. (Ed.) Edinburgh, Edinburgh University Press, Vol. 5, p: 465 – 585 3. Uysal T (2008) Ann Bot Fennici 45: 135 – 137 4. Baytop T (1999) Türkiye’dede Bitkilerde Tedavi (Gerçekte ve Bugünün), Nobel Tip Kitabevleri, İstanbul, 2.baskı, s:316 5. Arif R, Küpel E, Ergun F (2004) GUI Science 17(4): 149 – 164
investigation of in vivo anti-inflammatory and analgesic effects of rose hip powder (Rosa canina L.)
Saaby L1, Jüger AK2, Heegaard A3, Christensen SB1
1Department of Medicinal Chemistry, Faculty of Pharmaceutical Sciences, Universitésparken 2, 2100 Copenhagen, Denmark; 2Department of Pharmaceutics and Pharmacotherapy, Faculty of Pharmaceutical Sciences, Universitésparken 2, 2100 Copenhagen, Denmark

The standardized rose hip powder LitoMove® (Rosa canina L.) is a widely used herbal remedy. Consumption of LitoMove® has been shown to reduce pain in patients with osteoarthritis (1). The dichloromethane extract of LitoMove® possess in vitro immunomodulating effects which have been correlated to the presence of triterpene acids (2). To establish if the clinical effect of LitoMove® is caused by anti-inflammatory or analgesic effects, the dichloromethane extract was tested in the paw edema model of inflammation and the 1:1 methanol: dichloromethane extract in the hot plate test of acute pain. In both models, extracts were administered orally once daily in the indicated period. Treating rats with 100 mg dry extract/kg for three weeks did not result in a significant reduction of the paw edema compared to the control group. In the hot plate test, mice were treated with 500 mg dry extract/kg for five days. No significant difference in pain threshold between the treatment and the control group could be observed. It thus appears that the tested extracts neither possess anti-inflammatory nor analgesic effects in the chosen animal models. However, the paw edema model and the hot plate test are general models of inflammation and pain and thus, may not represent the inflammation and pain in arthritis. Therefore, further studies in specific animal models of arthritis inflammation and pain are needed before the clinical effects of LitoMove® can be understood. Keywords: Rosa canina, arthritis, inflammation Acknowledgement: HybenVital and the Danish Rheumatism Association are thanked for financial assistance. References: 1. Christensen R et al. (2008) Osteoarthritis and Cartilage 16: 965 – 972, 2. Saaby L et al. (2011) Phytother Res 195 – 201.

Anti microbial activities of leaves and stems of Ulmus minor Mill. subsp. minor
Tag O1, Yasa F2, Polat E3, Özgöç F4, Karayıldırım T5
1Ege University, Faculty of Science, Department of Chemistry, Bornova, 35010, İzmir, Turkey; 2Department of Biology, Faculty of Science, Ege University, Bornova, 35010 İzmir, Turkey; 3Department of Biology, Faculty of Science and Art, Yüzüncü Yıl University, 65080 Van, Turkey

Ulmus L. (Ulmaceae) is represented by three species in Turkey. Ulmus minor Mill. subsp. minor is a tree to 30 m but often much smaller, suckering. Twigs glabrous or sparingly pubescent [1]. Although there are no reports of the medicinal uses of Ulmus minor Miller subsp. minor in Turkey, the bark of the root and stem of Ulmus davidiana var. japonica has been used as a traditional Korean medicine to treat inflammatory disorders. This plant reportedly exhibits antioxidant, anticancer, and anti-inflammatory effects [2]. Ulmus species contain biologically active compounds, such as sesquiterpenoids, triterpenes and flavonoids [3,4]. In this study, n-hexane, dichloromethane, ethyl acetate, methanol, and methanol:water (20:80) extracts of leaves and stems of Ulmus minor subsp. minor were tested separately against selected Gram-positive, Gram-negative bacteria, and Candida albicans, an unicellular yeast, using a broth microdilution broth susceptibility assay. All of the extracts exhibited antimicrobial activity against Enterococcus faecalis and Salmonella typhimurium resulting MIC values 0.81 and 25 mg/mL. Ethyl acetate extract of the stems of the plant was found to be active against all tested microorganisms with a range of MIC values extended from the concentration of 0.25 – 0. 2 mg/mL. Keywords: Ulmus minor, Ulmaceae, anti-microbial activity References: 1. Davis PH (1982) Flora of Turkey and East Aegean Islands, University Press, Edinburgh. 2. Choi SY et al. (2010) J Med Food 13: 1019 – 1023. 3. Zheng MS et al. (2010) Biomol Ther 18: 321 – 328. 4. Lee CY et al. (2008) Planta Med 74: 1800 – 1802.

Antimicrobial studies on Semecarpus kathalekanensis
Husakadl P1, Harashiti MK2, Hegde H3
1Department of Pharmaceutical Biotechnology, KLE University College of Pharmacy, Nehrungagar, Belgaum-590 010, Karnataka, India; 2Department of Pharmaceutical Biotechnology, KLE University College of Pharmacy, Nehrungagar, Belgaum-590 010, Karnataka, India; 3Regional Medical Research Centre, ICMR, Belgaum-590 010, Karnataka, India

Semecarpus kathalekanensis Dassapa & Swaminath, an evergreen tree with very large simple leaves, which attains a height of about 30 m belonging to the family Anacardiaceae which is critically endangered swamp tree and consists major chemical compounds like phenols, bio-flavonoids and traditionally having high medicinal importance also used as an antimicrobial, antioxidant, and as an anticancer. The endophytic fungi were isolated from plant species and subjected for antimicrobial studies which showed significant results against gram positive and gram negative bacteria. Keywords: Endemic, Endophytic and Semecarpus kathalekanensis

The influence of extracts from Potentilla species on normal human colon cells
Paduch R1, Tomczyk M2, Wiatr A3, Hlezczynska M4, Kandefer Szerzen M5, Szczodrok J6
1Department of Virology and Immunology, Institute of Microbiology and Biotechnology, Maria Curie-Skłodowska University, ul. Akademicka 19, 20 – 033 Lublin, Poland; 2Department of Pharmacognosy, Faculty of Pharmacy, Medical University of Białystok, ul. Mickiewicza 2a, 15 – 230 Białystok, Poland; 3Department of Industrial Microbiology, Institute of Microbiology and Biotechnology, Maria Curie-Skłodowska University, ul. Akademicka 19, 20 – 033 Lublin, Poland

The biological activity of extracts obtained from aerial parts of Potentilla species: P. erecta (L), Rauschel, P. anserina L., P. argentea L., P. grandiflora L. was analyzed. Extracts were tested using MTT, NR and DPPH tests on two human normal cell lines: CCO 841 CoT and CCO-18Co. Fluorescence staining of the cellular cytoskeleton after rhodamine-phalloidin addition and IL-6, IL-10 (ELISA) in culture supernatants after 24h of incubation with Potentilla extracts and nitric oxide (NO) analysis with a Griess method were performed. Extracts were tested at the range of 25 – 225 µg/mL concentrations while to the ELISA two non-toxic doses (15 and 30 µg/mL) were chosen. We found that all extracts stimulated metabolism of epithelial cells while myofibroblasts’ mitochondriaal dehydrogenase activity was stimulated at concentrations higher than 125 µg/mL. The exception was P. grandiflora which activated succinyl dehydrogenase just at low extract dose (25 µg/mL). Extracts from P. erecta and P. argentea had no toxic effect on colon epithelial cells while other extracts significantly decreased viability of cells even when added at 25(g/mL concentration. Only P. grandiflora and P. argentea significantly decreased viability of myofibroblasts. All extracts showed free radical scavenging effect in a concentration dependent manner. Potentilla extracts inhibited IL-6 and IL-10 production by myofibroblasts while in epithelial cells slightly induced or had no effect on the cytokine level. Potentilla extracts influenced F-actin filament composition and changed the cellular cytoskeleton and morphology of cells. Modulation of NO production after plant extracts addition has also been observed. Keywords: Potentilla, cytotoxicity, normal human colon cells

Antimicrobial and cytotoxic activities of roots of Centaurea cadmea Boiss.
Alizadeh Astari K1, Baykan Erel S2, Koksal Ç3, Aydn Kose P4, Karaçep S5
1Department of Pharmaceutical Botany, Faculty of Pharmacy, Ege University, 35000 Bornova-Izmir, Turkey; 2Department of Biology, Faculty of Science, Ege University, 35000 Bornova-Izmir, Turkey; 3Department of Biochemistry, Faculty of Pharmacy, Ege University, 35000 Bornova-Izmir, Turkey

Centaurea cadmea Boiss. is an endemic taxon for Anatolia, growing wild in N, W & SW of Turkey (1). Phytochemical studies revealed the presence of a sesquiterpene lactone, ivalin, which is known cytotoxic co-
pound on several tumor cell lines (2), together with eudatolin, 5-hydroxy-3',4',6,7-tetramethoxyflavone and β-sitosterol from the aerial parts of C. cadmea (3). In vitro anti-inflammatory, antioxidant, antiproliferative and antimicrobial activities of the aerial parts of C. cadmea extracts have been reported before (4, 5), but no bioactivity study has been performed on roots of the plant, yet. The present study aims at investigating the antimicrobial and cytotoxic activities of roots of C. cadmea. The antimicrobial activities of extracts of the plant were investigated by MİC method. The antimicrobial activities of the extracts were tested against four main components investigated. The most sensitive microorganism to perilla aldehyde (54.3%) and limonene (30.1%) were the main constituents. The oil was rich in oxygenated compounds (73.1%) and hydrocarbons (25.0%). Monoterpenes including perilla aldehyde, limonene, P. patientia, Rosmarinus officinalis L., antioxidant, anti-inflammatory activity, natural products, target-based assays

Rumex patientia L., a member of Polygonaceae family, is a perennial plant widely distributed and cultivated in Eastern Europe. Roots of R. patientia have been used extensively in traditional medicine worldwide for treatment of different disorders due to their laxative, diuretic, antipyretic, wound cure and anti-inflammatory properties. It has been reported that R. patientia contains anthraquinones, tannins, flavonoids and phenolic acids. Some of these compounds have shown anti-inflammatory and antioxidant effects. Therefore the present study was undertaken to evaluate the chemical composition, antioxidant and anti-inflammatory properties of ethanolic extracts of aerial plants and roots of R. patientia. Phytochemical profile was determined by measuring total phenolic and total flavonoid content and by qualitative (LC-MS/MS) and antioxidant activity of the extracts. The antioxidant activity was evaluated by measuring ferric reducing ability (FRAP) of the extracts and their radical scavenging capacity towards DPPH, OH, NO and superoxide radicals (1). The anti-inflammatory activity considering inhibitory potency toward production of 12-HETE, 12-HHT, PGE2 and TXB2 was investigated. (2) Experimental data, obtained by the study of R. patientia specimens collected at three different locations in Serbia, showed that ethanolic extracts of aerial parts have higher total phenolic and total flavonoid contents, reducing capacity and OH scavenging ability in comparison to root extracts. On the other hand, root extracts exhibited higher DPPH and superoxide scavenging activity. Both root and herb extracts showed dose-dependent inhibition of 12-HETE, 12-HHT, PGE2 and TXB2 production. Herb extract exhibited higher COX/LOX pathway inhibitory activity than root extract. Keywords: Rumex patientia L., antioxidant, anti-inflammatory, LC-MS/MS. Acknowledgement: Ministry of science and technological development, Republic of Serbia, grant No. 0172058 References: [1] Beara IN et al. (2009) J Agric Food Chem 57:9268–9273 [2] Beara IN et al. (2010) J Pharm Biomed Anal 52:701–706.

Antibacterial activity of the essential oil and main components of two Dracocephalum species from Iran

Sobahi A*, Cholipour A*, Yousefzadeh M^1
^1Department of Biology, Medicinal Plants and Drugs Research Institute, Shahid Beheshti University, Tehran, Iran; ^2Department of Biology, Payne Noor University (PNU), Sari, Mazandaran, Iran; ^3Department of Marine Biology, Hormozgan University, Bandar Abbas, Iran

Antibacterial activity of Dracocephalum polychaetum Bornm. and D. sur- mangium Royle ex DC. essential oils and two main components were investigated. Essential oils of the plants were analyzed by GC and GC-MS (1). The antibacterial activities of the extracts were tested against four main components investigated. The most sensitive microorganism to perilla aldehyde (54.3%) and limonene (30.1%) were the main constituents. The oil was rich in oxygenated compounds (73.1%) and hydrocarbons (25.0%). Monoterpenes including perilla aldehyde, limonene, P. patientia, Rosmarinus officinalis L., antioxidant, anti-inflammatory activity, natural products, target-based assays

Rumex patientia L., a member of Polygonaceae family, is a perennial plant widely distributed and cultivated in Eastern Europe. Roots of R. patientia have been used extensively in traditional medicine worldwide for treatment of different disorders due to their laxative, diuretic, antipyretic, wound cure and anti-inflammatory properties. It has been reported that R. patientia contains anthraquinones, tannins, flavonoids and phenolic acids. Some of these compounds have shown anti-inflammatory and antioxidant effects. Therefore the present study was undertaken to evaluate the chemical composition, antioxidant and anti-inflammatory properties of ethanolic extracts of aerial plants and roots of R. patientia. Phytochemical profile was determined by measuring total phenolic and total flavonoid content and by qualitative (LC-MS/MS) and antioxidant activity of the extracts. The antioxidant activity was evaluated by measuring ferric reducing ability (FRAP) of the extracts and their radical scavenging capacity towards DPPH, OH, NO and superoxide radicals (1). The anti-inflammatory activity considering inhibitory potency toward production of 12-HETE, 12-HHT, PGE2 and TXB2 was investigated. (2) Experimental data, obtained by the study of R. patientia specimens collected at three different locations in Serbia, showed that ethanolic extracts of aerial parts have higher total phenolic and total flavonoid contents, reducing capacity and OH scavenging ability in comparison to root extracts. On the other hand, root extracts exhibited higher DPPH and superoxide scavenging activity. Both root and herb extracts showed dose-dependent inhibition of 12-HETE, 12-HHT, PGE2 and TXB2 production. Herb extract exhibited higher COX/LOX pathway inhibitory activity than root extract. Keywords: Rumex patientia L., antioxidant, anti-inflammatory, LC-MS/MS. Acknowledgement: Ministry of science and technological development, Republic of Serbia, grant No. 0172058 References: [1] Beara IN et al. (2009) J Agric Food Chem 57:9268–9273 [2] Beara IN et al. (2010) J Pharm Biomed Anal 52:701–706.

Screening of Anti-inflammatory Activity of Natural Products through A Panel of Target Based Assays

Nalbantsoy A^1, Khan P^2, Khan S^3,4
^1Ege University, Faculty of Engineering, Bioengineering Department, 35100 Bornova, Izmir, Turkey; ^2National Center for Natural Products Research, Research Institute of Pharmaceutical Sciences; ^3Department of Pharmacognosy, The University of Mississippi, MS, 38677, USA; ^4Department of Pharmacognosy, College of Pharmacy, King Saud University, 11451 Riyadh, Saudi Arabia

Inflammation is considered as a risk factor for several types of cancers, obesity and metabolic disorders. Chronic inflammation has been linked to various steps involved in tumorigenesis, including cellular transform-
Long-term effects of the rhapontic rhubarb extract ERF 731® on estrogen-regulated targets in the uterus and on the bone in ovariectomized rats

Keiler A, Kretzschmar G, Zierau O, Vollmer G
Technische Universität Dresden, Molecular Cell Physiology & Endocrinology, Zellescher Weg 20b, 01217 Dresden, Germany

The efficacy of the commercially available extract ERF 731® from Rheum rhaponticum L. regarding attenuation of menopausal complaints like hot flushes, depression, anxiety and vaginal dryness has been proven in a two-year clinical study. Further, no undesired side effects like uterotrophy or proliferation of the endometrium became apparent while testing ERF 731® in a 3-day uterotrophic assay. The present study aimed at further substantiating the safety of application of ERF 731® regarding endometrial hyperplasia and at the same time test for potential bone sparing effects in the preclinical ovariectomized (ovx) rat model. For this purpose we performed a 90d dietary feeding study in ovs rats. The impact of exposure on uterine proliferation was investigated by assessing the mRNA levels of proliferation marker genes (Mki67, Pcnal) in comparison to the expression of the mRNAs of the estrogen receptors ESRI and ESRII and the estrogen response gene C3. To test for potential effects on the bone, we additionally performed densitometry analysis of the proximal tibia metaphysis using peripheral computed tomography and quantified bone homeostasis marker in the serum. With this study design, neither an uterotrophic response nor a modulation of mRNA levels of proliferation markers was detected after 90d of dietary exposure with the rhapontic extract. Furthermore, no effect of the two adiministered doses of ERF 731® on E2 deprivation induced bone loss became apparent. In conclusion, the observations from previous trials regarding the endometrial safety of ERF 731® application were substantiated, but no effect on the Bone Mineral Density could be observed. Keywords: Rheum rhaponticum, ovariectomized rat, bone loss, endometrium, proliferation

PM158 Phenolic profile and biopotential of Plantago schwarzenbergiana Schw. Bara I, Lesjak M, Orcic D, Simin N, Jovin E1, Anacak G2, Mimica Dukic N1
1Department of Chemistry, Biochemistry and Environmental Protection, Faculty of Sciences, University of Novi Sad, Trg D. Obradovica 3, 21000 Novi Sad, Serbia
2Institute of Medical Science, Jeju National University, Jeju, South Korea

Ancient use of plantains (genus Plantago L., Plantaginaceae) as herbal remedies is a consequence of their antiseptic, anti-toxic, antimicrobial, expectorant and diuretic properties. Plantago schwarzenbergiana Schur. is distributed in the Balkan Peninsula, but there is no data about biological activity of this species. In order to valorize medicinal use of this species, in order to valorize medicinal use of this plantain, some tests on antioxidative and anti-inflammatory activities of methanolic extract of this plantain, collected from area of Schwarzenbergiana/C210, were determined using assays which measure the inhibitory capacity towards COX-1 and 12-LOX enzymes in human platelets, by novel optimized method which was based on method previously described [2]. Extracted assay is using LC-MS/MS technique for the quantification of three products (12-HPTE, TXB2, and PGF2α) of COX-1 and one product (12-HETE) of 12-LOX metabolism. Furthermore, no effect of the two administered doses of ERF 731® on E2 deprivation induced bone loss became apparent. In conclusion, the observations from previous trials regarding the endometrial safety of ERF 731® application were substantiated, but no effect on the Bone Mineral Density could be observed. Keywords: Phenolic profile, biological activity, extracts, essential oils, LC-MS/MS

PM159 Anti-inflammatory property of Juniperus communis L. var. communis needles and cones extracts and essential oils
Lesjak M, Bara I, Orcic D, Simin N, Jovin E, Franciskovic M, Mimica Dukic N
Department of Chemistry, Biochemistry and Environmental Protection, Faculty of Sciences, University of Novi Sad, Trg D. Obradovica 3, 21000 Novi Sad, Serbia

All over the world plants from the Juniperus genus have always been regarded as a well-known traditional remedy and spice. These plants are extensively used in the folk medicine for healing various disorders: common cold, urinary and kidney infections, dermatological disorders, bronchitis, pneumonia, dysentery, hemorrhage, rheumatic arthritis, stomachache, diarrhea and for regulation of the menstruation and in relieving menstrual pain [1]. However, there are only few literature data about their pharmaceutical activity and chemical composition. In this study anti-inflammatory properties of methanol extracts and essential oils of leaves and cones of the Juniperus communis L. var. communis, were determined using assays which measure the inhibitory capacity towards COX-1 and 12-LOX enzymes in human platelets, by novel optimized method which was based on method previously described [2]. Extracted assay is using LC-MS/MS technique for the quantification of three products (12-HPTE, TXB2, and PGF2α) of COX-1 and one product (12-HETE) of 12-LOX metabolism. Furthermore, no effect of the two administered doses of ERF 731® on E2 deprivation induced bone loss became apparent. In conclusion, the observations from previous trials regarding the endometrial safety of ERF 731® application were substantiated, but no effect on the Bone Mineral Density could be observed. Keywords: Juniperus communis, bioactivity, anti-inflammatory activity, extracts, essential oils, LC-MS/MS


PM161 Regulatory effect of 4-O-methylhonokiol on TGF-β1-induced cell cycle arrest in human keratinocyte cell line (HaCat)
Kang J1, Kim S1, Kim E1, Park D2, Koh Y1, Yoo E1, Kang H1
1Department of Pharmacology, School of Medicine, Institute of Medical Science, Jeju National University, Jeju, South Korea
2Department of Histology, School of Medicine, Institute of Medical Science, Jeju National University, Jeju, South Korea

Transforming growth factor-β (TGF-β) signal pathway has a pivotal role in the progression of catagen phase in hair growth cycle. 4-O-Methylhonokiol, a neolignan compound from Magnolia Officinalis L. var. officinalis, is regarded as a well-known traditional remedy and spice. This plant has been reported for its antibacterial, anti-inflammatory and anti-tyrosinase activity. Recently we have reported the biological activities such as anti-inflammatory, neurite outgrowth activity and anti-acetylcholinesterase activity. 4-O-Methylhonokiol inhibited TGF-β1-induced cell cycle arrest in human keratinocyte cell line (HaCat) cells. When HaCat cells were pretreated with 4-O-methylhonokiol, the expression of TGF-β1-induced p21 was decreased. Moreover, 4-O-methylhonokiol attenuated the nuclear translocation of Smad2/3, Smad4 and Smad7 activation. 4-O-Methylhonokiol reduced TGF-β1-induced activation of ERK. On the other hand, TGF-β has been reported to increase reactive oxygen species (ROS), and TGF-β1-induced growth arrest have been known to be mediated by oxidative stress. 4-O-methylhonokiol inhibited TGF-β1-induced ROS production and suppressed mRNA expression of NOX4. These results suggest that hair-growing activity of 4-O-methylhonokiol might be at least related to its modulatory activity, Anti-inflammatory activity, Phenolic profile, LC-MS/MS

Acknowledgement: Autonomous Province of Vojvodina – Provincial Secretariat for Science and Technological Development, Grant No. 114-451-PM158


PM160 Regulatory effect of 4-O-methylhonokiol on TGF-β1-induced cell cycle arrest in human keratinocyte cell line (HaCat)
action on TGF-β-induced cell cycle arrest and ROS production. Keywords: 4-O-methylhonokiol; Magnolia officinalis; TGF-β; HaCaT cells; cell cycle arrest, NOX4

PM162

Chemical composition, antioxidant and antimicrobial activities of the lichen Toninia candida (Weber) Th. Fr [Cetrariales]. Manojlovic N1, Maksic P1, Manojlovic I2, Vasiljevic P3, Bogdanovic Dusanovic G4, Juskovic M6, Aleksić M6, Zabar A6
1Department of Pharmacy, Medical Faculty, University of Kragujevac, 34000 Kragujevac, Serbia; 2Faculty of Agronomy, University of Kragujevac, Cara Dulana 34, 32 6000 Cacak, Serbia; 3Faculty of Science, Radioja Damanovica 12, University of Kragujevac, 34 000 Kragujevac, Serbia; 4Department of Biology, Faculty of Science, University of Niš, Visegradska 33, Niš, Serbia; 5College of Applied Professional Studies, 17000 Vranje, Serbia; 6Department of Biology and Ecology, Faculty of Sciences and Mathematics, University of Niš, 18000 Niš, Serbia

In the present investigation, methanol, chloroform and petroleum ether extracts of the lichen Toninia candida (Weber) Th.Fr. were assayed for their antioxidant and antimicrobial activities. The phenolic composition of the extracts was determined by HPLC-UV analysis. The predominant phenolic compound in all the extracts was the depsidone norstictic acid. Apart from the norstictic acid, the tested extracts of T. candida contain different amounts and ratios of artranin and stictic, protocetraric and usnic acids. The lichen extracts showed comparable and strong antioxidant activity, exhibited higher DPPH and hydroxyl radical scavenging capacity, chelating activity and inhibitory activity towards lipid peroxidation. The lichen extracts demonstrated major antimicrobial activity against 8 strains with MIC values ranging from 16.62 to 62.50 µg/ml. This is the first report of the chemical composition, antioxidant and antimicrobial activities of the lichen Toninia candida. Keywords: Tonia candida, HPLC-UV, chemical composition, antioxidant activity, antimicrobial activity Acknowledgement: This work was supported by the Serbian Ministry of Science and the Environment, Project No. 172015

An in vitro approach to neuroprotective activity of Rosa damascena Mill., a medieval age traditional medicine used for memory enhancement
Senol F5, Orhan I1, Kürkçüoğlu M6, Khan MH1, Alituntas A4, Şener B3, Başer KH4
1Department of Pharmacognosy, Faculty of Pharmacy, Gazi University, 06530 Ankara, Turkey; 2Department of Pharmacognosy, Faculty of Pharmacy, Anadolu University, 26470 Eskisehir, Turkey; 3Department of Medical Biology, Faculty of Health Science, University of Tromsø, N-9037 Tromsø, Norway; 4Department of History of Medicine, Catharina Faculty of Medicine, Istanbul, Turkey; 5Department of Botany and Microbiology, College of Science, King Saud University, Riyadh, Saudi Arabia

Rosa damascena Mill. was recorded to be used traditionally for memory enhancement in the medieval age. Therefore, neuroprotective effects of the essential oil and aromatic waters of R. damascena was investigated by in vitro and in silico methods. The essential oil and its components (citronellol, geraniol, nerol, and phenylethyl alcohol), and two samples of the aromatic water (Eau de rose) of R. damascena were tested for their inhibitory activity against acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) at 100, 200, 500, and 1000 µg/ml. Since oxidative damage is associated with neurodegeneration, antioxidant activity of the samples was determined by DPPH radical scavenging, metal-chelation, and ferric-reducing antioxidant power (FRAP) assays. Chemical composition of the samples was elucidated by GC-MS. The rose essential oil showed a noteworthy inhibition against AChE (60.86 ± 1.99%) and BChE (51.08 ± 1.75%) at 1000 µg ml-1, whereas the aromatic waters did not have any inhibition. The essential oil exhibited moderate activity in antioxidant assays. Phenylethyl alcohol exerted higher cholinesterase inhibition than other components. None of the double and triple combinations of citronellol, geraniol, nerol, and phenylethyl alcohol could reach at inhibition level of phenylethyl alcohol. Phenylethyl alcohol was theoretically studied utilizing molecular docking simulations into the active site gorge of AChE and BChE and the data revealed that this compound is more selective towards BChE than AChE. Our findings confirmed traditional use of R. damascena for memory enhancement, which is suggested to come into view through mainly cholinesterase inhibition, and antagonistic interaction presumably exists between phenylethyl alcohol and other components. Keywords: Rosa damascena, rose water, memory enhancement, enzyme inhibitory activity

PM164

Studies on anticholinesterase and DPPH radical scavenging effects of 41 species of Fritillaria L. genus of Turkish origin
Seniv D1, Senol F5, Orhan I1, Şener B3, Kaya E2, Rastegeli U3, Kesici A4, Aslay M4
1Department of Pharmacognosy, Faculty of Pharmacy, Gazi University, 06530 Ankara, Turkey; 2Department of Ornamental Plant Breeding and Agronomy, Atatürk Central Horticultural Research Institute, 77102 Yalova, Turkey

The genus Fritillaria L. (Liliaceae) is a member of geophytes with attractive flowers, which are cultivated as ornamental plants. Many of the European Fritillaria species are found in the Alps, the Pyrenees, the Balkans, and northern Turkey. There are 41 Fritillaria species growing in Turkey, 26 of which are endemic. The research carried out on Fritillaria species are focused on the alkaloid content of the plant. In the present study, the dichloromethane and methanol extracts prepared from the 59 samples belonging to 41 Fritillaria cultivated in Turkey have been investigated for their cholinesterase inhibitory activity against acetylcholinesterase (AChE) and butyrylcholinesterase (BChE), the enzymes linked to Alzheimer’s disease, at 50, 100, and 200 mg mL-1 using ELISA microplate reader. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging effect of the extracts was also tested. 25, 50 and 50 mg mL-1 final concentrations. According to our results; the highest inhibition against AChE was caused by the dichloromethane and methanol extracts of F. persica L. (21.03 ± 0.34% and 27.39 ± 2.26%, respectively). The most active extracts against BChE were found as the dichloromethane extract of F. pinardinii Boiss. (49.72 ± 2.56%) and two samples of F. persica (48.27 ± 1.98% and 47.29 ± 1.72%). Among the methanol extract, the best BChE inhibitions were found to be caused in F. minima Rix (54.69 ± 3.40%), F. persica (51.85 ± 4.68%), and F. caucasica J.F. Adam (46.14 ± 2.96%). On the other hand, all of the extracts displayed low profile of DPPH scavenging effect below 25%. The present results indicate that especially F. persica could be a potential source of natural compounds with anticholinesterase activity. Keywords: Fritillaria, anticholinesterase activity, free radical scavenging effect

PM165

Methanolic Alnus glutinosa bark extract affect ROS and TNF-α production
1Department of Pharmacochemistry, Faculty of Pharmacy, Universidad Ceu San Pablo, Fac Farmacia, Urb. Monternprincible, 28660 Boadilla del Monte, Madrid, Spain

Alnus glutinosa (L.) Gaertn., commonly known as ‘European alder’, presents several traditional secondary plant metabolites, including anthraquiones, phenolic glycosides, flavonol glycosides, terpenoids or xanthones, that had been previously reported in barks, buds, leaves and pollens. Alnus glutinosa stem bark (AGSB) is traditionally used as alternative, astringent, cathartic, febrifuge, emetic (fresh), haemostatic and tonic. In addition, a decoction of AGSB is used to treat swelling, inflammation and rheumatism. These traditional uses suggest that AGSB may contain active metabolites related to the inflammation process. Inflammation is associated with the progression of numerous diseases and is accompanied by the chronic release of cytokines and reactive oxygen species (ROS), which may be involved in tissue injury increment. Moreover, ROS, as well, contribute to the expression of a variety of different inflammatory cytokines such as TNF-α, which is considered to be a primary mediator of the inflammatory response. In this sense, the present study was designed to evaluate the capacity of the AGSB extract to reduce ROS generation in H2O2-induced oxidative stress in HeLa cells. In addition, the extract effect on TNF-α production using HL60 cell line, was also tested. Results show that the AGSB extract is able to protect cells from induced oxidative stress and may be able to decrease TNF-α production. These biological properties are linked to a successful reduction in inflammatory processes and may support, in part, its ethnopharmacological use. Keywords: Alnus glutinosa, TNF-α, ROS
The genus *Paeonia* L. (*Paioniaceae*), known as “şakayik, ayi gülü, bucor, etc.” in Turkey, was recorded to be used in Chinese traditional medicine against amnesia. *Paeonia* species were recorded in Turkey, which is the most important gene center worldwide for this genus. Consequently, the ethanol extracts of the defatted seeds of 7 *Paeonia* taxa; (*P. orieitana* Andrews, *P. dactyлина Andrews, P. mascula Miller subsp. bodurii N. Ozhatay, *P. cf. mascula* L. Mill.) subsp. *pajera* P. renepperler Miller, *P. tenuifolia* L., and *P. kayaye* N. Ozhatay) were screened against acetylcholinesterase (ACHE), butyrylcholinesterase (BChE), linked to Alzheimer’s disease and tyrosinase (TYRO), connected to Parkinson’s disease using ELISA microplate reader. As amnesia is a neurodegenerative situation associated with oxidative damage, antioxidant activity of the extracts was also measured by radical scavenging activity tests against 2,2-diphenyl-1-pircilydrylazo (DPPH), N,N-dimethyl-phenylendiamine (DMPD), and nitric oxide (NO) as well as metal-chelation capacity and ferric-reducing antioxidant power (FRAP) tests. Total phenol and flavonoid contents were determined spectrophotometrically. All of the extracts strongly inhibited ACHE (85.69 ± 0.58%, 96.68 ± 0.44%), BChE (73.34 ± 1.92%, 98.87 ± 1.08%), and TYRO (60.50 ± 1.68% - 76.16 ± 0.30%) at 200 µg ml⁻¹. The extracts displayed scavenging activity below 40% against DPPH and DMPD radicals, whereas they were not able to quench NO. They exhibited moderate FRAP values and very low metal-chelation capacity. As conclusion, our findings reveal that *Paeonia* species possess potent anti-amnesic activity in vitro via enzyme inhibition associated with neurodegeneration. The present study confirms the claimed utilization of the plant against amnesia in traditional medicine.

**PM167** Spasmodic response and neurogenic mechanism of water extract of *Vernonia cinerea* (L.) Less. on rat duodenum

The pharmacological study was conducted on adult female albino rats (175 – 225 g). Rats were anesthetized with pentobarbitone sodium (40 mg/kg, IP) and sacrificed by cervical dislocation and exsanguination. A small piece of duodenum 4 cm apart stomach was removed, cleaned and mounted on an organ bath containing Tyrode solution. The bathing fluid was continuously bubbled with air and maintained at constant temperature (37 ± 0.5 °C). The tissue was allowed to equilibrate for a period of 30 min under a resting tension of 0.5 gm. During this period the tissue was washed every 15 min. After equilibration, drugs were administered in bath fluid to record their effect on physiograph. Every time response indicates that the plant contains an active ingredient, which has spasmodic response on rat duodenum smooth muscle. The responses of VWE were recorded directly in Tyrode solution and mixed properly with the help of an Ultra-Sonicator. Effect of cumulative doses (0.25 to 25 mg/ml) of VWE on rat duodenal smooth muscle was recorded. This response indicates that the plant contains an active ingredient, which has spasmodic response on rat duodenum smooth muscle. The responses of VWE were fully reversible on wash. This finding suggests that VWE’s responses were mediated through an agonistic action without blockade of any receptor or enzyme. Extract possibly stimulates presynaptic cholinergic nerve endings to produce spasmodic response in rat duodenal smooth muscle. Keywords: Vernonia cinerea, Rat duodenum, Aqueous extract, spasmodic response Acknowledgement: Thanks are due to authorities of G.B. Pant University of Agriculture & Technology, Pantnagar, India for providing necessary research facilities.

**PM168** Estrogenic activity of the methanolic extract of *Ebenus cretica* L.

Kounadi S 1, Aligiannis N 1, Pongratz I 2, Lelovas P 3, Ismini D 3, Skaltsounis AL 1

1Department of Pharmacognosy, Faculty of Pharmacy, University of Athens, Zografou, 15771, Athens, Greece; 2Department for Biosciences and Nutrition, Karolinska Institutet, Huddinge, 17177, Stockholm, Sweden; 3Laboratory for the Research of the Musculoskeletal System, School of Medicine, University of Athens, Goudi, 11527, Greece

Plant derived compounds when friendly to the human organism tend to become very important ingredient of our nutrition. Members of the Leguminosae family are known to exhibit a mild estrogenicity due to their rich phenolic profile. In the present study, methanolic extracts of *Ebenus cretica* L. (ECME), *Ebenus sibthorpii* DC, *Medicago marina* L. (MMME) and *Medicago falcata* L. have been investigated for their estrogenic activity on ERα. All extracts were subjected to ERβ-binding screening using stably transfected HeLa cells. Luciferase assay was used as a tool to monitor the ERβ-binding. At the concentration of 400 µg/ml ECME and MMME exhibit 2.7-fold and 3.2-fold respectively stronger binding with the ERβ compared to its natural agonist, estradiol. Following, in order to isolate bioactive compounds from E. cretica, ECME was submitted to fast centrifugal partition chromatography and sephadex analysis and was proved rich in flavonoids such as rutin and isoflavones such as 4', 8-dimethyltheter-7-O-J-D-glucopyranosyloflavone. An aurone, maesopin glucoside, was also isolated. Additionally, an in vivo experiment was performed in order to evaluate ECME’s osteoprotective role. Female Wistar rats were treated with the ECME dissolved in drinking water. Rats were submitted to ovariectomy prior to the treatment. Natural products from plants traditionally provide the pharmaceutical and food industry with one of the most important sources of “lead” compounds. Findings of our study offer valuable information on the beneficial effects of *E. cretica*, which could be used as the basis of food supplement, functional food or even drug. Keywords: Ebenus, Medicago, estrogenic activity Acknowledgement: The present study has been funded by the project IARALETOS II

**PM169** Larvicidal activities of selected Nigerian plants

Famuyiwa FG, Okuyeeye AI, Oluosia FI

Department of Pharmacognosy, Faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife, Nigeria

The methanol extracts of *Bryophyllum pinnatum* (Lam.) Kurz leaves, *Diosyoxylon lenticellare* Gillespie leaves, *Newboldia laevis* Seem stem bark, *Markhamia tomentosa* K.Schum, ex Engl. stem bark, *ethylacetate extract of Jatropha multifida* L. leaves and *Jatropha gossypifolia* L. stem bark were investigated for larvicidal activity against the larvae of *Aedes aegyptii*, the vector of dengue and yellow fever. After 48hours, *D. lenticellare* extract with LC50 and LC90 values of 1.54 ± 0.31 mg/ml and 3.52 ± 0.15 mg/ml respectively and *J. gossypifolia* extract with LC50 and LC90 values of 1.96 ± 0.26 mg/ml and 3.50 ± 0.25 mg/ml respectively were the most active. Work in is progress to purify these most active extracts and isolate their active constituents. Keywords: plant extracts, *Aedes aegyptii*, larvicidal activity

**PM170** Appraisal of *in vitro* neuroprotective effects of Turkish *Pinus* L. species and pycnogenol and essential oil analyses

Ustyin O1, Senol F2, Kürkçüoğlu M2, Orhan I1, Kartal M3, Bauer KW4

1Department of Pharmacognosy, Faculty of Pharmacy, Gazi University, Ankara, Turkey; 2Department of Pharmacognosy, Faculty of Pharmacy, Ankara University, Eskisehir, Turkey; 3Department of Pharmacognosy, Faculty of Pharmacy, Anadolu University, Eskisehir, Turkey; 4Department of Botany and Microbiology, College of Science, King Saud University, Riyadh, Saudi Arabia

Pycnogenol, the French maritime pine bark extract, has been popular recently for its various health effects including memory enhancement. Therefore, we aimed to determine neuroprotective effect of the acetone, ethyl acetate, and ethanol extracts and essential oils of the shoots and needles of *P. brutia* Ten., *P. halepensis* M.Bieb., *P. nigra* Link, *P. pinea* L., and *P. sylvestris* L., which are the Pinus species growing in Turkey, and...
Pycnogenol by in vitro experiments using enzyme inhibition and antioxidant assays. Inhibitory activity of the extracts, essential oils, and pycnogenol was assessed against acetylcholinesterase (AChE) and butyrylcholinesterase (BChE), connected to Alzheimer’s disease. Since neurodegeneration is associated with oxidative damage caused by free radicals and metal accumulation, antioxidant activity of the extracts, essential oils, and pycnogenol was measured using the methods: 2,2-diphenyl-1-picrylhydrazyl (DPPH) and N,N-dimethyl-p-phenylenediamine (DMPD) radical scavenging activity as well as ferric ion-chelation capacity and ferric-reducing antioxidant power (FRAP) tests. Chemical compositions of the essential oils were analyzed by gas chromatography-mass spectrometry (GC-MS). Accordingly, the best AChE inhibition was caused by the shoot essential oil of P. halepensis (83.91 ± 1.95%), while the needle ethanol extract displayed a high profile of BChE inhibition (82.47 ± 5.27%) at 200 μg/mL. AChE and BChE inhibitions by pycnogenol were 63.33 ± 0.22% and 83.67 ± 0.22%, respectively. The extracts and essential oils exerted moderate activity in antioxidant tests. However, many of them displayed similar or greater activity ferric ion-chelation capacity (26.49 ± 4.47% – 67.77 ± 3.33%) than that of pycnogenol (29.14 ± 2.00%). Our findings revealed that the Turkish pine and pycnogenol possess neuroprotective effects by the in vitro methods applied herein. Keywords: Antioxidant activity, Pinus sp., essential oil.

### PM171

**Black Cohosh and the protective effects on bone metabolism as measured by computer tomography (qCT) in ovariectomized (ovx) rats**


Osteoporosis is a major disease in postmenopausal women. There is compelling evidence that the special extract from *Cimicifuga racemosa* (L.) Nutt. (CR) BNO 1055 influences positively bone metabolism and prevents induced osteoporosis by ovariectomy (ovx). Aim of the study was the investigation of CR BNO 1055 extracts and their protective effects on bone metabolism. Via liquid-liquid extraction, extracts were separated into two groups, i.e. a lipophilic fraction rich in saponins and a hydrophilic rich in sugars and phenylpropanoids. Preparative fractions were characterized by TLC and by HPLC-UV-ELSD and HPLC-MS. Analytical data clearly prove the presence of triterpene glycosides in the lipophilic fraction and the presence of carbohydrates and phenylpropanoid acid derivatives in the hydrophilic fraction. To investigate the activity on bone metabolism of CR, ovx rats received either nutrition containing CR, 17β-estradiol or food without any active component. All types of food were soy-free food. QCTs were performed at the level of the metaphysis of the tibia and the trabecular density measured prior to the ovariectomy and at the end of the four weeks after the extracts. Within these four weeks the bones of the ovx rodents lost nearly half of their cancellous density. This effect did not appear by rats fed with nutrition including 17β-estradiol and CR. The lipophilic fraction shows a significant high activity on bone metabolism while the hydrophilic fraction was inactive. The QCT revealed that the prevention effect of CR BNO 1055 on bone is up to the lipophilic compounds of the extract. Keywords: Black Cohosh, Cimicifuga racemosa, bone metabolism

Acknowledgement: This work was funded by the Bayerische Forschungsförderung AZ-838 – 08, Germany

### PM172

**Biologically active lupane triterpenoids from Anatolian Salvia species**

**Calhuoa B, Topçu G**

Istanbul Technical University, Faculty of Science and Letters, Department of Chemistry, 34469, Maslak, Istanbul, Turkey

Salvia species are used in perfumery, food and drug industry, belonging to Lamiaceae family which contain annual or perennial plants with nice fragrance. Totally 900 Salvia species grown widely all over the world, and Turkey has over 90 Salvia species, half of them being endemic [1]. Since ancient times, Salvia species have been traditionally used as antioxidant, antiseptic, digestive, carminative, and sedative as well as in the treatment of bronchitis, tuberculosis, menstrual and neurological disorders. Salvia species are rich in terpenoids [2,3], steroids, flavonoids and other phenolics. In our continuing studies on *Salvia* species, we have isolated many oleane and ursane type triterpenoids, namely oleanolic and ursolic acids in addition to diterpenoids, flavonoids and phenolics. Besides oleane and ursane triterpenoids, we have also isolated lupane triterpenoids from Salvia species, but with poor biodiversity. In this presentation, a number of lupane triterpenoids, obtained from the extracts of several Salvia species (S. montbretii Benth., S. cedrenella Boiss., S. macrochlamys Boiss. & Kotschy ex Boiss., S. strachilada Benth., S. hypargyra Fisch. & Mey.), [4,5] will be presented with their promising activity results. Structures of the pure triterpenoids were identified by spectroscopic analysis using extensive NMR (1D and 2D), UV, IR, and mass spectroscopic techniques. Antioxidant activity of the triterpenoids was established by β-carotene bleaching and radical scavenging methods. The most promising activity was found for globolone and globolone acetate against AChE and BChE enzymes by Ellman Method [6]. Monogynol A and its three natural derivatives, isolated from *S. macrochlamys* were also found to be highly active in a metal chelating test system on ferrous ion [5]. Keywords: Salvia species, S. montbretii, S. cedrenella, S. macrochlamys, S. strachilada, S. hypargyra, antioxidant activity, Ellman Method

**REFERENCES**


Cancer is one of the diseases with increased prevalence in the 21st century. Since the 1970s a class of transmembrane proteins called ABC transporters are known, being P-Glycoprotein (Pgp) the most representative member. They are often involved in the efflux of drugs preventing their accumulation in the cytoplasm and thus decreasing the therapeutic effect. This key factor strongly contributes to the phenomena of resistance to anticancer drugs, preventing the success of chemotherapy regimens [1]. Despite the recent publication of the murine P-gp crystallographic structure, the characterization of its binding site is still limited and, therefore, the theories for the protein’s functioning cannot be validated and more suitable modifiers for the effective inhibition of the multidrug-resistance phenomenon cannot be developed. In this case, pharmacophores can give important input on the subject matter. Several pharmacophores were already published that identified hydrophobic and acceptor/donor groups as essential characteristics for the recognition by the transporter [2,3]. However, the majority of the already published pharmacophores only cover a small variety of compounds, frequently derived from a primary scaffold or to select from a database only the active ones. In addition, the literature-derived pharmacophores fail to detect our in-house macrocyclic diterpenes [4]. Inspired on the published literature and based on the lathyrane-type scaffold, we developed a new pharmacophore (Figure 1) capable of detecting not only the literature (84.2%) but also all in-house compounds, with higher detection of inactive molecules, in a database comprising 272 compounds.
Keywords: P-glycoprotein; pharmacophore; modulators; lathyrane; macrocyclic diterpenes
Acknowledgement: This work was supported by Fundação para a Ciência e Tecnologia (FCT) (Project PTDC/QUI-QUI-098815/2008)

Enhancement of MHC-II peptide loading by low-molecular weight chemicals is of twofold interest in immunological research. Compounds that elicit an increased loading of MHC-II molecules with immunoactive peptides (MLEs) may be involved in the pathophysiology of autoimmune diseases. On the other hand, such compounds might be of potential use to enhance the activity of vaccines and of antitumour immunotherapies [1]. We have now discovered that some natural essential oils and their constituents are able to increase the loading of MHC-II allele HLA DR1 to a very significant extent. In a screening based on Dissociation-Enhanced Lanthanide Fluorescent Immunoassay (DELFIA) [2], we found that a variety of essential oils as well as isolated constituents could increase the spontaneous loading of soluble HLA DR1 [3] with an influenza A haemagglutinin peptide (HAA 306 – 318) [4]. Quite interestingly, structurally simple and widespread monoterpenes (citronellol, geraniol) showed the strongest activity among > 40 pure compounds tested. Of 28 essential oils tested so far, farangol (German Chamomile, Matricaria recutita L.) showed the strongest effect, comparable with the reference compound, adamantylethanol, a potent MLE [1]. Activity-directed isolation led to the identification of E-ene-yne-dicycloether as the strongest MLE compound, about 3 times stronger than the Z-isomer. Bisabololoxides A and B were also significantly active but much less potent than the E-spiroether. These findings indicate that MHC-II loading enhancement might be involved in the immunological activities of essential oils and may also open new perspectives with respect to potential applications. Keywords: Major histocompatibility complex-II; MHC-loading enhancers MLE; essential oil, German Chamomile, Matricaria recutita References: 1. Hoppen S et al. (2006) J Biol Chem 281: 38535 – 38541. 2. Khadkodayan S et al. (2007) Assay Drug Dev Technol 5: 38535 – 42. 3. HLA-DR1 (DRB1*0101) in house production S. Günther, MDC Berlin. 4. Lamb JR et al. (1982) Nature 300: 66 – 9.

Saponins from Astragalus pycnocephalus var. pycnocephalus FISCHER and their α/β-glucosidase inhibitory effects
Kož O1, Ekinci D2, Şentürk M3, Perrone A4, Piaceante S4, Alaknaš Kaštun P5, Bedir E1
1Ege University, Faculty of Science, Department of Chemistry, Bornova, 35100 Izmir, Turkey; 2Onodokuz Mayıs Univ. Fac. of Agriculture, Dept. of Agricultural Biotech., Enzyme and Microbial Biotech. Division, 53139, Samsun, Turkey; 3Astragalus pycnocephalus var. mutense (32.7%, 40.3%), Astragalus pycnocephalus var. mutense (37.4%, 70.5%) demonstrated the highest activities on the both wound models.

The leaves, flowers, and whole aerial parts of Verbascum L. (Scrophulariaceae) species are used to treat eczema and other types of inflammatory skin conditions for desiccating wounds in traditional Turkish medicine. The methanolic extracts prepared with thirteen Verbascum species growing in Turkey, including V. chionophyllum Hub.-Mor., V. ciliicolum Boiss., V. dudleyeanum (Hub.-Mor.) Hub.-Mor., V. lasioglossum Boiss., V. latipespalum Hub.-Mor., V. mucronatum Lam., V. olympicum Boiss., V. pterocaulcyum var. mutense Hub.-Mor., V. pycnostachyum Boiss. & Heldr., V. salviitfolium Boiss., V. splendidium Boiss., V. stachyfolium Boiss. & Heldr and V. uschackense (Murb.) Hub.-Mor. were assessed for their in vivo wound healing activity. In vivo wound healing activity of the plants were evaluated with linear incision and circular excision experimental models subsequently histopathological analysis. The healing potential was comparatively assessed with a reference ointment Madecassol®, which contains 1% extract of Centella asiatica. The methanolic extracts of V. olympicum (36.6%, 75.6%), V. stachyfolium (40.1%, 79.1%) and V. uschackense (37.4%, 70.5%) demonstrated the highest activities on the both wound models. Moreover, the methanolic extracts of V. latipespalum (32.7%, 40.3%), V. mucronatum (21.2%, 26.4%) and V. pterocaulcyum var. mutense (26.7%, 56.6%) were found generally highly effective. On the other hand, the rest of the species did not show any remarkable wound healing effect. Results of the present study support the continued and expanded utilization of these plant species employed in Turkish folk medicine. The experimental study revealed that Verbascum species display remarkable wound healing activity.
PM177

Protective Effects of Astragaloside IV and Cycloastragenol in 6-hydroxydopamine (6-OHDA)-Induced Neurotoxicity in PC12 Cells

Nesil T1, Nesil T2, Sendemir Urkmez A2, Bedir E3
1Ege University, Graduate School of Natural and Applied Sciences, Department of Biotechnology, 35100 Bornova, Izmir, Turkey; 2Ege University, Faculty of Engineering, Bioengineering Department, 35100 Bornova, Izmir, Turkey

Astragaloside IV (AST-IV), one of the bioactive constituents of Radix Astragali, was extracted from the roots of Astragalus trojanus Bunge (Leguminosae). Cycloastragenol (CG), which is a minor metabolite mostly found in its glycosidic form, was obtained from AST-IV via hydrolysis reaction. CG has been shown to extend T cell proliferation by increasing telomerase activity showing that it may also help delay the onset of cellular aging [1]. Indeed, recently, CG has been introduced to the market as a new generation anti-aging molecule. Moreover our studies proved CG as an extraordinary wound healing agent [2]. Although AST-IV’s neuroprotective effects on Parkinson’s disease was reported previously, there has been no data for CG. The aim of this study was to investigate the protective effects of AST-IV and CG on neurotoxicity in vitro model for Parkinson’s disease. The cells were seeded on tissue culture plates for 24h. After 24 hours, they were incubated with AST-IV (0.1 µM-1 µM) and CG (0.1 µM-1 µM) for 30 min before the insults with 200 µM 6-OHDA. The cells were incubated for 24h. Cell viability and cell death were assessed by (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) MTT assay and lactate dehydrogenase (LDH) assay kit, respectively. AST-IV and CG inhibited the apoptosis of PC 12 induced by 6-OHDA at 6001 and 0.001 µM concentrations. On the basis of these results, we propose AST-IV and CG as potential neuroprotective agents in the treatment of Parkinson’s disease.

Figure 1


PM178

A QASAR study of macrocyclic diterpenes with P-gp inhibitory activity isolated from Euphorbia species

Santos B1, Molnar J2, Ferreira MU3, Fernandes MX4
1Centro de Química da Madeira, Universidade da Madeira, Campus da Penteada, 9000 – 390 Funchal, Portugal; 2Department of Medical Microbiology, University of Szeged, H-6720 Szeged, Hungary; 3Research Institute for Medicines and Pharmaceutical Sciences (Med.UL), Faculdade de Farmácia, Universidade de Lisboa, Av. das Forças Armadas, 1600 – 083, Lisboa, Portugal.

A set of 50 compounds, constituted mainly by macrocyclic diterpenes of the jatrophane and latyrane-type, isolated from Euphorbia species, was used in the present study. These compounds were found to be P-glycoprotein-mediated multidrug-resistance (MDR) reversing agents in cancer cells. The reversal of MDR was investigated by flow cytometry, at two different concentrations, 4 and 40 µg/mL, by measuring rhodamine-123 accumulation, a fluorescent substrate analogue of doxorubicin [1, 2]. The quantitative structure-activity relationship (QSAR) methodology was applied to the whole set of diterpenes and to the jatrophanes subset at both concentrations studied. We used multiple linear regression (MLR) with forward features (from five classes of molecular descriptors: constitutional, topological, geometrical, electrostatic and quantum-chemical) selection to establish QSAR models. The best model obtained, at 4 µg/mL, for the diterpenes set was constructed using 4 descriptors with R² of 0.80 for a training set of 40 compounds and R²pred of 0.71 for a test set of 9 compounds. The best model obtained, at 4 µg/mL, for jatrophenes subset was constructed using 5 descriptors with R² of 0.82 for a training set of 28 compounds and R²pred of 0.54 for a test set of 6 compounds. All models were statistically valid with high predictability, and in both models the descriptors used (logP and quantum descriptor related to resonance energy) can easily be translated into the design of novel diterpene derivatives with a forecasted improved P-gp inhibitory activity. Keywords: QSAR, P-gp inhibitory activity, macrocyclic diterpenes Acknowledgement: This study was supported by FCT (PTDC/QUI/QUI/098915/2008). Portugal. References: 1. Molnár J et al. (2006) Curr Pharm Des 12: 287 – 311. 2. Duarte N et al. (2008) Bioorg Med Chem 16: 9323 – 30.

PM179

Antidepressive mechanisms of action of willow bark extract STW 33-I

Kelber O1, Okpanyi SN2, Freischmidt A2, Heinrich EU2, Müller J3, Heilmann J3, Ulrich Menzenich G2, Weiser D1
1Scientific Department, Steigerwald Arzneimittelwerk GmbH, Darmstadt, Germany; 2Pharmazeutische Biologie, Institut für Pharmakologie und Toxikologie, Universität Regensburg, Germany; 3Medizinische Klinik III, Universitätsklinikum, Rheinische Friedrich-Wilhelms-Universität Bonn, Germany

Therapies in chronic musculoskeletal pain can be supplemented by antidepressants [1]. For willow bark extracts, known for their anti-inflammatory and analgesic properties [2], additional antidepressant effects have been suggested [3]. To elucidate the underlying potential mechanisms of action, the willow bark extract STW 33-I (Proaktiv®) and four of its fractions separated by polarity (4) have been studied. Male Sprague Dawley rats (n = 6/group) were treated for two weeks once daily per os with different doses of the extract and with fractions produced by liquid/liquid partition, in comparison to a control and reference group (imipramine 20 mg/kg b.w.). On day 15, a forced swim test according to Porsolt was performed and the locomotor activity was determined. Treatment was continued for further four days and neurotransmitter concentrations were determined in frontal cortex, hypothalamus, hippocampus and striatum. A significant shortening of the cumulative period of immobility was seen after treatment with 15, 30, 60 mg/kg b.w. of the extract whereas the locomotor activity did not increase. Higher doses of STW 33-I were ineffective. Fractions were characterized analytically by HPLC and tested in doses resembling their yield in the extract, with best effects in doses equivalent to the lowest extract dose tested. 5-HT levels in the hippocampal tissue were increased significantly. STW 33-I and its fractions showed an antidepressant like effect. The serotonergic system seems to be involved. This central effect of the willow bark extract STW 33-I may contribute to its clinical efficacy in musculoskeletal pain. Keywords: Willow bark extract, pain, FST, analgesia, depression, hippocampus, serotonin, 5-HT Acknowledgement: This contribution is dedicated in memoriam to Prof. Dr. Ilke Winterhoff, Institut für Pharmakologie und Toxikologie, Westfälische Wilhelms-Universität, Münster, Germany, and former Chair of the Permanent Committee on Biological and Pharmacological Activity of Natural Products of GA, who passed away on May 9, 2010. References: 1. Perrot S et al. (2008) Rheumatology doi: 10.1093/rheumatology/ken110; 2. Nahrstedt A et al. (2007) Wien Med Wochenschr 157: 348 – 351; 3. Hegger S et al. (2005) Kongress Phytopharmaka Phytotherapie, Berlin 2005-S19, (4) Bonnaterre GA et al. (2010) Phytotherapy 2017: 1106 – 1113.

PM180

Evaluation of diterpene compounds as inhibitors of multidrug resistance on human colon adenocarcinoma cells

Res M1, Serly J2, Madureira AM1, Duarte N1, Molnar J2, Ferreira MU1
1Research Institute for Medicines and Pharmaceutical Sciences (Med.UL), Faculdade de Farmácia, Universidade de Lisboa, Av. das Forças Armadas, 1600 – 083, Lisboa, Portugal; 2Department of Medical Microbiology, University of Szeged, H-6720 Szeged, Hungary

One of the most promising strategies for overcoming multidrug resistance (MDR) is to use compounds that can modulate P-glycoprotein (Pgp) and restore the cytotoxicity of anticancer drugs. A large variety of compounds have been shown to be MDR-modulators, and some of them have undergone clinical trials. They are a large chemical and structurally diverse group that includes among others, natural products and their semi-synthetic derivatives. However there are currently no reversal agents clinically available. Therefore, a great need for new reversal
agents with higher specificity and efficacy still remains [1]. In previous studies, we have reported the isolation of several macrocyclic lathyrane and jatrophane-type diterpenes, which were found to have a potent inhibitory activity against P-glycoprotein of human MDR1 gene-transfected mouse lymphoma cells [2–4]. The purpose of this work was to study the ability of lathyrane and jatrophane derivatives to modulate the transport activity of P-glycoprotein on human colon adenocarcinoma cell lines (COLO 205 and COLO 320). The reversal of MDR was investigated by flow cytometry, measuring rhodamine-123 accumulation. Several of the compounds tested have shown to be strong Pgp inhibitors. Furthermore, some of these modulators, which presented a significant MDR reversal activity, were assayed, in vitro, for their antiproliferative effects in combination with doxorubicin. Some of the compounds synergyistically enhanced the effect of the antitumor drug. According to these results, macrocyclic diterpenes may be valuable as lead compounds for the development of Pgp modulators in different multidrug-resistant cancer cells and to further study their effect in animal experiments.

Keywords: Cancer, multidrug resistance, macrocyclic diterpenes, adeno-carcinoma cells


Richness of extremophile plants in phenolics with interesting biological activities

Ksouri R1, Medini F2, Oueslati S1, Trelais N1, Megdiche W1, Waffo Teguo P1, Richette A1, Legault J3, Abdelly C3
1Laboratoire des Plantes Extremophiles, Centre de Biotechnologie de Borj-Cédria, Tunisia; 2Laboratoire des Sciences Végétales, Mycologie et Biotechnologie, U.V. Segalen Bordeaux; 3Laboratoire LASEVE, Université du Québec à Chicoutimi, Québec, Canada G7H 2B1.

Extremophile plants are remarkable plants that tolerate severe environmenal and physiological conditions. They may absorb more toxic compounds to overcome oxidative stress. These species have developed potent antioxidant systems. Among them, polyphenols constitute the main powerful compounds, owing to their strong biological activity. Therefore, the broader profile of STW 5, possibly mediating its therapeutic effects in irritable bowel syndrome, is in part, but not entirely, based on the effects of STW 6. Keywords: Iberis amara, Colon, Colon irritabile, Irritable bowel syndrome, Intestinal hypersensitivity, Bradykinin.

References:

Iberis amara L. desensitizes low-threshold mechano-sensitive afferents of the colon

Müller S1, Müller MH2, Kasparek MS3, Kelter O3, Weiser D4, Kreis MB5
1Walter-Bredel Institute, University of Munich, Munich, Germany; 2Surgery, University of Munich, Munich, Germany; 3Scientific Department, Steigerwald Arzneimittelwerk GmbH, Darmstadt, Germany

Medicinal plants used in irritable bowel syndrome include Iberis amara L. (1). Its extract (STW 6) is component of the herbal combination preparation STW 5 (Iberogast®) (2), for which an antagonizing effect on gastrointestinal hypersensitivity has been shown (3). The colon of C57Bl6 mice was isolated and recordings of the mesenteric nerve were taken in an organ bath, while perfusing lumen and bath with Krebs solution (32 °C, 10 ml·min⁻¹) containing 1.1 μM nifedipine to eliminate contractility. STW 5 (lyophilized, 5.71 x 10⁻⁷ mg·ml⁻¹) or STW 6 (lyophilized, 21.2 x 10⁻⁷ mg·ml⁻¹) were applied 10 minutes prior to stimulation with bradykinin (0.5 μM), 5-HT (10 μM) or luminal ramp distension from 0 to 80 cm H₂O. Intestinal afferent nerve discharge to 5-HT was reduced to 0.2 ± 0.2 imp·s⁻¹ after STW 6, compared to 5 ± 2 imp·s⁻¹ following vehicle and 3 ± 1 imp·s⁻¹ following STW 5 (p < 0.05 STW 6 vs. vehicle). The response to bradykinin was 36 ± 5 imp·s⁻¹ after STW 6 and 38 ± 6 imp·s⁻¹ after vehicle (n.s.). Following STW 5 it was reduced to 9 ± 2 imp·s⁻¹ compared to vehicle (p < 0.05). Values at 80 cm H₂O were 14 ± 3 imp·s⁻¹ after STW 6, 22 ± 3 imp·s⁻¹ after vehicle and 4 ± 3 imp·s⁻¹ after STW 5 (p < 0.05 for both versus vehicle). The Iberis amara extract STW 6 has a particularly desensitizing effect in low-threshold mechano-sensitive afferents, while STW 5 acts on both low- and high-threshold afferents. Therefore the broader profile of STW 5, possibly mediating its therapeutic effects in irritable bowel syndrome, is in part, but not entirely, based on the effects of STW 6. Keywords: Iberis amara, Colon, Colon irritabile, Irritable bowel syndrome, Intestinal hypersensitivity, Bradykinin.

References:

Larvicidal activity of Eugenia uniflora in Aedes aegypti

Famuyiwa FC, Adeboye AC, Aladeseomi JA
Department of Pharmacognosy, Faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife, Nigeria

In Brazil, the leaves of Eugenia uniflora L. are crushed and spread on the floor for its flavour and fly-repellent property [1]. Insecticidal activity of the oil [2] and larvicidal activity of the extract has been reported[3]. The larvicidal activity of the leaf methanol extract was therefore investigated to determine the most active subfraction from which the active compounds could be isolated. The leaf methanol extract was partitioned into n-hexane, chloroform, ethylacetate, butanol and aqueous phase and tested for larvicidal activities, using the larvae of Aedes aegypti. The most active n-hexane fraction (B₁) was successively subfractionated from which the active compounds were isolated. The calculated percentage mortalities of the extract, fractions and subfractions of the leaf as well as of endosulphan, positive control, were used to determine their LC50 and LC90 values. The result showed that methanol extract had larvicidal activity that was statistically comparable (P > 0.05) to that of endosulphan. Fractions B₃ and C₉ had LC50 and LC90 values that were insignificantly different from those of methanol extract and endosulphan. Fractions C₉ and C₄ had significantly greater activity than B₁ and comparable to that of endosulphan, making them to be the most active fractions. Work is in progress in order to isolate the active compounds from these most active fractions. Keywords: Eugenia uniflora, larvicidal, extract, leaf References: 1. Morton J (1987) Surinam Cherry. In: Fruits of Warm Climates, JF Morton, Miami Florida. 2. Gbolade AA, Adebayo T (1993) Insect Sci Appl 4(5/6): 631 – 636. 3.Luna JS et al. (2005) J Ethnopharmacol 97(2):199 – 206.
Effect of Matricaria chamomilla L. extract on fetal absorption, placenta structure and liver of diabetic pregnant rats.

Namjooyan F1, Panahi M1, Ahmadpour F2, Darvish A1, Azemi M1, Samaee H1, Khodaiyan M1
1Medical Plant Research Center, Pharmacognosy Department, School of Pharmacy, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran; 2 Anatomy Department, School of Medicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran.

Diabetes mellitus (DM) results in severe metabolic imbalances and pathological changes in many tissues (1). Diabetes was induced by Streptozotocin in this research. Mating condition was prepared by putting male rats and diabetic female rats together and vaginal plaque was as a positive sign of pregnancy and treatment started with three doses 100,300,500, mg/kg chamomile extract or vehicle from 1th to 17th day of gestation by gastric gavages. Blood glucose was measured during 17 days. At 17th day, rats were sacrificed. The fetuses were released from the yolk sacs and surrounding deciduas and were examined for absorption rate. Results shows that level of blood glucose was reduced about 1.62 fold (p < 0.00) in comparison to vehicle treated diabetic rat group. In diabetic group that received no treatment feta’s spontaneous abortion was 15%. Percentage of absorbed fetuses in chamomile groups received 100,300,500 doses and control group were 0.2%, 0.6% and 0% respectively, the percentage of absorptions was significantly elevated in vehicle-treated diabetic rats, in the diabetic group, all signs, such as separated necrosis of hepatocytes, anachism of liver plates, and lymphocytic inflammation were improved. Matricaria chamomilla was found to have protective effects on spontaneous abortion and histopathological changes of placenta and liver associated with STZ diabetes in chamomile treated pregnant female rats. Keywords: Matricaria chamomilla, fetal absorption, placenta, liver, diabetes, pregnancy, Ultrastructure, STZ


Polycytylates from Notopterygium incisum as a novel class of specific PPARgamma activators

Blunder M1, Fakhrudin N2, Liu X1, Kunert O2, Atanasov AG2, Diusch TF1, Bauer K1
1Institute of Pharmaceutical Sciences, Department of Pharmacognosy, Karl-Franzens-Universitat Graz, 8010 Graz, Austria; 2 Department of Pharmaceutical Sciences, University of Vienna, 1090 Vienna, Austria; Institute of Pharmaceutical Sciences, Department of Pharmaceutical Chemistry, Karl-Franzens-Universitat Graz, 8010 Graz, Austria.

In the course of our search for PPARγ active anti-inflammatory natural products we have investigated the dichloromethane extract of the dried rhizome and root of Notopterygium incisum Ting ex H. T. Chang (Umbelliferae). PPARγ is one of the three Peroxisome Proliferator Activated Receptor (PPAR) subtypes and is involved in the regulation of glucose and lipid metabolism and therefore an important target for metabolic diseases. Additionally, PPARγ plays a role in other chronic diseases such as inflammation, cancer and atherosclerosis. We have isolated six polycytylates, which were structurally characterized by means of multidimensional NMR and mass spectrometry, and which were shown to inhibit NO production in RAW 264.7 macrophages. Now, the potency of these polycytylates as PPARγ agonists has been evaluated. The EC50 of 8- acetoxycalcolin, falcariol, 9-epoxy-falcariol, crithmumidiol, 9-heptadecene-4,6-diyne-1-ol and 29,29-heptadecadiene-4,6-diyne-1-ol were determined as 2.36-fold activation (EC50 of 3.59 μM), 2.29-fold activation (EC50 of 4.25 μM), 1.88-fold activation (EC50 of 2.03 μM) and 2.29-fold activation (EC50 of 4.56 μM), 1.92 fold activation (EC50 of 11.31 μM) and 1.73-fold activation (EC50 of 4.18 μM), respectively, whereas the positive control pioglitazone exhibited 7.96-fold activation (EC50 of 0.31 μM). Therefore, these polycytylate derivatives contribute to the anti-inflammatory activity of Notopterygium incisum by selectively activating PPARγ without affecting the other two PPAR subtypes.2

PM184

PM185

PM186

PM187

Figure 1: Chemical structures of the polycytylates from N. incisum.


Figure 1: Chemical structures of the polycytylates from N. incisum.


Characterization of glycosidic triterpenoids of Scilla litardieri and investigation of its potential cytotoxic effects

Arjune S, Klar F
Flurepha, Division of Natural Product Research, Buer-Gladbecker Str. 78, 45894 Gelsenkirchen, Germany

Scilla litardieri Breitst. (syn. Chouardia litardieri Speta) (Hyacinthaceae) is a bulbous geophyte, which is part of the original flora of southeast Europe and the Balkans. In this study the separation and purification of saponin-rich fractions obtained by partition of bulbous extracts from S. litardieri was carried out. The glycosidic natural products were elucidated by chromatographic and spectroscopic methods. Pure substances were gained by purification using preparative HPLC. Sugar moieties were identified after hydrolysis and derivatisation by gas chromatography. Several triterpenoids, which differ in the aglycons, were detected. African green monkey kidney cells (COS-7) and human embry-
oral kidney (HEK-293) cells were treated with isolated triterpenoids. In order to elucidate possible antiproliferative and cytotoxic effects of these substances the commonly used MTT assay was utilized. Keywords: Scilla litardierei, Glycidosid saponins, Hyacinthaceae, Triterpenoids References: 1. Müller B and Klar F (2010) Planta Med 76: 1234.

**PM188**

Effect of fumarprotocetraric acid isolated the Lichen Cladonia verticillaris on tracheobronchial phenol red excretion in mice 
Duarte GP1, Alves GD2, Franco ED1, Melo RC1, Cordeiro DP1, Pereira EC1, Silva NH1, Maia MD1  
1Department of Biochemistry and Physiology, Federal University of Pernambuco, Recife-PE, Brazil; 2Department of Physiology and Pharmacology, Federal University of Pernambuco, Recife-PE, Brazil.

The lichen Cladonia verticillaris is very common in the northeast of Brazil. It has as essential biologically active secondary metabolite the fumarprotocetraric acid. Similar chemical composition is found in Cetraria islandica used in Turkish folk medicine for treatment of bronchitis and tuberculosis. This study shows the effect of FUM isolated from C. verticillaris on tracheobronchial phenol red excretion in mice. FUM administered by oral gavage (50 and 100 mg/kg) or intraperitoneal (25 and 50 mg/kg) and Ambroxol (1 mg/kg) were administered in male Swiss mice (n=6 animals/group) thirty minutes before the administration of phenol red (200 mg/kg; i.p.). Administration of vehicle (solution saline 0.9%) was used as control. Sixty minutes after the drug administration, the mice were euthanized and a bronchial lavage (BL) was realized with saline. The lavage fluid was mixed with NaOH 0.01N, and the quantification of phenol red in BL was analysed photometrically at 535 nm. The expectorant effect was determined by comparing the phenol red concentration (mg/mL) in BL of treated and control group. It was shown that oral gavage (50 mg/kg and 100 mg/kg) or intraperitoneal (25 and 50 mg/kg) administration of FUM increased phenol red excretion in BL in a dose-dependent manner in comparison to control group (p < 0.05). However, there was no statistical difference between phenol red excretion in the BL after intraperitoneal (3.2 ± 0.38 ng/mL) or oral gavage (4.0 ± 0.68 µg/mL) administration of the same dose (50 mg/kg). The results suggest that expectorant action of FUM is not mediated by a vagal reflex initiated by stimulation of the gastric mucosa following oral administration. Keywords: Cladonia verticillaris, fumarprotocetraric acid, excretion Acknowledgement: CAPES References: [1] Dülger B et al. (1998) TJB 22: 111 – 118. [2] Santos N. P, et al. (1997) RUASCB 1(2): 23 – 43.

**PM189**

Non-host interactions to detect anti-Fusarium substances 
Schump O1, Bruderhofer N1, Gindro K2, Wolfender J1  
1Agroscope Changins-Wädenswil ACW, Route de Duller 50, 1260 Nyon, Switzerland; 2School of Pharmaceutical Sciences EPFL, University of Geneva, University of Lausanne, 30 Quai Ernest-Ansermet, 1211 Geneva 4, Switzerland

Fusarium are ubiquitous filamentous fungi and a major threat for numerous plant or animal species [1]. They are often considered as highly resistant to conventional antifungal treatments. The Fusarium genus also contains isolates considered as endophytopic or saprotrophic strains. These lifestyles are dispersed over a large phylogenetic spectrum [2], which suggests either multiple evolution of the same trait or enormous capacity of each strain to adapt to the available environment or host. We've shown that Fusarium strains have genuine capacity to develop on various host and we made use of these adaptation capacities to establish non-host interactions on vine leaves. Some Fusarium strains developed efficiently as necrotrophic pathogens while others adopt less aggressive mode of interaction on susceptible vine varieties as Chasselas. Correspondingly, some Vitis species or varieties were more resistant and restrained efficiently fungal growth. To identify natural substrates involved in the control of fungal development, we set-up a sensitivity test in 96 well plates to screen for anti-Fusarium activity of natural extracts. The test on solid growth medium enables to screen natural extracts on several filamentous fungi and we compared fungal susceptibility to these extracts using various human and plant pathogens. Keywords: Fusarium, antifungal Acknowledgement: This work was supported by Swiss National Science Foundation Sinergia Grant CRSII1_127187 (to J-L. W. and K. G.) References: 1. Schuch S et al. (2010) Agrarforschung Schweiz 1: 442 – 445. 2. Zhang et al. (2006) J Clin Microbiol 44: 2186 – 2190

**PM190**

Coronilla varia L. nitrotoxins as defensive secondary metabolite against heavy metals pollution 
Noozi M, Amini F, Forough M  
Department of Biology, Faculty of science, University of Arak, P. O. Box: 38156 – 8-8349, Arak, Iran

Nitrotoxins or nitroglycosides are aliphatic nitro compounds, which chemically or structurally glucose esters of nitropionic acid and nitropropanol, which were detected in some legumes (Papilionoideae). They are important due to mammal toxicities, seed disperses repulsion or inhibition of herbivores and microorganisms and has a role in plant defense [1]. In this study six weeks aged grown Coronilla varia L. plants in equal growth condition were treated with different concentrations of Zn and Ni for 24 and 72 h. The Qualitative test and quantitative determination for aliphatic nitro toxins of control and treated plants examined was done [2, 3]. Increasing nitrotoxins concentration ranging from 4 – 25 mg NO2 mg g-1plant in all treated plants were observed in compared to control (1 mg NO2 mg g-1plant). This study showed nitro compounds may have a protective defensive role against some environmental stresses such as heavy metals pollution [4, 5]. Keywords: Nitrotoxins, Coronilla varia, heavy metals, Zn, Ni, legumes Acknowledgement: Authors wish to thank Biology Department of University of Arak. References: 1. Majak M (2001) Range Management 54: 494 – 498. 2. Cooke AR (1955) Arch Biochem Biophys 55: 114 – 120. 3. Williams MC and Parker R (1974) Weed Sci 22: 259 – 262. 4. Noozi M et al. (2007) Toxicol and Environ Chem 89 (3): 479 – 485. 5. Noozi M et al. (2010) Toxicol and Environ Chem 92 (1): 97 – 105.

The Importance of Anthocyanins for Human and Animal Health 
Poracova J1, Trakicova L2, Blascakova M3, Muchanickova A3  
1Excellence Centre of Animal and Human Ecology, Presov University in Presov, Faculty of Animal and Human Health Sciences, 1, 17, November Street, 081 16 Presov, Slovak Republic; 2University of Veterinary Medicine and Pharmacy in Kosice, 73, Komenskeho, 040 01 Kosice; 3Presov University in Presov, Department of Ecology, Faculty of Humanities and Natural Sciences, 1, 17, November Street, 081 16 Presov

Anthocyanins belong to the plant secondary metabolites causing pigmentation to flowers, fruits, seeds and leaves. They are abundant in red berries and fruits. They are phenolic compounds belong to the flavonoids, and occur mainly as glycosides. They have antioxidant effects, and they protect cells against oxygen radical-related damage at the basis of various diseases. The concept evolves that the human and animal health condition could be partly controlled through the dietary intake of plant polyphenols. Plant polyphenols are recognized as naturally occurring antioxidants but also catalyze oxidative DNA degradation of cellular DNA either alone or in the presence of transition metal ions such as copper. In this paper we show that similar to various other classes of polyphenols, delphinidin is also capable of causing oxidative degradation of cellular DNA [1]. The antimicrobial activity and major anthocyanins pigments were determined of Vitis vinifera L. and the extracts of Vitis vinifera were studied with association of its antiradical activity [2]. Anthocyanin content varied from 85.7 to 1914.0 mg/kg fresh berry weight. Assessment of the antiradical activity of extracts suggesting that other constituents are likely to exert strong antioxidant effects in grapes. Our work is focused to study antimicrobial and antioxidant activity of anthocyanins from the selected plant species in Slovakia. Keywords: anthocyanines, antioxidant activity, health, animal Acknowledgement: This research is supported by the Agency of Ministry of Education SR for the Structural Funds of the EU, the project: ITMS 2622012003, ITMS 2622012004, ITMS 2622022003. References: [1] Hanif S et al. (2008) DNA Toxicology 249: 19 – 25. [2] Kallithraka S et al. (2005)J Food Compon Anal 18: 375 – 386.
PM192

Two matrix metalloproteinase inhibitors from Scrophularia striata Boiss.
Monsef Esfahani H1, Hajighaei R2, Shahverdi A3
1Department of Pharmacognosy, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran; 2Department of Pharmacognosy & Pharmacoeconomics, Institute of Medicinal Plants, ACECR, Tehran, Iran; 3Department of Pharmaceutical Biotechnology and Pharmaceutical Research Center, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran; 4Department of Pathobiology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran; 5Department of Medicinal Chemistry, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran

Because the activation of metalloproteinases (MMPs) is a key factor in the metastatic process, compounds with the ability to inhibit MMP activity have a potential in treatment of tumor. The genus Scrophularia, consisting of about 300 Species, is one of the genera belonging to the Scrophulariaceae. In this study, two active substances of S. striata Boiss. were identified by using bioassay-guided fractionation and their chemical structures were deduced by nuclear magnetic resonance and mass spectrometry. Neptirin at lower doses showed progressed MMP inhibitory effect with negligible cytotoxicity, whereas acteoside 1 at higher doses (up to 80 μg/ml) revealed most MMPs inhibitory effect preserving low cytotoxicity. Keywords: Scrophularia striata, neptrin, acteoside 1, wehi-164, zymoanalysis.

PM193

Brazilian Amazon plant extract active against head-and-neck tumor cell-line tested for toxicity against Saffredini II1, Estork DM2, Casmão DP3, Bernardi MM4
1Centre for Research in Biodiversity, Universidade Paulista – UNIP, São Paulo, SP, Brazil; 2Graduate Program in Veterinary, Universidade Paulista – UNIP, São Paulo, Brazil

Plant extract obtained from Pentaclethra macroloba (Willd.) Kuntze [Ppem], Simaroubaceae, showed antiproliferative activity against head-and-neck human tumor cell line. Toxicity assays were carried out in order to obtain its toxic tendency, as well as its LD50. Acute toxicity assay was carried out using groups of three young-adult Balb-c male mice. Animals received i.p. administration of Ppem, suspended in almond oil, also used in the control group. Doses were tested starting up from 5 g/kg and subsequent ½-fold-decreasing doses until the observation of non-lethality. General activity including evaluation of more than 20 parameters was also accessed. Statistics was done using ANOVA. Parameters observed after i.p administration of Ppem showed that general activity was not significantly affected by treatment, as well as the surface-righting-reflex, body tone and grip reflex. Hindquarter-fall showed was significantly different from control after Ppem-administration of 5 and 2.5 g/kg. Significant differences between treatments were observed for Ppem administered at 5 g/kg for stimulation, tremor and ptosis, while cyanosis appeared in lower doses. Response to stimulation significantly decreased after administration of higher doses as well as the response to tail squeeze, and aural reflex. Defecation significantly diminished after administration of higher doses, but mortality was not elevated. LD50 for Ppem was 37 mg/kg (medium-toxicity). Toxicity of Ppem was being investigated and some degree of toxicity was found, although the effective cause of death in mice is unknown so far. Substances causing both the antiproliferative and the toxicity is being isolated and elucidated.

PM194

Effect of an alpha – glucosidase inhibitor from the seeds of Adenanthera pavonina on rodent weight and function in mice.
Eskendari Offi J1, Oony A2, Amiri MN3, Obideh OO4
1Nnamdi Azikiwe University, Awka, Nigeria; 2University of Nigeria, Enugu Campus, Enugu, Nigeria; 3University of Nigeria,Nsukka Campus, Nsukka Nigeria

The compound responsible for the alpha glucosidase inhibitory activity of seeds of Adenanthera pavonina L. was isolated from ethanolic extract (70% v/v), purified by ion exchange chromatography and partially characterized by UV, 1H NMR, and 13C NMR to be a unresolved nitrogenous five-membered lactone structure, that is possibly pavonin. In vivo treatment of mice with isolate at sub acute levels (10 – 100 mg/kg body weight) for 28 days decreased significantly the relative weight (expressed as percentage of body weight) of the testes and ovaries of male and female rats respectively at p < 0.05. The glutathione-S-transferase activity of both organs also decreased significantly (p < 0.05) with time when compared with the control, suggesting reduced gonadal function. Histopathological studies are being investigated and some degree of toxicity was found, although reversion of foetus was observed in pregnant mice in the course of the experiment. Conclusively, the isolate may interfere with gonadal function by affecting spermatogenesis, oogenesis and possibly the production of sex hormones in both sexes. Keywords: Adenanthera pavonina, five membered lactone, gonadal weight, function. Acknowledgement: Dr A.I. Gray, Department of Chemistry, University of Strathclyde, Glasgow, for spectral elucidation of isolate. References: 1. Ali MS (2005) Nat Prod Res 19: 37 – 40. 2. Awasthi S et al. (1993) Arch Biochem Biophys 301: 143 – 150. 3. Macedo MLR et al. (2010) Arch Insect Biochem Physiol 73: 213 – 231

PM195

Triterpenoids as inhibitors of Plasmodium liver-stage development
Ramalhete C1, Cruz A2, Mulhovo S3, Prudêncio M2, Ferreira MM1
1Research Institute for Medicines and Pharmaceutical Sciences (Med.UI), Faculdade de Farmácia, Universidade de Lisboa, Av. das Forças Armadas, 1600 – 083, Lisboa, Portugal; 2Unidade de Malária, Instituto de Medicina Molecular, Universidade de Lisboa, 1649 – 028, Lisboa, Portugal; 3Departamento de Ciências Agronômicas, Escola Superior Técnica, Universidade Pedagógica, Campus de Lhanguene, Av. de Moçambique, 21402161 Maputo, Mozambique

Malaria is one of the foremost public health problems in Africa. It is endemic in 90 countries, affecting nearly 40% of the global population. The increasing prevalence of drug-resistant Plasmodium falciparum strains is one of the greatest challenges in malaria control. In order to overcome drug-resistance, new antimalarial drugs are urgently needed. Most of the available antimalarials agents kill blood stage and only a limited number of drugs act on liver stages. In fact, the study of Plasmodium liver stage development has been hampered by limitations in the experimental approaches required to quantify hepatocyte infection by the parasite. Therefore, the development of new drugs targeting the Plasmodium liver stage represents an important and under-exploited site of intervention [1, 2]. Previously, bioassay-guided fractionation of the methanol extract of the aerial parts of Momordica balsamina L led to the isolation of several cucurbitane-type triterpenoids. Several of those compounds and acylated derivatives displayed in vitro antimalarial activity against blood schizonts of chloroquine-sensitive and -resistant strains of Plasmodium falciparum [3 – 5]. In this study some of the isolated compounds from Momordica balsamina and several alkanoyl and acyl ester derivatives were evaluated for their in vitro tissue-schizontocidal activity on Plasmodium berghei infected hepatoma cells. Inhibition of liver stage infection was determined by measuring the luminescence intensity in Huh-7 cells infected with a firefly luciferase-expressing P. berghei line, PbhGFP-LucTb, as previously described [1]. Most of the compound tested displayed a dose-dependent antimalarial activity with IC50 < 5μM. Keywords: Malaria, Plasmodium, liver-stage, Momordica balsamina Acknowledgement: This study was supported by FCT, Portugal (SFRH/BD/22231/2005). References: 1. Ploemen et al (2009) PLoS One 4: e7881. 2. Prudêncio et al. (2008) Cell Microbiol 10: 218 – 24. 3. Ramalhete et al. (2009) Bioorg, Med Chem 17: 6942 – 51. 4. Ramalhete et
PM196

Antihelminthic activity of Cymbopogon schoenanthus and Cymbopogon martini essential oils evaluated by four different in vitro tests
Katiki LM1, Chagas AS2, Bizzo HR3, Ferreira JF1
1Instituto de Zootecnia, Nova Odessa – SP, Brazil; 2Embrapa Pecuária Sudeste, São Carlos – SP, Brazil; 3Embrapa Agroindústria de Alimentos, Rio de Janeiro – RJ, Brazil; 4USDA-ARS – Appalachian Farming Systems Research Center, Beaver – WV, USA;

Antihelminthic resistance is a worldwide matter in small ruminant industry and new compounds derived from plants are being studied to be used as an additional tool to control nematodes [1,2]. Cymbopogon schoenanthus Spreng, and Cymbopogon martini (Roxb.), J. F. Watson [family Poaceae] essential oils were chosen to be evaluated against development stages of trichostrongylids from sheep by Egg Hatch Assay (EHA), Larval Development Assay (LDA), Larval Exsheathment Assay (LEA) and larval feeding inhibition assay (LFI).

Table 1: CL50 (μL/mL) and confidence limits of Cymbopogon schoenanthus and Cymbopogon martini essential oils in egg hatch assay (EHA), larval development assay (LDA), larval exsheathment assay (LEA) and larval feeding inhibition assay (LFI) against C. schoenanthus and C. martini.

<table>
<thead>
<tr>
<th>Assay</th>
<th>C. schoenanthus</th>
<th>C. martini</th>
</tr>
</thead>
<tbody>
<tr>
<td>EHA</td>
<td>0.05 (0.04 – 0.06)</td>
<td>0.15 (0.10 – 0.17)</td>
</tr>
<tr>
<td>LDA</td>
<td>0.07 (0.06 – 0.08)</td>
<td>0.18 (0.17 – 0.19)</td>
</tr>
<tr>
<td>LEA</td>
<td>27.10 (21.37 – 32.38)</td>
<td>32.02 (29.87 – 34.47)</td>
</tr>
<tr>
<td>LFI</td>
<td>0.01 (0.01 – 0.02)</td>
<td>0.04 (0.03 – 0.05)</td>
</tr>
</tbody>
</table>


PM197

Anti-Zygomycetes activity of 7-hydroxycalameleone isolated from Croton cajucara
Azevedo MM1, Almeida CA2, Bizzo HR3, Chaves FC2, Alviano DS2, Alviano CS2
1Instituto de Química, Universidade Federal do Rio de Janeiro, Rio de Janeiro RJ, Brazil; 2Instituto de Microbiologia Professor Paulo de Góes, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil; 3Embrapa Agroindústria de Alimentos, Rio de Janeiro RJ, Brazil; 4Embrapa Amazônia Ocidental, Manaus AM, Brazil

The leaves and bark from Croton cajucara Benth. (family Euphorbiaceae), a shrub from the Amazon, have been used locally used in folk medicine to treat diabetes, malaria, gastrointestinal and liver disorders [1]. The essential oil from the leaves is rich in linalool [2] and presented antifungal and antimicrobial activities [3,4]. A chromotype of this species was found, with an essential oil rich in 7-hydroxycalameleone [5]. This substance is reported to have antifungal activity against Botrytis cinerea, Closstridium coccum, Phytophtora infestans, Pycniciaria oryzae and Septoria tritic.[6]. During our studies with C. cajucara oil, we isolated 7-hydroxycalameleone by silicagel column chromatography followed by preparative TLC. The pure compound (98.5% by GC) was tested against some zygomycetes. A minimum inhibitory concentration (MIC) of 9.76 μg/mL was found to be Absidia corymbifera, Cunninghamella elegans and Mucor circinelloides I. circelelloides, while for Rhizopus microsporus and Rhizopus oryzae the MIC was 19.53 μg/mL. The reference drug used, amphotericin B, presented a MIC of 43.9 μg/mL for C. elegans and M. circinelloides, and 0.3 μg/mL for the other species tested. From these data, we observed that 7-hydroxycalameleone is a compound with good activity against zygomycetes. Keywords: Zygomycetes, Croton cajucara, essential oil, antifungal activity Acknowledgements: CAPES, FAPEPA, References: 1. Maciel MAM et al. (2000) J Ethnopharmacol 70: 41 – 45. 2. Lopes D et al. (2000) J Essent Oil Res 12: 705 – 708. 3. Rosa MSCS et al. (2003) Antimicrob Agents Chemother 47: 1905 – 1909. 4. Alviano WS et al. (2005) Oral Microbiol Immunol 20: 101 – 105. 5. Pereira AQ et al. (2010) J Essent Oil Res 23: 20 – 23. 6. Scher JM et al. (2004) Phytochemistry 65: 2583 – 2588.

PM198

Simultaneous determination of four auxins in cyanobacterial extracts using HPLC-ESI-MS
Seyed Hasrourdi M1, Shariatmadari Z2, Riahi H2, Ghassempour A3
1Department of Phytochemistry, Medicinal Plants and Drugs Research Institute, Shahid Beheshti University, G.C. Evin Tehran, Iran; 2Faculty of Biosciences, Shahid Beheshti University, G.C. Evin, Tehran, Iran

The prokaryotic cyanobacteria are important source of structurally bioactive secondary metabolites[1]. Besides having considerable pharmacological impacts such as antibacterial, antifungal and also cytotoxicity, the potential biofertilizer activity of cyanobacteria make them an attractive alternative to chemical fertilizers. Most paddy soils have a natural population of cyanobacteria. Treatment of rice seeds with collected cyanobacteria from paddy fields of Iran showed positive growth effect in vitro [2] which could be partly attributed to the possible presence of phyt hormons in their composition [3]. Herein, we report a new approach for the simultaneous determination of four important auxins in cyanobacteria. Under the optimized conditions, a complete separation of four auxins was achieved within a short time with a good reproducibility. The comparison of auxin chromatographic profile of this cyanobacteria with others and also their concentration levels will be reported and further discussed. Keywords: Cyanobacteria, Auxin, HPLC-ESI-MS -References: 1. Kiyota Y (2007) Phytochemistry 68: 971 – 979 2. Saadatnia H, Riahi H (2009) Plant Soil Environ 55:207 – 212 3. Sergeeva E, Liämer A, Bergman B (2002) Planta 215: 229 – 238

PM199

Comparison of the cytotoxicity and antimicrobial activity of several isohexenylnaphthazarins
Kretschmer N5, Damianakos H2, Chinchou I1, Andujar I1, Rios J1, Kunert O6, Boechelt H2, Bauer R7
1Institute of Pharmaceutical Sciences, Pharmacognosy, Karl-Franzens University, Universitätsplatz 4, 8010 Graz, Austria; 2Department of Pharmacognosy, School of Pharmacy, University of Athens, University Campus of Zografou, 157 71 Zografou Athens, Greece; 3Department of Pharmacology, Faculty of Pharmacy, University of Valencia, Av. Vicent Andrés Estelles s/n,46100 Burjasot, Spain; 4Institute of Pharmaceutical Sciences, Pharmaceutical Chemistry, Karl-Franzens University, Universitätsplatz 1, 8010 Graz, Austria; 5Department of Plant Materials Sciences and Utilisation – Institute Resources, Joanneum Research Forschungsgesellschaft mbH, Elisabethstrasse 16, 8010 Graz, Austria

Shikonins, alkannins and derivatives thereof are natural, lipophilic red pigments found in many species of the Boraginaceae family. Since many centuries, they are traditionally used for the treatment of wounds and have been shown to possess wound-healing, anti-inflammatory, anti-microbial, anti-tubercotic and anti-cancer activities [1]. The cytotoxicity of several shikonins (shikonin, acetylsichikonin, β-hydroxysovalerylshikonin, isobutyrylshikonin, 2-methyl-n-butyryl-shikonin, deoxyshikonin, dimethylacrylshikonin, epoxysichikonin, and isovalerylshikonin) and alkannins (alkannin, acetyllalkannin, β-hydroxysovalerylalkannin, isovalerylalkannin, α-methyl-butyryl-alkannin, dimethylacrylalkannin propionylalkannin and teracylalkannin) was determined using the XTT viability assay and human CCRF-CEM leukemia, MDA-MB-231 breast cancer, U251 glioblastoma and HCT 116 colon cancer cells. Most IC50 values of shikonins were in a range of 0.1 to 10μM, whereby, the highest activity was found for shikonin. IC50 values of alkannins varied from 0.4 to 70μM indicating that shikonin derivatives possess a higher cytotoxic potential than alkannins. Dimethylacrylalkannin exhibited no
activity up to 100 μg/ml in contrast to dimethylcysteikinon. Moreover, the anti-microbial activity of the alkannin derivatives and acetylshikonin against nine microorganisms (Staphylococcus aureus, S. epidermidis, Escherichia coli, Enterobacter cloacae, Klebsiella pneumonia, Pseudomonas aeruginosa, Candida albicans, C. tropicalis and C. glabrata) was examined by the disc diffusion method. The most active derivatives were alkannin, acetylshikonin, β-hydroxyisovaleralkannin and isobutyrylalkannin. Also in this case, acetylshikonin exhibited higher activity than the respective alkannin derivative. Keywords: Isohexenylnaphthazarins, shikonins, alkannins, cytotoxicity, antimicrobial activity. Acknowledgement: This work was supported by the “FWF – Fonds zur Förderung der Wissenschaftlichen Forschung” P21114. References: 1. Papageorgiou VP et al. (1999) Angew Chem Int Ed. 38: 270.

PM200

Evaluation of antioxidant capacity and L-ascorbic acid content in Brazilian tropical fruits acerola (Malpighia emarginata), mangaba (Harcona speciosa), siriguela (Spondias purpurea) and umbu (Spondias tuberosa) 

Ramalho SA, Guaitero NC, Oliveira GB, Gomes ED, Miranda RM, Narain N

Laboratory of Flavor Analysis and Chromatography, Federal University of Sergipe, Sao Cristovao, Sergipe, Brazil

Lately in Brazil there is an appreciate increase in the consumption of non-traditional tropical fruits. However, very little information is available on the presence of bioactive compounds and antioxidant properties in these fruits. Some locally grown tropical fruits such as acerola (Malpighia emarginata DC), mangaba (Harcona speciosa Gomez), siriguela (Spondias purpurea L) and umbu (Spondias tuberosa Arruda) were analyzed for their antioxidant capacity and L-ascorbic acid content. Antioxidant capacity was measured by using 2,2-diphenyl-1-picrylhydrazyl (DPPH) standard and L-ascorbic acid content was determined by ultrafast liquid chromatography using a ShimadzuTM, UFLC-20A chromatograph with a reversed-phase octadecysilane column XR-ODS TM, and 0.025 M of a dihydrogen potassium phosphate solution as the mobile phase. Antioxidant capacity, expressed as concentration of antioxidant required to reduce the original amount of free radicals by 50% and values expressed as g of pulp per g of DPPH, was 7,257.6 for acerola; 15,163.9 for the mangaba; 9,415.3 for the siriguela and 14,100.4 for the umbu fruit pulp. Ascorbic acid was not detected in siriguela pulp, and its content (in mg per 100 g of pulp) was 1,719.63 in acerola; 22.62 in mangaba and 19.53 in umbu. These results indicate the nutritional and therapeutic potential of these tropical fruits for their antioxidants properties. Among the fruits evaluated, the decreasing order of antioxidant activity was ranked as mangaba followed by umbu, siriguela and acerola fruits. Keywords: Bioactivity, bioactive compounds, food phenolics Acknowledgement: This work was supported by the INCT/CNPq (National Council for the Development of Science & Technology, Brazil) for the financial support received while the fourth and fifth co-authors also thank CAPES for fellowships.

PM201

Allopathic impact of some medicinal plants on Portulaca oleracea L. and Lepidium sativum L. seed germination: a study to find natural herbicide Fattahi M1, Fattahi B1, Seyed Shirazi S2, Mosavi F3, Moharram Zade M4, Nazeri V1

1Horticultural Department, College of Agriculture and Natural sciences, University of Tehran, Karaj, Iran; 2Institute of Higher Education of Mehran, Mahalat, Iran; 3Desert management section, College of Agriculture and Natural sciences, University of Tehran, Karaj, Iran

Recently by expose the health requirement as primal needs of mankind and people interested to use of organic production, investigation to find out effective natural compounds to replace of synthetic materials is increasing [1, 2, and 3]. In this context, the use of plant extracts as natural materials is a new strategy for management of weeds. In present study the allelopathic effects of aqueous and hydro-alcoholic extracts of Ruta graveolens L., Artemisia sieberi Besser, Allium sativum L., Tribulus terrestris L. and Peganum harmala L. were evaluated on Portulaca oleracea L. and Lepidium sativum at plant germination stage (pre-emergence). Percentage, germination rate (GR) and mean days to germination (MDG) obtained in 1-day intervals for 12 days. The results showed that hydro-alcoholic were effective in prevention of seed germination in compare with aqueous extract. Aqueous extract of R. graveolens with

PM202

Chemical composition, antioxidant and antimicrobial properties of Frankenia thymifolia Desf. shoot extracts

Ksouri WM1, Chaouachi F2, Medini F1, Zaouali Y1, Ksouri R2

1Laboratoire des Plantes Extrêmedéphiles, Centre de Biotechnologie à la Technopole de Borj-Cédria, BP 901, 2050 Hammam-lif, Tunisie; 2Laboratoire de Biotechnologie Végétale, Institut National des Sciences Appliquées et Technologie (INSAT). BP 676, 1080 Tunis Cedex, Tunisie

Frankenia thymifolia Desf. is an endemnic xero-halophyte species in the salted and arid region of Tunisia [1]. In this study, two shoot fractions (methanolic and chloroformic) of Frankenia were assessed on their polyphenol contents and biological activities [2]. Then, the main phenolic and fatty acid compositions were identified. Results showed that polar fraction contain a highest amount of polyphenol, flavonoid and condensed tannin contents (14.2 mg GAE g-1 DW, 4.8 and 4.6 mg CE g-1 DW respectively). The higher phenolic content in this fraction reflect the best total antioxidant capacity (8.8 mg GAE g-1 DW), antiradical activity against DPPH, β-carotene bleaching and Fe-reducing tests with the lowest IC50 and EC50 values as compared to apolar fraction. However, chloroformic fraction was more efficient against human pathogen strains. In fact, this fraction was active against all strains. The HPLC analysis showed that salicylic and trans-cinnamic acids were the major phenolics. The major fatty acids identified by GC/MS were palmitic, elaidic and linoleic acids. Such variability in biological capacities between the 2 fractions can be explained by different bioactive compounds contain in each fraction and might be of great importance in terms of valorizing this halophyte as a source of bioactive molecules for cosmetic and pharmaceuticas industries. Keywords: biological activities, fatty acids, Frankenia thymifolia, FPLC, OPLC, IC50, EC50, Haba H, Harconia H, Marcourt L, Long C, Benkhaled M (2007) Biochem Syst Ecol 35: 176 – 179. 2 Mout-Duros L, Le Floch G, Magne C (2008) J Ethnopharmacol 116: 258 – 262.

PM203

Solvent effects on Antioxidant and biological activities of the halophyte Nitraria retusa (Forsk.) Asch Zaouali Y1, Ksouri WM1, Saada M2, Cheidy A2, Ksouri R2

1Laboratoire de Biotechnologie Végétale, Institut National des Sciences Appliquées et Technologie (INSAT). BP 676, 1080 Tunis Cedex, Tunisie; 2Laboratoire des Plantes Extrêmedéphiles, Centre de Biotechnologie de Borj-Cédria (CBBC). BP 901, 2050 Hammam-lif, Tunisie.

Nitraria retusa (Forsk.) Asch is a traditional medicinal species widely used as anti-inflammatory and cicatrizant agent. In this work, two fractions (non-polar and polar) of Nitraria leaves, after fractionation by solvent mixture (chloroform/methanol/water, 12/5/3), were investigated on their phenolic content, antioxidant activity (using several tests) and antimicrobial capacity against human pathogen strains. Moreover, phe- nolic and fatty acid compositions were identified using RP-HPLC and GC/MS, respectively. Results showed that phenolic contents and antioxidant activities varied considerably as function of solvent polarity. Polar fraction (methanol/water) led the highest antioxidant (7.89 mg GAE/g DW) and tannin (1.78 mg CE/g DW) contents, while chloroform fraction (non-polar) exhibits the highest content of flavonoids (2.74 mg CE/g DW). Moreover, antiradical activity against DPPH, and β-carotene bleaching test (IC50 values were 39, 700 μg/mL, respectively) and Fe-
Reducing power (EC50 = 410 μg ml−1) were more important in leaf non-polar fraction as compared to polar fraction. Besides, chloroform fraction was more efficient against all human pathogen strains mainly Escherichia coli and Staphylococcus aureus. The HPLC analysis showed two major phenolic compounds: trans-4-hydroxy-3-methoxybenzoic acid (ferulic acid) and p-coumaric acid. The major fatty acids identified by GC/MS were palmitic acid (28.04%), and polyunsaturated acids (48.78%) are characterized by linolenic acid (29.69%) and α-linolenic acid (omega 3) (19.08%). These results indicate that selective extraction of bioactive molecules from natural sources as halophyte species, by appropriate solvents, is important for obtaining fractions with high biological activities which can be used as preservative ingredients in food, cosmetic and/or pharmaceutical industries. Keywords: Nitraria retusa, phenolic content, biological activities, phenolic composition, fatty acid, GC/MS.

**References:**

The evaluation of *Teucrium persicum* methanolic extract and its fractions in Pavlovian Fear Conditioning

**Moncef Esfahani H1, Sharifizadeh M2, Moattari M1, Miri A1, Nezireslami E2**

1Department of Pharmacognosy, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran 14155 – 6451, Iran; 2Department of Department of Toxicology & Pharmacology, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran 14155 – 6451, Iran

In this research, the effects of total methanolic extract and related fractions (chloroform, ethyl acetate, aqueous) of *Teucrium persicum* Boiss. on conditional memory were evaluated. The methanolic extract of *T. persicum* aerial parts and related fractions were administrated orally in doses of 100 mg/kg, 300 mg/kg, 400 mg/kg every 24 hours for 10 days to male rats. 24 hours later animals were trained in Pavlov Fear Conditioning. Training consists of 5 tone (30 s, 2.3 kHz, 80 dB)-shock (2 s, 1 mA) pairings. Fear to the context and the tone were evaluated by measuring freezing in separate tests. The results showed that chloroform fraction of the methanolic extract had a positive effect on fear conditioning. According to positive effects of chloroform fraction on fear conditioning, possible cholinergic properties of the chloroform fraction (consisting of terpenes) was tested by inducing conditional memory via scopolamine (a non-selective cholinergic antagonist) before training. It was found that the chloroform fraction had a protective effect against cholinergic disruption. All the doses had more freezing duration(s) compared to control group and there was a significant difference between 400 mg/kg and control group (P value 0.01 for context test, P value 0.05 for tone test). It is concluded that chloroform fraction has a memory enhancing effect according to cholinergic properties of the component. Keywords: Teucrium persicum, Alzheimer's disease, pavlovian fear conditioning, contextual conditioning, tone conditioning, chloroform fraction

Fatty acid composition and antioxidant activity of *Pistacia lentiscus* L. fixed oil

**Mezni F1, El Kharchani A1, Boussaïd M2, Khouja M1**

1National Institute for Researches on Rural Engineering, Water and Forests, INRBGF, BP 10 Ariana 2080, Tunisia; 2National Institute of Applied Sciences and Technology, INSAT. BP 676 – 1080, Tunis Cedex, Tunisia

*Pistacia lentiscus* L. is known by its essential oil and its mastic. The fixed oil extracted from fruits is used in traditional medicine for stomach diseases and wound healing. This study aims to determine the fatty acid composition and the antioxidant activity of *Pistacia lentiscus* fixed oil extracted from different parts of mature fruits. The work was performed on three different parts of the fruit: the envelope, seed and whole fruit. The work was performed on three different parts of the fruit: the envelope, seed and whole fruit. The work was performed on three different parts of the fruit: the envelope, seed and whole fruit.

PM204

The evaluation of *Teucrium persicum* methanolic extract and its fractions in Pavlovian Fear Conditioning

Moncef Esfahani H1, Sharifizadeh M2, Moattari M1, Miri A1, Nezireslami E2

1Department of Pharmacognosy, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran 14155 – 6451, Iran; 2Department of Department of Toxicology & Pharmacology, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran 14155 – 6451, Iran

In this research, the effects of total methanolic extract and related fractions (chloroform, ethyl acetate, aqueous) of *Teucrium persicum* Boiss. on conditional memory were evaluated. The methanolic extract of *T. persicum* aerial parts and related fractions were administrated orally in doses of 100 mg/kg, 300 mg/kg, 400 mg/kg every 24 hours for 10 days to male rats. 24 hours later animals were trained in Pavlov Fear Conditioning. Training consists of 5 tone (30 s, 2.3 kHz, 80 dB)-shock (2 s, 1 mA) pairings. Fear to the context and the tone were evaluated by measuring freezing in separate tests. The results showed that chloroform fraction of the methanolic extract had a positive effect on fear conditioning. According to positive effects of chloroform fraction on fear conditioning, possible cholinergic properties of the chloroform fraction (consisting of terpenes) was tested by inducing conditional memory via scopolamine (a non-selective cholinergic antagonist) before training. It was found that the chloroform fraction had a protective effect against cholinergic disruption. All the doses had more freezing duration(s) compared to control group and there was a significant difference between 400 mg/kg and control group (P value 0.01 for context test, P value 0.05 for tone test). It is concluded that chloroform fraction has a memory enhancing effect according to cholinergic properties of the component. Keywords: Teucrium persicum, Alzheimer’s disease, pavlovian fear conditioning, contextual conditioning, tone conditioning, chloroform fraction

PM205

Protective effect of (-) α-bisabolol on markers of oxidative stress in erythrocytes subjected to oxidative stress

Lugman S, Sriwastava S

Molecular Bioprospection Department of Biotechnology Division, Central Institute of Medicinal and Aromatic Plants (Council of Scientific and Industrial Research), Lucknow-226015, India

(-)α-bisabolol is a sesquiterpene alcohol found as a major component of essential oil of chamomile (*Matricaria recutita* L., *Chamomilla recutita* L., *Matricaria chamomilla* L.; Family Asteraceae). Chamomile, one of the most ancient and widely recognized herbs to mankind, has been used traditionally for centuries as an anti-inflammation, antispasmodic, carminative, mild astringent and healing medicine [1,2]. It is also known to be very helpful as an external agent for encouraging the rapid healing of ulcers and burns without infection, as well as persistent skin problems such as eczema and psoriasis [3]. Since clinical trials and human studies are limited, we have investigated the effect of (-)α-bisabolol on markers of oxidative stress in human erythrocytes by incubating with hydrogen peroxide (2 mM) and tert-butyl hydroperoxide (10 μM). Subjecting erythrocyte to oxidative stress caused a significant alteration in reduced glutathione (GSH), malondialdehyde (MDA) concentration as well as superoxide dismutase and catalase activity compared to control. Presence of (-)α-bisabolol as low as 0.1 μM in incubation medium protected the erythrocytes from oxidative stress and helps to maintain the basal level of GSH and MDA. The activity of superoxide dismutase and catalase were also restored in a concentration-dependent manner (0.01 – 100 μM). The effect was also compared with L-Ascorbic acid, quercetin and BHT. Our findings provide evidence for the protection of oxidative stress in erythrocytes by (-)α-bisabolol that could be considered for further studies. Keywords: (-)α-bisabolol, GSH, MDA, Superoxide dismutase, Catalase, Hydrogen peroxide, tert-buty1 hydroperoxide

Acknowledgement: Council of Scientific and Industrial Research, New Delhi, Council of Science and Technology, Government of Uttar Pradesh, India.


PM206

Cytotoxic activity of selected plants extracts on normal and cancerous oral mucosal cells

Abdul Razak F, Mohd Majid Z, Ab Rahman M, Zuraida M

Faculty of Dentistry, University of Malaya, Kuala Lumpur, Malaysia

Oral cancer is associated with cells of the oral mucosa that have become malignant and destroys healthy tissues of the lips, tongue, gingiva and other intra-oral locations (1). Many plants from the tropical forest of Malaysia have been used in local medicaments in the prevention and treatments of various types of cancer. The active compounds of these plant materials are often obtained through vigorous heating and then consumed in the form of decoction. The objective of this study was to determine the toxic effect of extracts from three plants on oral mucosal cell lines. Fibroblast and two cancerous oral mucosal cell lines which include the KB (ATCC) and ORL-48 (CARIF, Malaysia) cells were tested for their responses to the extracts of *Brueca* sp., *Typhonium* sp. and *Azadirachta* sp. using the neutral red cytotoxicity assay (2). The effective concentrations (EC50) of the extracts and the cytotoxic response of the cells at various concentrations of the extracts was observed using Giemsa staining procedure. Results revealed the extracts from all three plants to exhibit cytotoxic activity towards KB cells with EC50 at less than 100 μg/ml. Potent cytotoxic activity on ORL-48 cells at an EC50 of 0.67 ± 0.11 μg/ml was only displayed by *Brueca* sp. extract. Extracts of all plants did not produce toxic effect on normal fibroblast cells. Based on its strong cytotoxic activity, *Brueca* sp. extract is worthy of further investigation to isolate and identify the active compounds. Keywords: *Brueca* sp.; *Typhonium* sp.; *Azadirachta* sp. Acknowledgement: The research was financial supported by the University of Malaya Research Grant (RG020/09HTM) and Post Graduate Grant (PS164/2010B). References: 1. Kumar V, Cotran RS, Robbins S.L (2010) *Basic Pathology* 6th Edn. W.B. Saunders and Company. USA. 2. Fathilah AR, Sujata R, Norhanom WA, Ilham MI (2010) *J Med Plants Res* (2006) Basic Pathology 6th Edn. W.B. Saunders and Company. USA. 2. McKay DL, Blumberg JB (2006) Phytother Res 20(7): 519 – 530. 3. Martens D (1995) Chiropractic Acud Homeopathy 6:15 – 18.
determined and the highest antioxidant activity value was reached by oil extracted from whole fruit with a percentage of inhibition of DPPH about 9.67% and about 7% for both envelope and seed oil. Similarly, the highest value of TEAC was reached by whole fruit oil with about 20.0% for both envelope and seed oil. The results of histopathological evaluation supported the outcome of the wound healing effect was comparatively evaluated with a reference ointment Madecassol®. Noteworthy wound healing activity was observed for the ointment formulation prepared with 1% methanol extract. The results of histopathological evaluation supported the outcome of both incision and excision wound models. The methanol extract of T. pratense var. pratense L. var. anatolicum (Boiss.) Boiss., T. hybridum L. var. hybridum, T. pannonicum Jacq., T. pratense L. var. pratense, T. purpureum Lois. var. purpureum, T. repens L. var. repens, T. resupinatum L. var. microphloem Zoh., T. spadiceum L., T. trichophyllum Boeck. Results were also evaluated histopathologically. The wound healing effect was comparatively evaluated with a reference ointment Madecassol®. Noteworthy wound healing activity was observed for the ointment formulation prepared with 1% methanol extract. The results of histopathological evaluation supported the outcome of both incision and excision wound models. The methanol extract of T. pratense var. pratense and T. canescens exerted wound healing activity. T. canescens which contains 0.1% genistein and 0.05 biochanin A is found effective than T. pratense var. pratense which contains 0.04% genistein and 0.01% biochanin A, according to our previous studies which contains % 0,13 genistein and % 0,05 biochanin A. Inhibitors of this enzyme derived from the population ancestors. In the nursery, it was found that the seeds of Quina have low germination. The seedlings and plants show a high degree of adaptation to the environment and the medical use of the plant. The medicinal plant “Velame” (Macrocyphonia velame (A. St.- Hil.) Müll. Arg. – Apocynaceae) is a medicinal plant used as aphrodisiac. Biocidal effect of A. arvensis was evaluated on the larvae of Spodoptera frugiperda. Plant leaves were collected in Cáceres (MT, Brasil) to make a Catuaba crude methanolic extract (EMeC). Five 50 mL concentrations were used to make the treatments: T0 (0,00, distilled water; T1 = 2,250; T2 = 4,503; T3 = 9,000 and T4 = 18,000 ppm). It was used 120 third instar larvae of the second generation, raised at 30 ± 3 °C and relative humidity of 60 ± 10%. Under the effect of the treatments, the larvae were distributed in three classes of larval duration (Gi: days): C1: < 10 (10,26%), C2: 10 – 15 (64%), C3: > 15 (25,64%). The most significant differences between the observed and expected frequencies of larvae in C1, C2 and C3 occurred in T2 (50,0%), T3 (30,67%) and T4 (3,33%), respectively. Treatment T1 (25%) showed no significant difference in the larvae mortality (C1, C2 and C3) occurred in T2 whereas the largest contribution to C1 was T2 (26,08%) and 0 by T1. The treatments which contributed the least to C3 class were T1 and T2. The mean larval duration in T4 (13,87 ± 1,512A) and T0 (13,70 ± 2,176A) are similar and longer in T2 (11,65 ± 2,308B) whereas those of T1 (12,62 ± 1,055AB) and T3 (12,77 ± 2,287AB) are among those with a higher mortality. The results suggest that, at a certain concentration, the EMeC is bioactive, accelerating the larval cycle of S. frugiperda, because T1 and T2 tend to shorten (C1) and to center (C2) over the larval period, respectively, whereas T4 showed no equating effect for T0. Keywords: Anemopaegma arvens (Vell) Stellfeld FJ & de Sousa (Bignonia-ceae) is a medicinal plant used as aphrodisiac. Biocidal effect of A. arvenses was evaluated on the larvae of Spodoptera frugiperda. Plant leaves were collected in Cáceres (MT, Brasil) to make a Catuaba crude methanolic extract (EMeC). Five 50 mL concentrations were used to make the treatments: T0 (0,00, distilled water; T1 = 2,250; T2 = 4,503; T3 = 9,000 and T4 = 18,000 ppm). It was used 120 third instar larvae of the second generation, raised at 30 ± 3 °C and relative humidity of 60 ± 10%. Under the effect of the treatments, the larvae were distributed in three classes of larval duration (Gi: days): C1: < 10 (10,26%), C2: 10 – 15 (64%), C3: > 15 (25,64%). The most significant differences between the observed and expected frequencies of larvae in C1, C2 and C3 occurred in T2 (50,0%), T3 (30,67%) and T4 (3,33%), respectively. Treatment T1 (25%) showed no significant difference in the larvae mortality (C1, C2 and C3) occurred in T2 whereas the largest contribution to C1 was T2 (26,08%) and 0 by T1. The treatments which contributed the least to C3 class were T1 and T2. The mean larval duration in T4 (13,87 ± 1,512A) and T0 (13,70 ± 2,176A) are similar and longer in T2 (11,65 ± 2,308B) whereas those of T1 (12,62 ± 1,055AB) and T3 (12,77 ± 2,287AB) are among those with a higher mortality. The results suggest that, at a certain concentration, the EMeC is bioactive, accelerating the larval cycle of S. frugiperda, because T1 and T2 tend to shorten (C1) and to center (C2) over the larval period, respectively, whereas T4 showed no equating effect for T0. Keywords: Anemopaegma arvens (Vell) Stellfeld FJ & de Sousa (Bignonia-ceae) is a medicinal plant used as aphrodisiac. Biocidal effect of A. arvenses was evaluated on the larvae of Spodoptera frugiperda. Plant leaves were collected in Cáceres (MT, Brasil) to make a Catuaba crude methanolic extract (EMeC). Five 50 mL concentrations were used to make the treatments: T0 (0,00, distilled water; T1 = 2,250; T2 = 4,503; T3 = 9,000 and T4 = 18,000 ppm). It was used 120 third instar larvae of the second generation, raised at 30 ± 3 °C and relative humidity of 60 ± 10%. Under the effect of the treatments, the larvae were distributed in three classes of larval duration (Gi: days): C1: < 10 (10,26%), C2: 10 – 15 (64%), C3: > 15 (25,64%). The most significant differences between the observed and expected frequencies of larvae in C1, C2 and C3 occurred in T2 (50,0%), T3 (30,67%) and T4 (3,33%), respectively. Treatment T1 (25%) showed no significant difference in the larvae mortality (C1, C2 and C3) occurred in T2 whereas the largest contribution to C1 was T2 (26,08%) and 0 by T1. The treatments which contributed the least to C3 class were T1 and T2. The mean larval duration in T4 (13,87 ± 1,512A) and T0 (13,70 ± 2,176A) are similar and longer in T2 (11,65 ± 2,308B) whereas those of T1 (12,62 ± 1,055AB) and T3 (12,77 ± 2,287AB) are among those with a higher mortality. The results suggest that, at a certain concentration, the EMeC is bioactive, accelerating the larval cycle of S. frugiperda, because T1 and T2 tend to shorten (C1) and to center (C2) over the larval period, respectively, whereas T4 showed no equating effect for T0. Keywords: Anemopaegma arvens (Vell) Stellfeld FJ & de Sousa (Bignonia-ceae) is a medicinal plant used as aphrodisiac. Biocidal effect of A. arvenses was evaluated on the larvae of Spodoptera frugiperda. Plant leaves were collected in Cáceres (MT, Brasil) to make a Catuaba crude methanolic extract (EMeC). Five 50 mL concentrations were used to make the treatments: T0 (0,00, distilled water; T1 = 2,250; T2 = 4,503; T3 = 9,000 and T4 = 18,000 ppm). It was used 120 third instar larvae of the second generation, raised at 30 ± 3 °C and relative humidity of 60 ± 10%. Under the effect of the treatments, the larvae were distributed in three classes of larval duration (Gi: days): C1: < 10 (10,26%), C2: 10 – 15 (64%), C3: > 15 (25,64%). The most significant differences between the observed and expected frequencies of larvae in C1, C2 and C3 occurred in T2 (50,0%), T3 (30,67%) and T4 (3,33%), respectively. Treatment T1 (25%) showed no significant difference in the larvae mortality (C1, C2 and C3) occurred in T2 whereas the largest contribution to C1 was T2 (26,08%) and 0 by T1. The treatments which contributed the least to C3 class were T1 and T2. The mean larval duration in T4 (13,87 ± 1,512A) and T0 (13,70 ± 2,176A) are similar and longer in T2 (11,65 ± 2,308B) whereas those of T1 (12,62 ± 1,055AB) and T3 (12,77 ± 2,287AB) are among those with a higher mortality. The results suggest that, at a certain concentration, the EMeC is bioactive, accelerating the larval cycle of S. frugiperda, because T1 and T2 tend to shorten (C1) and to center (C2) over the larval period, respectively, whereas T4 showed no equating effect for T0. Keywords: Anemopaegma arvens, Spodoptera frugiperda, biocidal effect.
The medicinal herb Sarsaparilla (Herreria sarsaparilla Mart. [Herreriaceae]), native in Céres (MT, Brazil), was studied in our laboratory and the field, between 2003 – 2009. The species also has ornamental potential. It has roots thickened tuberiforms and elongated stem cylinders. The roots and leaves of the plant, in tea form, are used in folk medicine for many different diseases, such as sweat, blood purifying, diuretic, and also for the treatment of skin diseases, gout, syphilis, gonorrhea, arthritis, fevers, coughs, and hypertension. They mention that the root tea also help fight obesity; tea leaves and branches are used to aid digestion and relieve stomach pains. To treat influenza, colds and rheumatism use the infusion. The healers in the city of Céres sell the plant root, especially, for purifying and anti-rheumatic. There are few scientific studies of this species relationship with the indicated uses in folk medicine. In the laboratory, to evaluate the digestive tract in various concentrations, the effect of the raw methanol extract of leaves of the plant on mining of Spodoptera frugiperda not was no larvicidal effect “and also non-interference in the remaining duration of the larval stage. Alert treatments, that even though natural medicine, without guidance from qualified health professionals may harm due to inadequacies in its implementation.关键词: Medicinal Plant, Bioactivity, Mato Grosso, Brazil，Acknowledgement: For Fapemat – financial support, and for UNEMAT – institutional support; To the collaborators colleagues from the research group FLOBIO – (Plants carrying Bioactive substances)

Xanthine oxidase-inhibitory and hypouricemic action of Black poplar bud extract

Havel J, Rado V, Plachy

Department of Microbiology, Nutrition and Dietetics. Faculty of Agrobiology, Food and Natural Resources. Czech University of Life Sciences Prague, Prague, Czech Republic

Our study aimed to investigate the effect of the extract from Black poplar (Populus nigra L.) buds on xanthine oxidase (XO) activity in vitro and its hypouricemic action in rats. Poplars have been traditionally used in gout- and arthritis-treatment practices in medieval Europe. Besides, phenolic-rich resins from poplar buds are usually the main constituents of honey bee propolis that has a wide traditional use. To our preliminary in vitro evaluation, extract was administrated for 3 consecutive days to potassium oxonate-induced hyperuricemic rats in concentrations of 100 and 500 mg/kg. Allopurinol (10 mg/kg), was used as a reference drug. Uric acid/serum urate, urea, creatinine and electrolytes Na+, K+, Cl- were determined in daily collected urine and in serum at the end of the experiment. ALT, AST activities in serum and XO activities in the liver homogenate were determined. The extract inhibited XO activity in vitro, showing a mixed-mode inhibitory action and IC50 value of 8.2 μg/mL. In rats, poplar extract at 500 mg/kg significantly (P < 0.05) reduced serum urate levels by 27% compared to hyperuricemic control group which the effect similar to that of allopurinol at a dose 10 mg/kg. The mode of action still needs to be further elucidated as it did not exhibit effect on liver XO but the observed effect on Na+, K+, and Cl- excretion suggest the uricosuric action. Further research is needed to fully elucidate the potential of poplar extract in management of hyperuricemia. Keywords: Xanthine oxidase, hypouricemic effect, hyperuricemia, gout, enzyme inhibition, Populus nigra, black poplar extract Acknowledgement: This research was supported by MSM 6046070801 and GACR 525/08/P503

Bioassay guided fractionation of extracts from flowers of Bells perennis L. for their anticancer activity

Pehlivan Karakas F, Karakas A, Mesviladze V, Legault J, Picthet R, Ucar Turkner A

1Department of Biology, Faculty of Arts and Sciences, Abant Izzet Baysal University, Bolu 14280, Turkey; 2Laboratoire LASEVE, Universite du Quebe a Chicoutimi, 555, Boulevard de l’ Universite, Chicoutimi, Que., G7 H 2B1, Canada

Common daisy (Bells perennis L.) is a member of the cosmopolitan family Compositae (Asteraceae). It is native to western, central, and northern Europe, but is commonly found as an invasive plant in North America [1]. B. perennis has been used in the treatment of gastritis, enteritis, diarrhoea, bleeding, rheumatism, inflammation and infections of the upper respiratory tract (2 – 3). In this study, anticancer activity of crude hexane, dichloromethane, methanol, water extracts, also n-butanol and ethyl acetate fractions (after separation of methanol extract) of many species of the genus Salvia, for their 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging activities. Among them, Salvia officinalis L. leaf extracts have been shown to be the most active with effective dose (EC50) of 17 μg/mL followed by the genus Phlomis by (EC50) 32 μg/mL. This is for the first time studied in our search. For its high antioxidant activity, S. officinalis is being used commercially in foodstuffs (3). The air-dried and powdered overground parts of each plant (500 g) were macerated with MeOH (5000 ml) over night and successively extracted with MeOH at 40 °C. After filtration, the remaining plant material was then extracted with MeOH (2000 ml) at 40 °C for a second time. The combined methanolic extracts were evaporated under reduced pressure to give the crude methanolic extract.Total flavonoid compound amount in extracts was determined by Bhorun et al., 1996. The total phenolic compound amount in extracts was determined by Folin-Ciocalteu method, using the procedure of (Price and Butler, 1977). Keywords: antioxidant activity, DPPH radi, linoleic acid peroxidation, Salvia officinalis, Phlomis samia References: Price MP and Butler LG (1977) Agric Food Chem 25: 1268 – 1273.

n-Hexane extracted compounds of Bromus inermis with excellent anti-MRSA activity

Alahmadi A1, Roghanian R2, Emtiazi G1, Ghassempour A3, Mirzaei F1

1Department of Biology, Faculty of Sciences, University of Isfahan, Isfahan, Iran.; 2Department of Phytochemistry, Medicinal Plants and Drug research Institute, Shahid Beheshti University, Tehran, Iran.

Bromus inermis L. is a grass which belongs to family Poaceae. It is a potent weed. Weeds could be a potent source for finding antibacterial compound regarding their characteristics such as living in a divers conditions and situations. In a large screening program for antibacterial activity screening of some weed plants extracts, we finally find a great anti-MRSA activity of B. inermis (MIC of smaller than 100 μg/mL for crude extract in triplicate assays). We used HPTLC and a simple biographical assay in parallel for initial characterization and identification of effective substance(s) in the extract, and finally it was concluded that the effective anti-MRSA substance is a relatively polar substance which has MIC of smaller than 100 μL/Mg. Susceptibility tests with standard antibiotics had been shown the MIC of 35 for chloramphenicol against this strain. Further investigations with HPLC Fractionation and subsequent NMR analysis could reveal the identity of the effective substances in our continuing work.

The antioxidant activity and free radical scavenging potential of two medicinals plants Salvia officinalis L. and Phlomis samia L.

Fatima K, Nacira A, Safia I, Wahiba K, Awatef B, Arrar L

Dept of Biology, Faculty of Nature and Life, Mentouri University, Constantine, Algeria

In recent years considerable attention has been devoted to medicinal plants with antioxidant properties. The properties are commonly postulated to play an important role in preventing diseases caused by oxidative stress, such as cancer, coronary arteriosclerosis, and the ageing process. And there is much literature concerning the antioxidant properties of many species of the genus Salvia, for their 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging activities. Among them, Salvia officinalis L. leaf extracts have been shown to be the most active with effective dose (EC50) of 17 μg/mL followed by the genus Phlomis by (EC50) 32 μg/mL. This is for the first time studied in our search. For its high antioxidant activity, S. officinalis is being used commercially in foodstuffs (3). The air-dried and powdered overground parts of each plant (500 g) were macerated with MeOH (5000 ml) over night and successively extracted with MeOH at 40 °C. After filtration, the remaining plant material was then extracted with MeOH (2000 ml) at 40 °C for a second time. The combined methanolic extracts were evaporated under reduced pressure to give the crude methanolic extract. In our study, we performed bioactivity screening of some weed plants extracts, we finally find a great anti-MRSA activity of B. inermis (MIC of smaller than 100 μg/mL for crude extract in triplicate assays). We used HPTLC and a simple biographical assay in parallel for initial characterization and identification of effective compound(s) in the extract, and finally it was concluded that the effective anti-MRSA compound(s) is a relatively polar substance which has MIC of smaller than 100 μg/mL. Susceptibility tests with standard antibiotics have been shown the MIC of 35 for chloramphenicol against this strain. Further investigations with HPLC Fractionation and subsequent NMR analysis could reveal the identity of the effective compounds in our continuing work.
In the Serbian flora, genus Teucrium is represented by six species. In this work, for the first time, antimicrobial properties of methanol, ethyl acetate and acetone extracts of T. scorodium, T. scordium and T. botrys were examined [1].

Antimicrobial activity was tested by microdilution method determining minimum inhibitory (MIC) and microbicidal concentration (MMC) against standard and clinical strains of bacteria and fungi: Staphylococcus aureus ATCC 25923, Escherichia coli ATCC 25922, Staphylococcus epidermis, Candida albicans ATCC 10231, Candida albicans and Aspergillus niger [2]. The activity of extracts varied depending on the microorganism species, plant species and type of extract. The extracts of T. scorodium subsp. scorodion and T. scordium subsp. scorodion showed inhibitory effects towards tested bacteria while T. botrys inhibit only S. aureus ATCC 25923. Antifungal activity was recorded for ethyl acetate extracts of T. scorodion subsp. scorodion and T. botrys. MIC and MMC values were in range from 0.3 mg/ml to >20 mg/ml. Among tested extracts, the methanolic showed the greatest inhibitory effects towards tested bacteria and fungi. The inhibitory and bactericidal concentrations were lower than 1 mg/ml. Sulphate of S. aureus and P. aeruginosa was moderate with MIC values between 5 mg/ml – 20 mg/ml. E. coli, clinical and standard strain, exhibited susceptibility to the extracts at higher concentration or resistance. Also, tested fungi showed low sensitivity to the extracts. Keywords: Teucrium, antimicrobial activity, MIC, MMC.

Acknowledgement: Ministry of Science and Education, Republic of Serbia (II/410/10).


The Study on the Effect of Different Manure and Plants Density on the Growth and some Quantitative Characteristics of Milk Thistle (Silybum marianum L.)

Arouiee H, Mohammad S, Farzaneh A, Fatemi H, Nezami S, Aminifar M

Horticultural sciences, College of Agriculture, Ferdowsi University of Mashhad, Mashhad, Iran

Milk thistle (Silybum marianum L.) is an annual medicinal plant. Silybum marianum has been recognized as an antihepatotoxic plant. The active constituents of S. marianum include a group of flavonolignans known collectively as silymarin. To investigate the effects of different manure and plant density and the interaction between manure and density on the growth and some quantitative characteristics of this plant an experiment were examined. The completely Randomized design was installed in the experimental field, College of Agricultural of Ferdowsi University located in Mashhad. The treatments were included of two factors. The first factor included 3 different manure: cow, ship and multiple manure (1.65 kg/m²) and the second factor was three level of plant density (5, 7 and 10 pl/m²) which 3 replications. At the end of full bloom stage, morphological characteristics included plant height, number of shoots, number and diameter of capitule, percentage of leaf dry matter, percentage of healthy and free seeds, mass of 1000 grains of main and secondary capitule and chlorophyll content of leaf. Mean comparison was carried out using LSD test (at 5% level). Results showed a significant effect.
on number of capitula and shoots and plant height. By increase in plant density: plant height, number of shoots, number of capitula and mass of 1000 grains were decreased. The results showed that the best kind of density: plant height, number of shoots, number of capitula and mass of

PM221

Chemical profile and biological activities of Allium melantheranum Panc. extracts

Simin N, Orlic D, Mimica Dukic N, Jovicin E, Bajic I, Lesjak M, Bozin B

1Department of Chemistry, Biochemistry and Environmental Protection, Faculty of Sciences, University of Novi Sad, Serbia; 2School of Pharmacy, Faculty of Medicine, University of Novi Sad, Novi Sad, Serbia

Members of the genus Allium have been used and cultivated for thousands of years for their medicinal properties and characteristic flavor. Only two species of genus Allium (A. sativum L. and A. cepa L.) are well researched, while data on chemical composition and biological activities of other species, including Allium melantheranum Panc. (subgenus Allium, sect. Codonoprasum) are very scarce. In the present study we investigated chemical composition, antioxidative and anti-inflammatory properties of methanolic extracts of Allium melantheranum wild growing in Serbia. Phytochemical profile was determined by measuring total phenolic, total flavonoid and total anthocyanin contents and by LC-MS/MS analysis of the extracts and headspace GC/MS analysis of fresh bulbs volatiles. The antioxidant activity was measured by measuring radical scavenging capacity towards 2,2-diphenyl-1-pycrylhydrazile (DPPH) and NO radicals and effect on lipid peroxidation (LO) [1]. In addition, the anti-inflammatory activity considering inhibitory potency toward production of 12-HETE, 12-HHT, PGE2 and TXB2 was observed [2]. High contents of total phenolics (5.13 – 5.31 mg gallic acid equivalents/g of dry extract), total flavonoids (1.52 – 2.82 mg quercetin equivalents/g of dry extract) and total monomeric anthocyanins (33 – 169 µg cyanidine-3-glucoside equivalents/g of dry extract) were found. The dominant phenolic compounds in the extracts are ferulic and p-cumaric acids and flavonoids quercitin, quercetin-3-O-Glc and kaempferol-3-O-Glc. D-methyl-disulphide was detected as only volatile compound. The extract inhibited production of 12-HETE, 12-HHT, PGE2 and TXB2 in a dose-dependent manner. Antioxidant activity was weak compared with synthetic antioxidants. Keywords: Allium melantheranum, antioxidant, anti-inflammatory. Acknowledgement: This research was supported by Science and Technological Development, Republic of Serbia, grant No. 172058


PM222

Tectona grandis Linn. (Verbenaceae) leaf ethanol extract in renal artery occluded hypertensive rats

Ajayi GO, Olowo JA, Ajolahuchukwu JN

Department of Pharmacognosy, Physiology and Medicine University of Lagos, Lagos, Nigeria

Hypertension is one of the principal health problems and leading cause of cardio-vascular deaths in various communities worldwide. An elevated arterial pressure is an important public health issue. Although it is common, asymptomatic and readily detectable, but it can often lead to lethal complications, if left untreated. Many new drugs have been introduced which may demonstrate better efficacy but possess side effects. Recently attention has been focused towards herbal preparations which are traditionally used as potential therapeutic agents in the prevention and management of cardiovascular diseases. Ethanol extract of Tectona grandis Linn. leaf (family: Verbenaceae) was evaluated for its antihypertensive activity in renal artery occluded hypertensive rats. Wistar rats (160 – 250 g) were pretreated with ethanol extract of T. grandis for 6 weeks. Hypertension was induced in animals by clamping the renal artery with renal bulldog clamp for 4 h. Ischemia of the kidney caused elevation of blood pressure by activation of the renin-angiotensin system. Elevated blood pressure of the animals was significantly (p < 0.05) decreased by the ethanol extract of T. grandis at the dose levels of 20, 40 and 80 mg/kg, i.v. Captopril, angiotensin converting enzyme inhibitor (ACE-I) at the dose of 1 mg/kg, i.v. showed significantly (p < 0.05) reduced in the elevated blood pressure. The antihypertensive activity of ethanol extract of T. grandis may be due to the action on rennin-angiotensin system. This result would tend to justify the tradi-
In Turkish traditional medicine, the species under Juniperus section are frequently employed to treat several diseases [12]. This work was designed to define and compare the antiproliferative and antimicrobial activities of berries methanol extracts of five Juniperus species from Turkey: Juniperus communis L. var. communis (Jcc), J. communis L. var. saxatilis (Pallas) A.E.Murray (Jcs), J. drupacea L. spp. oxycedrus (Joo), J. oxycedrus L. spp. macrocarpa (Sibth. et Sm.); J. ballii (Jom). The effect of Juniperus extracts on cell proliferation was tested “in vitro” on human hepatocellular carcinoma (HepG2) cells. A decrease in HepG2 cells viability after 24-h exposure to Jcc, Jcs, and Jd extracts was observed. Based on IC_{50} values, the activity of the extracts decreased in the order Jcs > Jd > Jcc (IC_{50} = 6.62 ± 0.61 μg/mL, 7.61 ± 2.25 μg/mL, and 8.42 ± 1.32 μg/mL, respectively). Joo and Jom extracts inhibited the growth of HepG2 cells approximately 40% at the lowest tested dose (1.25 μg/mL), while the activity diminished with increasing concentrations, resulting close to zero at the dose of 10 μg/mL. The antimicrobial activity was evaluated by standard methods on gram-positive, gram-negative bacteria and fungi. The efficacy was appreciable on gram-positive only. Among the extracts Jd showed the higher bacteriostatic activity (MIC: 78.12 – 312.50 μg/mL) than Jcs and Jcc (156.25 – 1250.00 μg/mL), followed by Joo and Jom (25.00 – 1250.00 μg/mL). The obtained results give support to the ethnopharmacological use of these Turkish Juniperus species and suggest their potential utility in the prevention and/or treatment of infections and cancer. Acknowledgement: The authors are grateful to Federica Messina for financial support.

References:

PN3

2Department of Microbiology, Faculty of Veterinary Medicine, University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Manastur street no 3 – 5, 400372, Romania; 3Department of Microbiology, Faculty of Veterinary Medicine, University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Manastur street no 3 – 5, 400372, Romania

Acaricidal activity of the essential oil from Tetradenia riparia (Lamiaceae) on the cattle tick Rhipicophalus (Boophilus) microplus (Acarac; Ixodidae)

Gazim ZC1, Rezene CM2, Cacze LE3, Cortes DN4
1Department of Pathology, Faculty of Veterinary Medicine, University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Manastur street no 3 – 5, 400372, Romania; 2Department of Pathology, Faculty of Veterinary Medicine, University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Manastur street no 3 – 5, 400372, Romania

This experiment was carried out to study the bioacaricidal activity of Tetradenia riparia (Hochst.) Cord essential oil against engorged females of Rhipicephalus (Boophilus) microplus (Acarac; Ixodidae). For this purpose, nine serial concentrations (12.50, 6.25, 3.75, 1.80, 0.90, 0.45, 0.22, 0.11, and 0.055% w/w) of T. riparia were used for the adult immersion test (AIT). For the larval packet test (LPT), we used 14 serial concentrations (100.00, 50.00, 25.00, 12.50, 6.25, 3.15, 1.82, 0.91, 0.45, 0.22, 0.11, 0.057, 0.028, and 0.014% w/w). The results for AIT showed that the LC_{50} and LC_{99.9}, calculated using the Probit test, were for mortality (%): 1.34 g/mL (0.436 – 0.632) and 1.552 g/mL (1.183 – 1.922); for total number of ovispositions were 0.449 g/mL (0.339 – 0.558) and 1.76 g/mL (1.27 – 2.248); and for hatchability inhibition were 0.114 g/mL (0.0 – 0.31) and 2.462 g/mL (1.501 – 3.422), respectively. The LPT showed that the LC_{50} and LC_{99.9} were 1.222 g/mL (0.655 – 1.788) and 11.382 g/mL (7.85 – 14.91), respectively. A positive correlation between T. riparia EO concentration and tick control, was observed by the strong acaricidal effects against R. (B.) microplus, and the mortality rate of ticks was dose-dependent. Our results showed that T. riparia is a promising candidate as an acaricide against resistant strains of R. (B.) microplus. Keywords: Tetradenia riparia, Ixodidae, Tick; Rhipicephalus (Boophilus) microplus

Acknowledgement: The authors are grateful to CNPq for providing a research grant and fellowships References: 1. Gazim ZC et al. (2010) Molecules 15: 5509 – 5524.

PN4

In vitro activity of different plants essential oils against the yeast-like alga Prototheca Cosimina BM1, Nicidin F2, Adrian G1, Sorin R2, George N3, Pompei B4, Marian T1, Gabriel B1, Andras N1, Cornel C1
1Department of Pathology, Faculty of Veterinary Medicine, University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Manastur street no 3 – 5, 400372, Romania; 2Department of Microbiology, Faculty of Veterinary Medicine, University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Manastur street no 3 – 5, 400372, Romania

Species of the genus Prototheca (family Chlorellaceae) are unicellular achlorophyllous microalgae, spherical, oval or kidney shaped with diameters ranging from 3 to 30 μm. They are ubiquitous in nature and have a worldwide distribution [1]. Of the five known species of the genus, P. wickerhamii causes human infection and P. zopfii is considered pathogenic for animals, particularly cows and dogs [2,3]. These algae do not respond to classic therapy so introduction of new therapeutic agents for...
Inhibition of chemically induced mammary and non-mammary carcinogenesis by astaxanthin in Wistar rats

Adrian Florian G1, Sanda A2, Cosmina B2, Marian T1, Pompei B, Coriel C
1Department of Pathology, Faculty of Veterinary Medicine, University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Manastur street no 3 – 5, 400372, Romania; 2Department of Biochemistry, Faculty of Veterinary Medicine, University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Manastur street no 3 – 5, 400372, Romania

Astraxanthin is a fat-soluble, oxygenated pigment called a xanthophyll and a member of the carotenoid family. It has a unique molecular structure that gives it powerful antioxidant function. Astaxanthin is extracted from microalgae, salmon and Pteropus (a yeast) [1, 2]. The aim of the study is to follow up the effect of astaxanthin (ASTA) in chemoprevention of the chemically induced mammary carcinogenesis in immature Wistar female rats. There were established five groups of 37 days old Wistar rats: group I inoculated with the carcinogen MNU (N-methyl-N-nitroso-urea) (n = 9), group II with MNU and ASTA in diet (n = 8), group III which received oil in diet (oil was used as solvent for ASTA) (n = 4), and group IV with ASTA in diet (n = 4). The ASTA was administrated orally in a dose of 50 μg astaxanthin/rat/day, during 7 months. The experiment was finished at 14 months from MNU intake. Mammary tumor induction determined by MNU was reduced, representing 33.3% respectively 37.5% from all cases in groups I and II. There were diagnosed several other tumor types in several organs (nephroblastoma, liposarcoma, hemangio-sarcoma, squamous carcinoma, pulmonary carcinoma, cholangiocarcino-ma). Involvement of oxidative stress in (mammary and non-mammary) carcinogenesis was revealed by partial protection conferred by astaxanthin in cancer chemoprevention. Present study is one of the few long term experiments (420 days) that resemble the effect of astaxanthin in chemically induced carcinogenesis in rats. Concluding, a diet enriched in astaxanthin yield beneficial effects in cancer chemoprevention, minimizing the bad effects of oxidative stress induced by MNU. Keywords: astaxanthin, carcinogenesis, mammary, rat Acknowledgements: This work was supported by CNCSIS-UEFISCSU grant number PN II RU 185/2010. References: [1] Guerin M et al. (2003) Trends Biotechnol. 21(5):210 – 6. [2] Hoyouk N et al. (1999) Pure Appl Chem. 71(12): 2273 – 2278.

Researches Regarding in vitro Antimicrobial Effect of Some Types of Honey from Transylvania on Staphylococci Isolated from Animals and Humans

Nicodim F1, Flore C1, George N1, Luciu P1, Emoke P1, Pompei B, Cosmina B2
1Department of Microbiology, Faculty of Veterinary Medicine, University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Manastur street no 3 – 5, 400372, Romania; 2Department of Pathology, Faculty of Veterinary Medicine, University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Manastur street no 3 – 5, 400372, Romania; 3Adults Hospital, Croitorilor Street, no. 19 – 21, 400162 Cluj- Napoca, Romania

Despite the pharmaceutical industry development in recent years, resistance of microorganisms to antibiotics is increasing [1,2,3]. Under these conditions, the alternative of natural products with similar effect, must be considered. This study aimed to test in vitro antimicrobial activity of four types of honey obtained in Central Transylvania, on staphylococci isolated from lesions in both animals and humans, as well as to test comparatively the effect of the most frequently antibiotics used in treating the lesions caused by staphylococci. The products with potentially antimicrobial effect were represented by forest honey, multi-flower honey, lime honey and acacia honey. Tests were done on 38 strains of staphylococci form species S. aureus (8 strains), S. intermedius (10 strains), S. xylosus (7 strains), S. hominis (5 strains), S. chromogenes (4 strains) and S. sciuri (4 strains). Comparatively seven antibiotics commonly used to treat staphylococci were also tested. The sensibility was determined using the microdilution methods obtaining minimum inhibitory concentration (MIC) for each sample in accordance to CLSI standards. Forest honey had a good antimicrobial effect (MIC 15 μg/ml) on S. intermedius and S. chromogenes strains and multi-flower honey had good effect (MIC 15 μg/ml) against S. sciuri. Lime honey had a decreased
The complex therapeutic potential of honey and medicinal plants is well documented by the literature, but discrepancies may be observed while comparing research results [1, 2]. Twelve honeydew honey samples from different Transylvanian geographical locations were investigated for their antibacterial properties based on the results of two diffusion assays that included Pseudomonas aeruginosa (n = 10), strains isolated from bovine mastitis, and the reference strain Ps. aeruginosa ATCC 27853) as a relevant antibiotic resistant pathogen. Both screening tests indicated a strong inhibitory effect for eight samples when compared to the antibiotic ampicillin (MIC 8 μg/ml) on S. aureus, S. hominis, S. chromogenes and S. sciuri. These results show that the antibacterial effect of honey is variable it may depend on the type of used honey and tested microbial strains. Keywords: staphylococci, honey, antibiotics References: [1] Hancock EW (2005) Lancet Infect Dis 5(4): 209 – 218. [2] Levy SB et al. (2004) Nat Med 10: S122-S 129. [3] Nascimento GGF et al. (2000) Braz J Microbiol 31: 247 – 256.

The application of plant extracts into the feed of different farm animals has an importance in the health prevention of animals, in the aspect of immunostimulant effects and in the production of biofood, which is an important component of the food chain [1, 2]. In the model experiment a dry extract of Eleutherococcus senticosus Maxim. was applied to the layers of Hixex braun breed. The layers were divided into three groups, a control group (CG, n = 10), 1st experimental group (EG1, n = 10) with the addition of the extract in the concentration of 0.1%, the 2. experimental group (EG2, n = 10) with the addition of the extract in the concentration of 0.5%. The layers were bred in a three-storey terraced cage battery; feed and water were at disposal ad libitum. The dry extract contained Eleutheroside B (0.71%), Eleutheroside E (1.14%), and 30% ethanol was used as an extraction medium. The weight of the eggs and the layers was sampled weekly. The quality of the eggs was measured on the basis of the weight and shape of the eggs, the strength and thickness of the eggshells. In the 2nd experimental group, statistically significant changes (P < 0.05) in the strength (EGL = 30.93 ± 5.00) and thickness (EGL = 0.41 ± 0.03) were detected in comparison with the control group (CG = 26.71 ± 4.42 strength, CG = 0.39 ± 0.02 thickness of the eggshells). Keywords: Hixex braun, Eleutherococcus senticosus, egg production, quality. Acknowledgement: This research is supported by the Agency of Ministry of Education SR for the Structural Funds of the EU, the project: ITMS 26220120023, ITMS 26220120041, ITMS 26220220003. References: [1] Davydkov M, Krkorian AD (2000) Ethiopharmacol 72(2): 345 – 349. [2] Siwcki AK et al. (2004) Bull Vet Inst Pulawy 48: 489 – 492.

PN10 Comparison the anticoccidial effects of Artemisinin granule prepared from Artemisia sieberi extract with Monensin in experimental broiler chicken coccidiosis

Coccidiosis is the most important parasitic disease of poultry production industry [4]. Due to increasing resistance to conventional anticoccidial agents it is necessary to find out new anticoccidial compounds [1]. Herbal compounds are promising weapons in this regard. One of them is Artemisia sieberi Besser in which primary studies have shown its possible anticoccidial effect (2 – 3). To compare the anticoccidial effect of artemisinin granule prepared from Artemisia sieberi extract with Monensin in experimental broiler coccidiosis, 120 Ross 308 broiler chickens in 4 groups each with 3 replicates (n = 10) were used. Group 1 was separated as uninfected negative control and received no drug. Form the first day group 3 received Artemisinin granule 5 mg/kg, group 4 Monensin 110 ppm till 42 days of age as feed additive. At 21 days of age groups 2, 3, 4 were inoculated with a mixed suspension of 20 0000 oocyst of E. tenalla, E. maxima, E. acervulina & E. necatrix. Group 2 was kept as infected positive control which received no drug. One day after inoculation, oocyt per gram (OPG) of feces for 5 successive days and Mean Body Weight, Weigh Gain and Food Conversion Ratio (FCR) were determined weekly in each group. The data were presented as Mean ± SE and analyzed using Sigma stat (V 3.1) statistical software (P < 0.05). This study showed that both artemisinin granule and Monensin significantly (P < 0.05) decreased OPG (table1) also artemisinin granule improved performance included weight gain, mean body weight and FCR in infected broiler chickens which is comparable to Monensin. Keywords: Coccidiosis, Broiler chickens, Artemisinin granule, Monensin, Artemisia sieberi References: 1- Allen PC, Fetter RH (2002) Clin Microbiol Rev 15: 58 – 65. 2- Allen PC et al. (1997) Poul. Sci 76: 1156 – 1163. 3- Ara, HA et al. (2006) Trop Anim Health Pro, 38: 497 – 503. 4- Zhang Z, Zeng M (2005) Chin J Vet Parasitol 13: 29 – 36.

PN9 Effects of dry plant extract (Eleutherococcus senticosus Maxim.) on the quality of eggs laying hens Hixex Braun

The application of plant extracts into the feed of different farm animals has an importance in the health prevention of animals, in the aspect of immunostimulant effects and in the production of biofood, which is an important component of the food chain [1, 2]. In the model experiment a dry extract of Eleutherococcus senticosus Maxim. was applied to the

PN8 In vitro antimicrobial efficacy of honeydew honey and Calendula officinalis L. against Pseudomonas aeruginosa

Niculae M1, Spini M1, Rindt K1, Sandru CD1

1Department of Infectious Diseases, Faculty of Veterinary Medicine, University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Romania; 2Department of Beekeeping and Sericulture, Faculty of Animal Husbandry and Biotechnology, University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Romania; 3Department of Pharmaceutical Botany, Faculty of Pharmacy, University of Medicine and Pharmacy ‘Iuliu Hașeตำoaia’ Cluj-Napoca, Romania

In vitro antimicrobial efficacy of honeydew honey and Calendula officinalis L. against Pseudomonas aeruginosa. Marghitas LA1, Stan L2, Bobis O3, Brudascu GF1, Tamas M1

In vitro antimicrobial efficacy of honeydew honey and Calendula officinalis L. against Pseudomonas aeruginosa (n = 10), strains isolated from bovine mastitis, and the reference strain Ps. aeruginosa ATCC 27853) as a relevant antibiotic resistant pathogen. Both screening tests indicated a strong inhibitory effect for eight samples when compared to the artificial honey and also that the development of growth inhibition zones their antibacterial properties based on the results of two diffusion assays that included Pseudomonas aeruginosa (n = 10), strains isolated from bovine mastitis, and the reference strain Ps. aeruginosa ATCC 27853) as a relevant antibiotic resistant pathogen. Both screening tests indicated a strong inhibitory effect for eight samples when compared to the antibiotic ampicillin (MIC 8 μg/ml) on S. aureus, S. hominis, S. chromogenes and S. sciuri. These results show that the antibacterial effect of honey is variable it may depend on the type of used honey and tested microbial strains. Keywords: staphylococci, honey, antibiotics References: [1] Hancock EW (2005) Lancet Infect Dis 5(4): 209 – 218. [2] Levy SB et al. (2004) Nat Med 10: S122-S 129. [3] Nascimento GGF et al. (2000) Braz J Microbiol 31: 247 – 256.
Authors' Index

A
Ab Chani N 1334
Ab Rahman M 1451
Ab Rahman N 1402
Abaci O 1305, 1417
Abadia PJ 1401, 1407
Abbasi AM 1456
Abd Alla HI 1410, 1420
Ab el Aty AA 1359
Ab el Aziz HM 1368
Ab el Ehleem Said A 1399
Abel Azim NS 1250
Abel Aziz H 1244, 1423
Abel Hady NM 1289
Abel Megeed AA 1306
Abel Motaal A 1235
Abel Motaal AA 1388
Abel Salam RM 1368
Abelshaky NA 1379
Abdelgaleel SA 1311
Abdelly C 1430, 1445, 1450
Abdi G 1277
Abdollahi M 1373
Abdolmaleki P 1286
Abdul Manaf A 1322, 1329
Abdul Razak F 1326, 1451
Abdurahman EM 1331
Abolaji AO 1312
Abolfotouh OE 1398
Abou El Kassem LT 1331
Abou Elkassem LT 1415
Abou Zeid AH 1375
Abrahamyan A 1380
Abravesh A 1290
Abu Gabal N 1410
Acacovic Djokovic C 1342
Acero N 1440
Acevedo AC 1360
Acworth IN 1248, 1263, 1362
Adabao AC 1445
Adabayo AH 1312
Adabesin O 1308
Abdeloua EA 1403
Adejimi AS 1403
Adenigbe AO 1409
Ades M 1274
Adesanya SA 1309
Adesegun SA 1309
Adeyemi OG 1253, 1308, 1313
Adlassing W 1333
Adrian Florin G 1456
Adrian G 1456
Aberischer A 1404
Afiif S 1374, 1377
Afnani A 1322, 1329
Afolsab S 1253
Aghbelusi GA 1393
Aghboola I 1409
Aghboula OI 1309, 1376
Aghhavani Shajari M 1292, 1381, 1393
Agiang EA 1307
Aguiar EG 1306
Aguiar A 1313, 1331, 1336
Agure C 1264
Ahadi Dolatsara E 1284
Ahanjan M 1399
Ahmad MM 1456
Ahmad R 1335, 1400, 1434
Ahmad S 1434
Ahmad S 1434
Ahmadi Moghadam Y 1281
Ahmadi A 1315
Ahmadi K 1300, 1422, 1423
Ahmadpour F 1394, 1446
Ahmadu AA 1336
Ahmat N 1258, 1311, 1331, 1332, 1333, 1334, 1405, 1408, 1410
Ahmed EF 1278
Ahmed HH 1307
Ahmed KA 1305
Ahmed M 1279, 1358
Ahmed S 1250
Ahmed T 1348
Ahbidi V 1323
Abubidinov Y 1428
Aigbe FR 1313
Aigner L 1256
Airaiti N 1418
Ainsa JA 1318
Aizan G 1411
Ajayi GO 1309, 1398, 1455
Ajuluchukwu JN 1455
Akacha A 1402
Akah PA 1300, 1455
Akakabe M 1260, 1358
Akanide IS 1324
Akay Ş 1286, 1372
Akaydin G 1270
Akcan M 1231
Akdemir ZS 1443
Akgun IH 1351
Akhtar M 1259
Akindele Aj 1253
Akindele S 1308
Akkol EK 1435, 1452
Akrou A 1416
Al Ashaal HA 1425
Al Damen A 1380
Al Dwayri MA 1293
Al Fishawy A 1374, 1377
Al Khalidi K 1312
Al Okbi SY 1415
Al Rehaily Aj 1302
Al Sayed M 1386, 1392
Al Tahaniem R 1312
Al Taweel AM 1379
Al Yahya MA 1302
Alachkar A 1269
Aladesanmi Aj 1409
Aladesanmi JA 1445
Alali FQ 1293
Alamshahi L 1306
Alankus Çalıskan O 1443
Alarcón J 1257
Alban K 1442
Alban S 1245, 1359
Albert K 1346
Acamil J 1255, 1341
Aleksic M 1440
Aleha E 1288, 1289, 1365
Alexandrou V 1317
Agül D 1436
Alhwarini TA 1247
Ali A 1306
Ali B 1277
Ali HA 1267
Ali ME 1367
Aliabadi Farahani H 1293
Alihamidi A 1422, 1453
Alia S 1344
Alia F 1301
Aliashamghey F 1321
Alijani N 1272, 1282, 1347, 1441
Alizadeh Astari K 1437
Alizadeh H 1276
Alizadeh O 1293
Alldender C 1379
Allmaier G 1350
Allorge L 1386
Almassarani SM 1247
Almeida CA 1449
Almeida VL 1390
Almutsayev NM 1247
Alnouree S 1298
Alpak I 1372
Alqaisioui SI 1302
Almaik C 1230
Altnikin B 1372
Altintas A 1394, 1440
Alves CE 1401, 1407
Alves CD 1447
Alviano CS 1449
Alviano DS 1449
Aly AH 1262
Aly YS 1367
Amaral MT 1426
Amarante AF 1449
Amaya C 1360
Ambrosio SR 1393, 1397, 1416, 1417
Ameri A 1435
Amin G 1234, 1296, 1338, 1384
Amini Dahagh M 1294
Amini F 1447
Amini M 1338, 1448
Aminifar M 1454
Aminimoghadamfarourj N 1375
Amiri H 1294, 1399
Amiri J 1360
Amiri M 1393
Amnar N 1415
Ammoah S 1355
Amoogaheira R 1424
Amorim ML 1306
Amountzias V 1272
Amri H 1416
Anakov G 1439
Andrade PB 1416
Andras N 1456
Andriantsitohaina R 1246
Andujar I 1449
Anes E 1318
Angelis A 1347
Angst J 1244
Ankli A 1317
Antheaume C 1272
Anwar MI 1229
Anyika EN 1317
Apers S 1274, 1320, 1331
Appers S 1428
Aquino CF 1427, 1428
Aquino LC 1287
Arakawa NS 1393
Arar L 1453
Aradjus GS 1306
Aralvindaram K 1424
Arabini S 1242
Ardoso RF 1415
Arellano J 1249
Argypouloou A 1272
Ariburnu E 1343
Ariful D 1322
Arji I 1278
Arjune S 1446
Arkian E 1427
Arnason JT 1308
Armee de C 1244
Arrouiee H 1359, 1454
Arriogucci S 1372
Arroteia RF 1377
Arruda PC 1327
Arslan N 1298
Asadai AM 1381
Asakawa Y 1359
Asea A 1248, 1420
Asghari B 1367
Ashour WE 1375
Ashrafu M 1422
Asili J 1350
Aski H 1284
Aslan Erdem S 1322
Aslan C 1374
<table>
<thead>
<tr>
<th>Authors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kroyer G 1256, 1273</td>
</tr>
<tr>
<td>Kouiri R 1430, 1433, 1445, 1450</td>
</tr>
<tr>
<td>Kouiri WM 1450</td>
</tr>
<tr>
<td>Kftari L 1358</td>
</tr>
<tr>
<td>Kuban M 1285</td>
</tr>
<tr>
<td>Kuekahai P 1315</td>
</tr>
<tr>
<td>Kuehn S 1381</td>
</tr>
<tr>
<td>Kucik Markovic J 1270</td>
</tr>
<tr>
<td>Kukula Koch W 1396</td>
</tr>
<tr>
<td>Kula C 1285</td>
</tr>
<tr>
<td>Kulan EG 1312</td>
</tr>
<tr>
<td>Kulevanova S 1387</td>
</tr>
<tr>
<td>Kultur S 1396</td>
</tr>
<tr>
<td>Kumagai K 1260, 1358</td>
</tr>
<tr>
<td>Kumkrai P 1402, 1403</td>
</tr>
<tr>
<td>Kunert O 1333, 1338, 1446, 1449</td>
</tr>
<tr>
<td>Kune D 1387</td>
</tr>
<tr>
<td>Kwon Y 1346</td>
</tr>
<tr>
<td>Kwak D 1419</td>
</tr>
<tr>
<td>Kwak J 1350</td>
</tr>
<tr>
<td>Kwang Ho J 1284</td>
</tr>
<tr>
<td>Kwen H 1369</td>
</tr>
<tr>
<td>Kwon J 1346</td>
</tr>
<tr>
<td>Kwon Y 1376</td>
</tr>
<tr>
<td>Kyoko N 1335</td>
</tr>
<tr>
<td>L</td>
</tr>
<tr>
<td>Laakso I 1357</td>
</tr>
<tr>
<td>Laatsch H 1278</td>
</tr>
<tr>
<td>Labokas J 1295</td>
</tr>
<tr>
<td>Labou M 1339, 1389</td>
</tr>
<tr>
<td>Lacaille Dubois M 1322</td>
</tr>
<tr>
<td>Lachal M 1433</td>
</tr>
<tr>
<td>Lago JC 1377</td>
</tr>
<tr>
<td>Lahouel M 1299</td>
</tr>
<tr>
<td>Lai R 1339</td>
</tr>
<tr>
<td>Lajis NH 1344</td>
</tr>
<tr>
<td>Lako B 1457</td>
</tr>
<tr>
<td>Lai J 1407</td>
</tr>
<tr>
<td>Lai J 1336</td>
</tr>
<tr>
<td>Lim Y 1341</td>
</tr>
<tr>
<td>Lim J 1304, 1318</td>
</tr>
<tr>
<td>Lime LS 1361</td>
</tr>
<tr>
<td>Lim LG 1452</td>
</tr>
<tr>
<td>Lim S 1304, 1318</td>
</tr>
<tr>
<td>Lim J 1363</td>
</tr>
<tr>
<td>Lim C 1336</td>
</tr>
<tr>
<td>Lim G 1356</td>
</tr>
<tr>
<td>Lim Q 1334</td>
</tr>
<tr>
<td>Lim W 1262, 1353</td>
</tr>
<tr>
<td>Lin B 1321</td>
</tr>
<tr>
<td>Lin C 1336</td>
</tr>
<tr>
<td>Lin G 1356</td>
</tr>
<tr>
<td>Lin Q 1334</td>
</tr>
<tr>
<td>Lin W 1262, 1353</td>
</tr>
<tr>
<td>Lin Y 1410</td>
</tr>
<tr>
<td>Lindequist U 1253, 1261</td>
</tr>
<tr>
<td>Liatoudon M 1236, 1343, 1380</td>
</tr>
<tr>
<td>Liu SC 1334</td>
</tr>
<tr>
<td>Liu SC 1337</td>
</tr>
<tr>
<td>Liu X 1239, 1333, 1338, 1446</td>
</tr>
<tr>
<td>Liu Z 1338</td>
</tr>
<tr>
<td>Lizzano L 1407</td>
</tr>
<tr>
<td>Lobstein A 1272, 1274, 1446</td>
</tr>
<tr>
<td>Lopes C 1420</td>
</tr>
<tr>
<td>Lopes MC 1419, 1426</td>
</tr>
<tr>
<td>Lorencini M 1415</td>
</tr>
<tr>
<td>Lorsuvannarat N 1315</td>
</tr>
<tr>
<td>Lotfi H 1278</td>
</tr>
<tr>
<td>Loubna A 1277</td>
</tr>
<tr>
<td>Lozinski K 1295</td>
</tr>
<tr>
<td>Lozza GC 1234</td>
</tr>
<tr>
<td>Lu YH 1413</td>
</tr>
<tr>
<td>Lu YY 1256, 1381</td>
</tr>
<tr>
<td>Lubrano C 1268</td>
</tr>
<tr>
<td>Lucarini R 1416</td>
</tr>
<tr>
<td>Lucas RK 1404</td>
</tr>
<tr>
<td>Luci M 1342</td>
</tr>
<tr>
<td>Lucia P 1457</td>
</tr>
<tr>
<td>Luis MJ 1401, 1407</td>
</tr>
<tr>
<td>Lukas B 1361</td>
</tr>
<tr>
<td>Lundgren C 1279</td>
</tr>
<tr>
<td>Luo X 1318, 1350</td>
</tr>
<tr>
<td>Lugman S 1451</td>
</tr>
<tr>
<td>Lyamine M 1398</td>
</tr>
<tr>
<td>M</td>
</tr>
<tr>
<td>Maarouf M 1298</td>
</tr>
<tr>
<td>Maas W 1248</td>
</tr>
<tr>
<td>Macakova K 1387</td>
</tr>
<tr>
<td>Macedo FA 1282</td>
</tr>
<tr>
<td>Macedo JR 1342</td>
</tr>
<tr>
<td>Machado TF 1306</td>
</tr>
<tr>
<td>Mackenzie J 1435</td>
</tr>
<tr>
<td>Mackinaite R 1375</td>
</tr>
<tr>
<td>Mader E 1432</td>
</tr>
<tr>
<td>Madida KT 1219</td>
</tr>
<tr>
<td>Madureira AM 1349, 1377, 1378, 1444</td>
</tr>
<tr>
<td>Maes L 1330</td>
</tr>
<tr>
<td>Mafakheri S 1291</td>
</tr>
<tr>
<td>Magalhães L 1324</td>
</tr>
<tr>
<td>Magalhães LG 1393</td>
</tr>
<tr>
<td>Magbagbeola OA 1324</td>
</tr>
<tr>
<td>Mageed RA 1422</td>
</tr>
<tr>
<td>Magiatis P 1237, 1303, 1336</td>
</tr>
<tr>
<td>Magnano AR 1418</td>
</tr>
<tr>
<td>Mahbob EN 1434</td>
</tr>
<tr>
<td>Mahlo SN 1454</td>
</tr>
<tr>
<td>Mahmoodi Sourestani M 1291, 1295</td>
</tr>
<tr>
<td>Mahmoodi N 1264</td>
</tr>
<tr>
<td>Mahmood K 1331, 1420</td>
</tr>
<tr>
<td>Mahmood S 1291</td>
</tr>
<tr>
<td>Maia MD 1427, 1428, 1447</td>
</tr>
<tr>
<td>Maimone P 1456</td>
</tr>
<tr>
<td>Majidi E 1287</td>
</tr>
<tr>
<td>Makarenko IE 1411, 1414</td>
</tr>
<tr>
<td>Makarov VG 1397, 1411, 1414</td>
</tr>
<tr>
<td>Makarova MN 1411, 1414</td>
</tr>
<tr>
<td>Makisimovic M 1256</td>
</tr>
<tr>
<td>Malekshahi F 1291</td>
</tr>
<tr>
<td>Malekzadeh M 1282, 1295</td>
</tr>
<tr>
<td>Malik J 1425, 1434</td>
</tr>
<tr>
<td>Malik ZA 1250</td>
</tr>
<tr>
<td>Mambro V d 1377</td>
</tr>
<tr>
<td>Mambu L 1386</td>
</tr>
<tr>
<td>Mampunza M 1260</td>
</tr>
<tr>
<td>Man Soo C 1284</td>
</tr>
<tr>
<td>Man M 1418</td>
</tr>
<tr>
<td>Mangrayo TF 1405</td>
</tr>
<tr>
<td>Manzani R 1418</td>
</tr>
<tr>
<td>Manojlovic I 1440</td>
</tr>
<tr>
<td>Manojlovic N 1432, 1440</td>
</tr>
<tr>
<td>Mansour N 1317, 1318</td>
</tr>
<tr>
<td>Marangoni S 1416</td>
</tr>
<tr>
<td>Marcon F 1264</td>
</tr>
<tr>
<td>Maree JE 1455</td>
</tr>
<tr>
<td>Maregisi S 1331</td>
</tr>
<tr>
<td>Marenich M 1300, 1428</td>
</tr>
<tr>
<td>Marghitas LA 1458</td>
</tr>
<tr>
<td>Marian T 1456, 1457</td>
</tr>
<tr>
<td>Marino A 1456</td>
</tr>
<tr>
<td>Marichuk R 1339</td>
</tr>
<tr>
<td>Markovic A 1374</td>
</tr>
<tr>
<td>Marques A 1371</td>
</tr>
<tr>
<td>Marques C 1426</td>
</tr>
<tr>
<td>Marsik P 1425, 1434</td>
</tr>
<tr>
<td>Marston A 1236, 1336, 1354</td>
</tr>
<tr>
<td>Martel S 1274</td>
</tr>
<tr>
<td>Marti G 1391</td>
</tr>
<tr>
<td>Martin J 1345</td>
</tr>
<tr>
<td>Martinelli M 1377</td>
</tr>
<tr>
<td>Martins A 1422</td>
</tr>
<tr>
<td>Martins CG 1394, 1397, 1416</td>
</tr>
<tr>
<td>Martins CH 1393, 1417</td>
</tr>
<tr>
<td>Marzouk MM 1354</td>
</tr>
<tr>
<td>Marzouk MS 1379</td>
</tr>
<tr>
<td>Marzouk MM 1354</td>
</tr>
<tr>
<td>Marsik P 1425, 1434</td>
</tr>
<tr>
<td>Marti G 1391</td>
</tr>
<tr>
<td>Mat Soat S 1405</td>
</tr>
<tr>
<td>Mat A 1396</td>
</tr>
<tr>
<td>Mathewsson A 1330</td>
</tr>
<tr>
<td>Matloub AA 1315, 1323, 1357</td>
</tr>
<tr>
<td>Matos PM 1397</td>
</tr>
<tr>
<td>Mattioli F 1274</td>
</tr>
<tr>
<td>Mazzucato F 1370, 1372</td>
</tr>
<tr>
<td>Mbadinga B 1238</td>
</tr>
</tbody>
</table>
Mbabka GO 1310, 1311, 1317
Mbeye MS 1446
Meddour OS 1313
Meddour R 1313
Medeiros P 1249
Medeiros PL 1427
Medina S 1377
Medini F 1430, 1445, 1450
Meerow AW 1245
Megdiche W 1445
Mehrabanfar Z 1318
Mehrinia M 1399
Meier B 1322
Meier M 1275
Meiotti FC 1254
Mekidiche N 1274
Melchini A 1456
Melo MF 1393
Melo RC 1428, 1447
Melzig MF 1237, 1364, 1378, 1382
Memar A 1276
Memariani Z 1316
Mendonça FS 1420
Mentel R 1253
Mercedes B 1385
Merfort I 1346
Merkel K 1244
Mertens M 1247
Mesbah L 1265, 1401
Meslich M 1313
Mehdizadeh H 1300, 1423
Nakamura MS 1266
Nabih Rashed K 1399
Nabizadeh H 1300, 1423
Nasiri Mahallati M 1292
Nasolahi M 1399
Nasrallah I 1321
Nasser S 1325
Nawash O 1312
Nawash OS 1380
Nawfal T 1325
Nazarijadad K 1290
Nazari F 1298
Nazarian A 1423
Nazeri V 1301, 1385, 1450
Ndip RN 1310
Neagu E 1271, 1325
Nedialkov P 1351
Neftati M 1416
Negreanu CN 1453
Nejad Ebrahimi S 1354
Nejla B 1411
Nematollahi A 1375
Nesli T 1444
Nethengwe MF 1319
Neburger M 1234
Neuhaus E 1272
Nezami S 1454
Ng S 1274, 1396, 1446
Nguyen Thai H 1317
Nguyen T 1317
Ni YH 1337
Nicholas A 1309
Niciforovici N 1424, 1432
Nicolici D 1456, 1457
Niculae M 1458
Niebler K 1323, 1364, 1421
Nievregel A 1247
Nik Abdullah Zawawi N 1333
Niketic M 1270
Nikolic N 1280, 1286
Moeini A 1287
Mohamad Yusof M 1311
Mohamadrezza N 1287
Mohamed A 1401, 1402
Mohamed AM 1330
Mohamed MO 1354
Mohamed SM 1330, 1412
Mohammad Abadi A 1381
Mohammad Reza F 1382
Mohammadi A 1294
Mohammadi S 1296
Mohammadpour G 1399
Mohammady S 1454
Mohammed RS 1331, 1375
Moharram Zade M 1450
Mohd Majid Z 1451
Mohd Nazri N 1332
Mohd Yusof M 1409
Mohajab M 1353
Molavani M 1362
Molazem M 1321
Moldovan L 1316, 1418
Molgaard P 1314, 1421
Molnar J 1444
Molnar J 1422
Momokov G 1384
Mondello L 1252
Monod M 1234
Monsalve Z 1257
Monsef Esfahani H 1448, 1451
Monsef H 1289
Montamat Sicotte D 1383
Montanari C 1266
Montasser Khoussari S 1327, 1328
Monteagudo U 1304
Monteiro M 1345
Mosavi H 1389, 1430
Morad Abadi L 1328
Moradi M 1299
Moriaes TS 1416
Moriais GO 1304
Moriais LA 1304
Morazzoni P 1238
Moreira AN 1282
Moreira J 1378
Moreira Pd 1415
Moreno C 1345
Moreira M 1354, 1355
Mornjakovic Z 1340
Morhunfofuj Aj 1403
Morsi TA 1289
Mosavi F 1450
Mossa A 1412
Mostafa EA 1373
Mostapha MS 1398
Motaghasi E 1352
Motawea H 1330
Mothana RA 1253
Motta EM 1254
Moug T 1416
Mougios V 1233
Mouloud B 1398
Mourad B 1398
Mouavi F 1303
Mouavi S 1266
Movahgharnian S 1303
Moyano E 1282
Moraffafian V 1301
Mroczek T 1396
Mrrouch M 1324, 1325
Mshvildadze D 1453
Munandar T 1260
Munchanicova A 1447
Muhammad F 1259
Mujie E 1266
Mulabgovic N 1430
Mulhivo S 1318, 1324, 1350, 1448
Müller J 1251, 1444
Müller MH 1445
Müller V 1383
Munoz Mingarro D 1440
Munoz A 1255, 1341
Muradic H 1273
Muranaka T 1249
Muratspahic Pavlovic D 1348
Murillo R 1346
Murray AP 1323
Musazadeh M 1266
Mussalam L 1308
Muyemb T 1260
N
Nabih Rashed K 1399
Nacira A 1453, 1454
Nada SA 1367, 1409, 1413
Naghi M 1276, 1284
Nagy A 1369
Naharwar VP 1364
Naidoo KK 1307
Naidoo V 1309
Naim HY 1267
Najafi S 1325
Najaf M 1416
Najmizadeh H 1300, 1423
Nakamura MS 1266
Nalbantosy A 1418
Nam M 1419
Namjooyan F 1389, 1394, 1399, 1427, 1430, 1431, 1435, 1446
Nanayakkarawasam Masachighe CN 1234
Nantanong N 1403, 1406
Narain N 1265, 1287, 1412, 1450
Narender T 1308
Naseri HR 1297, 1318
Naseri R 1277, 1290, 1291
Naserirad H 1291
Nashiriyah M 1329
Nasirizad E 1451
Nasiri Mahallati M 1292
Nasolahi M 1399
Nasrullah I 1321
Nasser S 1325
Nawash O 1312
Nawash OS 1380
Nawfal T 1325
Nazaralizadeh K 1290
Nazar F 1298
Nazarieh A 1423
Nazeri V 1301, 1385, 1450
Ndip RN 1310
Neagu E 1271, 1325
Necula R 1317
Nedialkov P 1351
Neftati M 1416
Negreanu CN 1453
Nejad Ebrahimi S 1354
Nejla B 1411
Nematollahi A 1375
Nesli T 1444
Netshengwe MF 1319
Neburger M 1234
Neuhaus E 1272
Nezami S 1454
Ngom S 1274, 1396, 1446
Nguyen Thai H 1317
Nguyen T 1317
Ni YH 1337
Nicholas A 1309
Niciforovic N 1424, 1432
Nicolici D 1456, 1457
Niculae M 1458
Niebler K 1323, 1364, 1421
Nievregel A 1247
Nik Abdullah Zawawi N 1333
Niketic M 1270
Nikolic N 1280, 1286
Nikolic NC 1296
Nikolov S 1351
Nikolova MT 1391, 1392
Nischikas T 1260
Nixon PJ 1281
Nnamani OP 1374
Noba K 1446
Noga G 1383
Nöldeke M 1410
Nooriy M 1447
Noori S 1255
Nordin M 1326
Nordin NI 1422
Noritzan N 1334
Noronha VA 1306
Noroozi M 1384
Noté O 1272
Novak J 1361
Novelli A 1372
Ntamabalyiro N 1260
Ntente FR 1367
Nuhu H 1313
Nurisari N 1258
Nwodo JN 1332
Nworo SC 1309
O
Oben J 1367
Oberbauer E 1256
Obohia OO 1448
Odokoya OA 1393
Ogbonnin SO 1310, 1311, 1317
Oh B 1419
Oh M 1346
Ojha V 1408
Oke Aluntsan F 1368, 1372, 1414
Oko OO 1307
Okoli CO 1309, 1455
Okomenesi EO 1324
Okoye TC 1309
Okpanji SN 1244, 1444
Okpuzor JE 1308
Oku B 1231
Oladazad A 1287
Olawumi O 1311
Oliveira Junior HA 1373
Oliveira AB 1264, 1265
Oliveira AP 1427, 1428
Oliveira F 1355
Oliveira GB 1450
Oliveira N 1377
Oliveira SC 1412
Oliveira V 1349
Olusunfemi TB 1312
Olowo JA 1455
Olugbade TA 1434
Olugbenga IE 1403
Olukeye A 1441
Olsola FI 1441
Olutwosoyin AA 1403
Omar R 1313, 1335
Omar EA 1332, 1367, 1409, 1413
Omejoe EO 1309, 1332
Omer EA 1261
Omidibaqi R 1282, 1287, 1292
Omid M 1287
Omobuwajo OR 1376
Onay M 1314
O'Neil Johnson M 1248
Ongen G 1285
Onrubia M 1282
Onu MA 1379, 1382
Onus A 1295, 1296
Oommenelkwe NF 1448
Opetal L 1387
Opoku AR 1319
Orakci EE 1382
Oric D 1438, 1439, 1455
Orhan DD 1254
Orhan I 1440, 1441
Orhan N 1254
Orland A 1328, 1398
Oromiehie A 1299
Osada H 1249
Osadebo PO 1332
Osman C 1400
Osman NA 1267
Osorio AA 1341
Ostad SN 1324
Ostadahmadi P 1281
Ota DA 1317
Ottang WM 1310
Ottai ME 1373
Otus CF 1374
Ouchfoun M 1308
Oueslati S 1433, 1445
Ouyemni O 1398
Oyounou RN 1326
Ozay O 1428
Ozcelik B 1378
Ozek G 1300
Ozek T 1300
Ozegoke F 1437
Ozkan G 1312
Ozkan T 1372
P
Padsch R 1437
 Pai SR 1283, 1284
 Pako J 1395
 Palazon J 1385
 Palazon J 1282
 Palic A 1388
 Pallua J 1257
 Pallua JD 1275
 Palm GJ 1261
 Pan IH 1413
 Pan X 1279
 Panahi M 1394, 1446
 Panahian AR 1297
 Pandey AK 1408
 Pandey C 1441
 Panek D 1251
 Panossian A 1248, 1420
 Panzeri S 1234
 Panthong A 1404
 Papageorgiou VP 1295
 Papaspyridi LM 1282
 Parada K 1257
 Paramon PP 1379
 Paranaros A 1281
 Parashos S 1303
 Paradshett MK 1284, 1437
 Parastar H 1252
 Park D 1357, 1431, 1436, 1439
 Park K 1322, 1346
 Park S 1346
 Paschal N 1268
 Pazckuz AF 1254
 Patil AB 1285
 Patil DN 1371
 Pauo G 1271, 1325
 Pauo L 1242
 Pavlov A 1272, 1285
 Paz ST 1428
 Pazvala M 1266
 Pedro L 1350
 Pedro LG 1297, 1361
 Pevc C 1288
 Pevc DR 1391
 Pehlivan Karakas F 1453
 Pektras M 1254
 Pelosini MS 1396
 Peng S 1356
 Pereira AD 1264, 1265
 Pereira EC 1447
Pereira ES 1327
 Pereira OR 1352
 Pereira S 1371
 Peres AM 1352
 Perret D 1422
 Perrone A 1349, 1443
 Perrotey S 1396
 Peretz HH 1403
 Peschel W 1253
 Pescheli G 1257
 Petersen G 1245
 Petrova MI 1392
 Petrovic J 1457
 Petrovic S 1270
 Pezzi C 1257, 1275
 Perschky Wenzig E 1275
 Phiri P 1246, 1383
 Phoolcharoen W 1280
 Phuwapsrisaran P 1411
 Rachente S 1343, 1347, 1349, 1443
 Panowski LF 1254
 Rachette A 1445, 1453
 Penglis C 1244
 Peraccini G 1250
 Peters L 1260, 1274, 1330, 1331, 1428
 Pilipovic S 1365, 1371, 1430
 Ping TC 1255, 1345
 Pinnai K 1314
 Pinto JB 1264, 1265
 Pralhi Khair Abadi K 1458
 Pires C 1349
 Pires CT 1415
 Pires D 1318
 Piri K 1281, 1292, 1362
 Pittenauer E 1350
 Rachy V 1453
 Plante M 1263, 1362
 Plazcynska M 1431, 1437
 Poiana M 1365
 Polat E 1437
 Polepally PR 1243
 Polin Garcia L 1274
 Politie O 1397
 Pompei B 1456, 1457
 Pongratz I 1441
 Poorakbar L 1268
 Pop D 1369
 Pop G 1288, 1289, 1365, 1369
 Popescu R 1236
 Poracova J 1447, 1458
 Portet B 1268
 Porto TS 1393
 Pothe A 1231
 Pourrahimi M 1293
 Pourrezae J 1318
 Power JB 1279
 Pozhitariskayana ON 1357, 1411, 1414
 Pramudito TE 1258
 Prazina N 1259, 1307, 1429
 Preeprame S 1371
 Pretsch A 1257
 Priprem A 1371
 Prokshv P 1257, 1262, 1332, 1334
 Prokudina E 1343
 Prudêncio M 1448
 Putulan W 1265, 1279
 Puthong S 1411
 Q
Qazi MA 1250
Qi L 1239
Qiang KC 1255, 1345
Qin L 1258, 1321
Qin P 1338, 1381
Qin Z 1345
Quennou M 1369
Quitschau M 1234
R
Raaijmakers N 1361
Rabintossaporn P 1310
Racha K 1313, 1335
Rachida YZ 1277
Raclarius AC 1379
Radou V 1453
Rademehr B 1413
Radjojkovic M 1432
Radu GL 1271, 1325
Radulov D 1289, 1369
Radusiene J 1375
Ragac P 1389
Rahmati M 1299
Rai S 1308
Rajabi A 1234, 1384
Rajaei H 1303
Rajagopal M 1375
Rajasekar N 1308
Rakhmadieva SB 1300
Rakhmadieva S 1411, 1428
Ramalhete C 1324, 1448
Ramalho SA 1265, 1412, 1450
RamasyMY 1402, 1408, 1410
Ramirez K 1282
Ramos G 1304
Ramos PR 1452
Rao CV 1431
Rasamg 1294
Rasras M 1332
Rasoulinavov P 1236, 1380, 1386
Rastgeldi U 1440
Ratajad BD 1457
Rawat AS 1431
Realino PJ 1401
Redic A 1429
Redic S 1259, 1307, 1320, 1365, 1387, 1388, 1395, 1429
Regnier T 1326
Reich E 1275
Reis M 1349, 1444
Renda G 1452
Reshidi Monfred S 1276
Reyes F 1345
Rezadoust H 1284
Rezaei A 1362
Rezende CM 1456
Rezniec G 1236
Rezvani Moghadam P 1292, 1381, 1393
Riahi H 1449
Richomme P 1246
Rieder A 1327, 1452, 1453
Riepl H 1256, 1348
Rigbeschi C 1261, 1271, 1370
Rindt K 1458
Rios J 1449
Ro D 1279
Robin J 1268
Rocha V 1377
Rodilla J 1295
Rodrigues L 1297
Rodrigues RA 1373
Rodrigues V 1324, 1393
Rodriguez SA 1323
Roghani M 1426, 1427
Roghian R 1422, 1453
Rojas R 1300
Rollinger JM 1230, 1243, 1339, 1381
Romea C 1350
Roncales P 1407
Ransted N 1245, 1332
Ros G 1271
Rosas ST 1427
Rosini F 1266
Rostami S 1372
Rostock M 1406
Roht BL 1243
Rotenberg P 1325
Rotzschke O 1443
Roux S 1435
Roza O 1320
Rozena E 1236
Ruangrungrui N 1280
Rudaz S 1391
Ruiz Larrea M 1407
Ruiz Sanz J 1407
Ruiz C 1300
Rustayani A 1243, 1295, 1433
Ruyter Spira C 1249
Ryden A 1249
Ryu J 1376, 1377
Saaby L 1437
Saada M 1450
Sabau I 1288
Sahy GM 1307
Sahy R 1291
Sabuncucuolu SA 1388
Saciragic Boric S 1365
Sadeghinimako A 1384
Saed A 1400
Saed T 1400
Saedeh Mehrvaz S 1264
Saedian H 1433
Saedie S 1352
Saenihaweesuk S 1310, 1311, 1312
Safia I 1453, 1454
Saglam AC 1302
Sahari MA 1294
Sahiz N 1279
Sahin G 1260, 1279
Sahu SK 1408
Said M 1249
Said S 1393
Saida M 1277
Safian SN 1293
Salah El Din S 1331
Salama AB 1290, 1291
Salamanca E 1342
Salamat G 1245
Salami S 1284
Salamon I 1339, 1389
Saliari N 1300, 1423
Saliari J 1292
Salehi Sormagh M 1296, 1338
Salehi Sormagh M 1234
Salem AM 1307
Salih AI 1347
Saligh G 1347
Salimi A 1278
Salieh M 1409
Saller R 1406
Samadi N 1321
Samadi S 1329
Samaee H 1446
Samaei H 1394
Samanico C 1313
Samanai MA 1268
Samia A 1265
Samiee F 1411
Samiee J 1295
Samuel TA 1324, 1393
Sancaklaroglu S 1305
Sanchez J 1297
Sanda A 1457
Sandasi M 1306
Sandor C 1365
Sandru CD 1458
Sang X 1292
Sangvanich P 1315, 1319
Sanjaran F 1278
Santos EM 1420
Santos MM 1349
Santos VR 1282, 1306
Saufakwen N 1315
Sapcanin A 1266, 1365
Sarac F 1369
Saracoglu I 1242, 1353
Sarbania S 1297
Sarier E 1414
Sarg T 1386, 1392
Sarihaj G 1302
Sarker S 1353
Sarker SD 1314
Sarkhail P 1377
Sarkheil P 1377
Sasheva P 1384
Sathiyas B 1414
Saucedo Hernandez Y 1274
Saucedo Y 1304, 1428
Savedoroudi P 1276
Sawsan K 1313
Sayyad Mohtaim M 1400
Schatzmayr G 1432
Scherer B 1398
Schiemann S 1245
Schinkovitz A 1333, 1338
Schinnerl J 1349
Schmidt T 1443
Schmitt M 1306
Schnabelger EE 1432
Schneider D 1361
Schnieders A 1443
Schonbichler S 1257
Schonbichler SA 1275
Schramm A 1236
Schreiner CE 1433
Schripsema J 1276, 1325
Schneegans S 1381
Schulz B 1262
Schulz S 1272, 1285
Schumpo O 1234, 1447
Schuster D 1236, 1243
Schwabiger BV 1433
Schwanck B 1267
Scodro RB 1415
Scott N 1253
Scott NW 1243, 1361
Seabra Junior S 1452
Sebti M 1299
Seghidhi H 1268
Seidikon F 1297, 1385
Seidu AA 1396
Seidiova Wutke D 1259, 1260, 1366
Sejdic N 1388
Shekar Karatopok P 1374, 1382, 1383
Sendemir Ortmeze A 1444
Sfencheau CV 1446
Sfencheau CV 1274
Shener B 1440, 1441
Senol F 1441
Senol FS 1440, 1441
Senol SG 1347
Senturk M 1443
Seo M 1419
Sepahvand S 1435
Serier G 1269
Seresht H 1252
Seri G 1396
Serly J 1444
Sertav Z 1399
Setola V 1243
Seyhusana S 1244
Seylerman ME 1393
Sevim D 1440, 1441
Seyed Hashtroudi M 1449
Seyed Shirzazi S 1450
Seyran M 1417
Seyyed Rahmani S 1370
Sezik E 1285, 1412
Shaabani S 1298
Shaari K 1434
Shafee A 1373
Shahat A 1262

Authors’ Index | 59th International Congress of the GA | 4th–9th September 2011, Antalya, Turkey
Shalnia M 1294, 1413
Shahriz F 1413
Shahriari F 1277
Shahriari S 1293, 1306
Shahverdi A 1321, 1448
Shaker K 1330
Shakeri A 1352
Shakeri R 1297, 1318
Shalaby NM 1410
Shallan MA 1367
Shameeri Z 1334
Shamsal R 1287
Shao Y 1363, 1364
Sharaf W 1413
Shariatmadari Z 1449
Sharifi V 1245, 1268
Sharifzadeh M 1338, 1451
Sharma KK 1316, 1417
Sharma N 1417
Sharma V 1355
Shatha S 1313, 1335
Shehata HS 1290
Shehata IA 1399
Shen D 1353
Sheng Y 1345
Shikanga E 1254
Shikanga EA 1328
Shikov AN 1357, 1411, 1414
Shin D 1321
Shin S 1299, 1304, 1401
Shirani Rad A 1290
Shimatzk D 1266
Shokrpour M 1284
Shokrzadeh M 1315
Shonhali A 1319
Shoosharti L 1287
Shokroy MA 1399
Shoyama Y 1279
Shuddaf M 1312
Sukda R 1308
Shyur L 1424
Si C 1338, 1342
Si CL 1256, 1337, 1381, 1418
Siahpoosh A 1401
Sidat S 1291
Sievers H 1238
Silva AD 1396
Silva AF 1390
Silva AM 1352
Silva AN 1397
Silva CG 1279, 1390
Silva D 1274
Silva FF 1306
Silva JC 1306
Silva KB 1254
Silva L 1295
Silva NH 1447
Sim Y 1401
Simanek V 1288
Simão MR 1393
Simin N 1438, 1439, 1455
Simmonds M 1236
Simós Alfonso E 1304
Simonnet X 1369
Simonsen HT 1279
Singh M 1319
Singh N 1288
Singh SR 1364
Singh V 1288
Siqueira VL 1415
Sireeratatwong S 1310, 1314, 1404, 1406
Siero V 1394
Skaltsa H 1348
Skalsounis A 1233, 1237, 1268, 1272, 1303, 1347
Skalsounis AL 1282, 1346, 1390, 1391, 1441
Skalsounis LA 1252, 1344, 1350
Skrzypczak A 1275
Slater A 1243, 1253, 1361
Sleem AA 1375, 1399
Slovved HC 1314, 1421
Smith P 1319
Smith S 1253
Smynirovdis F 1303
Sobajic S 1286
Sobhoun P 1315
Socratous E 1243
Sodano UC 1418
Sofic E 1256, 1273, 1366
Sohn E 1321
Sophyrogou D 1388
Solimanani M 1362
Soleti R 1246
Sooleymanford A 1277, 1290, 1291
Soliman FM 1375
Soltani M 1396
Soltani S 1350
Solujic J 1348, 1374, 1424, 1432
Somporn N 1310, 1311, 1312
Son B 1430
Son M 1376, 1377
Sonboli A 1329, 1438
Sonderoccerer H 1226
Song C 1350
Song S 1419
Sootnornchareonnon N 1404
Sorin R 1456
Sosa S 1432
Sothea K 1346
Sousa IJ 1444
Sousa JP 1417
Sousa AB 1419
Souza MG 1397, 1417
Souza N 1355
Sowemimo A 1432
Sowemimo AA 1425
Soylu MK 1320
Speck UJ 1369
Sperl C 1432
Spies L 1425
Spinu M 1458
Spriano D 1322
Srisomsap C 1315
Sritularak B 1265
Sriubolmas N 1244
Srivastava S 1451
Stafford GI 1245
Stal IJ 1279, 1358
Stan L 1458
Stanislavjevic D 1301
Stanius Z 1375
Stankovic M 1424
Stankovic MN 1374
Stankovic MS 1374, 1397, 1454
Stapleton P 1383
Starks C 1248
Stefanovic A 1391
Stefanovic O 1454
Stefkov G 1387
Steingroewer J 1272, 1285
Steinhauer L 1346
Steinhoff B 1242
Stephen N 1363
Stiacini G 1418
Stiebing S 1396
Stojanov I 1437
Stojanovic D 1457
Stojovic C 1286, 1301
Stojiokovski K 1389
Storzini A 1367
Straub AL 1452
Student J 1268
Summer H 1243, 1250, 1339, 1381, 1395
Su Ling W 1339
Sudo R 1371
Sudo S 1371
Suffredini IB 1396, 1448
Sufan A 1410
Suganuma T 1311
Suk Ki L 1284
Sukroon S 1280
Sulyok E 1422
Sung Y 1385
Sunguroglu A 1372
Suntar I 1314, 1443
Suntar I 1452
Suntar JP 1435
Surer A 1249
Svidzinski TI 1420
Sytwala S 1382
Szczodrak J 1437
T
Tabana N 1302, 1305
Tabaran F 1369
Tafeiner B 1250
Tag O 1347, 1351, 1437
Taha KP 1367
Taheri S 1354, 1355
Taherian A 1389
Tahirovic I 1256, 1366
Talifeh Noori M 1370
Tahour F 1380
Taiwo OS 1312
Takaloozaedeh H 1423
Takeya K 1335
Tamas M 1458
Tamer K 1347
Tanaka H 1265, 1279
Tang C 1356
Tapeinos CG 1373
Tappenier J 1395
Tarmen K 1261
Tasdemir D 1230, 1246, 1317, 1318, 1380, 1383, 1426
Tasharioli N 1296
Tashlitsky V 1266
Tassanawat P 1265
Tatia R 1317
Tatke PA 1309, 1364
Tatli I 1443
Tautescu M 1369
Taura F 1359
Tava A 1295
Tavakoli Dinani E 1290
Tavcar E 1389
Taviano MF 1456
Thoumchoucha J 1252
Teh L 1311, 1409
Teichmann K 1432
Teilans A 1380
Texeira G 1297, 1377, 1378
Telic D 1435
Tenkerian C 1324
Teres P 1343
Termenzzi A 1268
Teyeb H 1416
Thakur M 1237
Thang T 1353
Theunis M 1274
Thomsen MO 1389
Thuppia A 1310, 1311, 1312
Ticona J 1447
Tittto RJ 1356
Tikhonov V 1266
Timoteo P 1271
Ting C 1340
Tinnam AB 1305
Tinoco T 1295
Tiralongo E 1247
Tito EA 1266
Tkacikova L 1447
Todorovic V 1286
Todorovic Z 1280, 1286
Tofghi Z 1321
Tohghare A 1290
Zafar MM 1456
Zafarani Moattar P 1264
Zahia K 1454
Zahra A 1382
Zahradnik C 1384
Zakaria I 1405
Zakaria Z 1311, 1408, 1409, 1410
Zaki HF 1244, 1423
Zamani Dehyaghobi R 1300, 1423
Zamani Z 1385
Zaouali Y 1450
Zapata Sudo G 1371
Zarei Kooshki M 1290
Zarei A 1294, 1399, 1456
Zaroori S 1359
Zatloukalova M 1269
Zayed R 1374, 1377, 1386, 1392
Zayed RA 1288
Zayova EG 1392
Zebarjadi A 1288, 1299
Zee O 1350
Zeitler H 1251
Zendehdel M 1293
Zeybek AU 1417
Zhang Q 1258, 1321
Zhang Y 1337, 1381
Zhao Y 1292
Zhen ZS 1255
Zheng C 1321
Zhou S 1356
Zhou Y 1262
Zhu M 1245
Zidek Z 1400, 1405
Zierau O 1439
Zietsman P 1336
Zimmermann S 1234
Zjawiony JK 1243
Zioh M 1249
Zohdi H 1422
Zorins A 1380
Zovko Koncic M 1267
Zupko I 1320
Zuraiza M 1451