Proanthocyanidins were isolated and purified from fruits of elephant apple (Dillenia indica Linn., commonly known as elephant apple, be-

Abstract: Proanthocyanidins were isolated and purified from fruits of elephant apple (Dillenia indica Linn.) and their structural and bioactive properties were examined. Bate-Smith alcoholysis, FTIR, and $^{13}$C NMR spectra revealed that elephant apple proanthocyanidins (EAPs) contained a dominant amount of B-type procyanidins (PC) with a minor amount of B-type prodelphinidins (PD) but no A-type interflavan linkage. $^{13}$C NMR spectrum indicated that the cis isomer was dominant in EAPs. The electron spray ionization and matrix-assisted laser desorption ionization time of flight mass spectra of EAPs showed the clear ion peaks corresponding to B-type PC dimer to B-type PD with degree of polymerization of 11. EAPs had strong antioxidant activity, which was evidenced by the high oxygen radical scavenging capacity at $1.06 \times 10^4$ μmol TE/g and ferric reducing antioxidant power of 2320 μmol Fe(II)/g. The results suggest that EAPs could be extracted to be used as promising functional food materials.

Keywords: Dillenia indica Linn., elephant apple, ferric-ion reducing antioxidant power, oxygen radical absorbance capacity, proanthocyanidins

Introduction

Dillenia indica Linn., commonly known as elephant apple, belongs to the Dillenia family. This evergreen shrub is widely distributed in India, Indonesia, Thailand, China, and other places, producing ripe sweet-sour fruits as a nutritious food which is eaten fresh or cooked, or made into jams and jellies although the unripe fruit is acid, astringent and seldom used as food (Shome and others 1980; Yazen and Armania 2014). Traditionally, the fruits, leaves, and barks of D. indica are used to treat the diseases such as fever, constipation, diarrhea, stomach pain, and so on (Yazen and Armania 2014). The fruit decoction had been adopted to treat hair loss and diabetes, and was also an immunity enhancer (Gandhi and Mehta 2014; Yazen and Armania 2014). Juice of elephant apple mixed with sugar and water can function as a cough syrup with cooling effect (Yazen and Armania 2014). Extracts of phytochemicals from D. indica possess several biological activities such as anti-inflammatory, antioxidant, anti-diabetic, anticholinesterase, antimicrobial, and cytotoxic activities (Abdille and others 2005; Kumar and others 2010; Kumar and others 2011; Migliato and others 2011). D. indica fruits showed different antileukemic activity against human leukemia cell lines, there into, the ethyl acetate extracts gave the highest cytotoxic activities (Kumar and others 2010). Ethyl acetate, methanol, and water extracts from D. indica fruits also gave different antioxidant activities (Abdille and others 2005). The glycolic extracts from mature D. indica fruits possessed a significant skin wound-healing activity when combined with microcurrent (Migliato and others 2011). The water extracts of the fruits can be used as a natural muco adhesive agent for formulation of patient friendly gel (Kuotsu and others 2007). In a methanolic extract of the D. indica fruit, the total phenolic content was about 34% (w/w) (Abdille and others 2005). A number of bioactive compounds, such as triterpenes (Banerji and others 1975), phenols (Das and others 2012), and lupeol, betulinaldehyde, betulinic acid, and stigmastanol (Parvin and others 2009; Kumar and others 2010) were found in D. indica species. To our knowledge, how the proanthocyanidins contribute to the functions of D. indica fruit has not been characterized yet.

Proanthocyanidins are a class of flavan-3-ol oligomers and polymers widely found in plant kingdom and exist in many foods and agro-byproducts (Wallace and Giusti 2010; Arimboor and Arumughan 2011, 2012; Travaglia and others 2011; Sarnoski and others 2012; Kruger and others 2014; Granato and others 2015). Both structural complexity and bioactive diversity coexist for proanthocyanidins. The variation in monomeric units, type of interflavan linkage and the stereochemistry categorize proanthocyanidins into different types and subclasses (Figure 1). B-type proanthocyanidins contain the single linkage (C4-C8 or C4-C6 bond) while A-type interflavan bond is doubly linked by an additional ether bond between C2→O→C7 (Figure 1). Regarding subclass, the proanthocyanidins consisting exclusively of (epi)catechin are designated as procyanidins (PC) while...
proanthocyanidins bearing (epi)gallocatechin or (epi)afzelechin as subunits are classed as prodelphinidins (PD) or propelargonidins (PP), respectively (Ferreira and Slade 2002). Numerous literatures have indicated the potential health benefits and applications of the crude and purified proanthocyanidin fractions, especially their antioxidant activities (Fu and others 2007; Chen and others 2014; Wang and others 2015). Proanthocyanidins from D. indica fruits may have similar activities, contributing to the health benefits of this fruit; however, their functional and structural properties remain unknown to date.

To determine the antioxidant function of various components from foods, several assays based on different principles were developed, which include predominant hydrogen atom transfer-based assay (HAT), single electron transfer-based assay (SET) and metal chelating mechanisms (Huang and others 2005). Because these assays assess antioxidant activity from different aspects, it is necessary to test antioxidant activities of functional compounds or foods in vitro by a parallel combination of methods based on different mechanisms.

In this study, we extracted and purified elephant apple proanthocyanidins (EAPs) from mature fruit of D. indica and elucidated their structural profiles by UV/vis, FT-IR, NMR spectroscopy, mass spectrometry (MS) including electron spray ionization (ESI) MS, and matrix-assisted laser desorption/ionization time of flight (MALDI-TOF) MS. The antioxidant activities of EAPs were determined by employing ferric reducing antioxidant power (FRAP) and oxygen radical absorbance capacity (ORAC) assays.

Materials and Methods

Materials and chemicals

All chemicals and solvents used were of analytical grade unless otherwise specified. Thirty kilograms mature elephant apple (D. indica Linn.) fruits were collected from Singapore in September, 2014. Sephadex™ LH-20 chromatographic matrix was purchased from GE Healthcare Bi-Sciences AB (Uppsala, Sweden). Commercial grape seed proanthocyanidins (cGSPs) (purity: 95%) were obtained from Chengdu SanHerb Plant Extract Co. Ltd. (Chengdu, Sichuan, China). Ferric chloride (FeCl₃), ferric ammonium sulfate, ferrous sulfate heptahydrate, 2,4,6-tri-pyridyl-s-triazine, 2,5-dihydroxybenzoic acid, Trolox, fluorescein disodium salt (FL), and 2,2′-azobis (2-amidinopropane) dihydrochloride (AAPH) were purchased from Sigma–Aldrich Chemical Company (St. Louis, Mo., U.S.A.).

**Extraction and purification of EAPs**

Proanthocyanidins were extracted and fractionated from elephant apple fruits according to a previous report (Wang and others 2012). The overall extraction and purification flow scheme of EAPs is shown in Figure 2(A). Fresh elephant apple fruits (2 kg) were cut into small pieces, and then ground to produce a paste which was then extracted by 2000 mL mixed solution of acetone/water (7:3, v/v) with shaking for 4 h at room temperature. The mixture was filtered and the extraction procedure was repeated 2 times. The collected filtrates were pooled and then evaporated to remove acetone. The water phase containing crude proanthocyanidins fraction (300 mL) was liquid-liquid extracted with chloroform (2 times, each time with 300 mL) to remove the nonpolar and lipophilic compounds. The water layer was concentrated by rotary evaporation at 45 °C for 2 h and then methanol/water (1:1, v/v, 5 mL) was added to the concentrated aqueous fraction. The mixture was filtered through a PTFE 0.20 μm membrane filter (Epsom) and then loaded on a pre-equilibrated Sephadex LH-20 column (50 g, equilibrated with 50% v/v) methanol aqueous solution (2 times, each time with 300 mL) to remove the nonpolar and lipophilic compounds. The water layer was concentrated by rotary evaporation at 45 °C for 2 h and then methanol/water (1:1, v/v, 5 mL) was added to the concentrated aqueous fraction. The mixture was filtered through a PTFE 0.20 μm membrane filter (Epsom) and then loaded on a pre-equilibrated Sephadex LH-20 column (50 g, equilibrated with 50% v/v, 5 mL) to concentrate the aqueous solution. The EAPs were recovered by eluting the column with 500 mL mixed solution of acetone/water (7:3, v/v). Acetone and part of water were removed via rotary evaporation at 45 °C for 3 h, and the resulting proanthocyanidin fraction was freeze-dried, which was presented as light brown powder.
UV/vis spectra and Bate-Smith alcoholysis
The UV spectra of EAPs detected with a Hewlett Packard 8452A UV/vis spectrophotometer were recorded in the range from 200 to 400 nm by adopting a 1-cm path length quartz cuvette. NaOH (2 mol/L) was added to 1 mg/mL EAPs in methanol to observe the bathochromic shift of proanthocyanidins. Bate-Smith alcoholysis giving information about the monomeric units was performed in a hydrochloric medium. The detailed procedures of the alcoholysis with ferric ammonium sulfate as a catalyst followed the methods of Ku and Mun (2007). Specifically, EAPs (1 mg) and 1 mL of methanol/water (1:1, v/v) were added to a PTFE screw capped vial. 1-Butanol (950 mL) was mixed with 50 mL concentrated HCl to produce the acid-butanol solution. 6.0 mL of the acid-butanol solution and 0.2 mL of the iron reagent (2% ferric ammonium sulfate in 2 M HCl) were added into the vial. Then, the mixture was reacted in a heating block set at 95 to 100 °C for 1 h. After the Bate-Smith alcoholysis, its visible spectrum was recorded in the range from 450 to 650 nm by the same UV/vis spectrophotometer.

FTIR and NMR
A Perkin Elmer Spectrum™ One FT-IR spectrophotometer was used to collect the FT-IR spectra of the KBr samples of EAPs. The spectra were scanned between 4000 and 400 cm⁻¹ and then recorded in the transmission mode. ¹H and ¹³C NMR spectra of EAPs in deuterated methanol were recorded at 300 MHz for ¹H and 75 MHz for ¹³C spectrum by adopting a Bruker AC300 spectrometer.

ESI and MALDI-TOF MS
The EAPs were dissolved in methanol and the solutions were used to collect the spectra of ESI-MS and MALDI-TOF MS.

Figure 2–Procedure flow chart and UV/visible spectra of the elephant apple proanthocyanidins (EAPs). (A) Procedure flow chart. (B) UV/Vis spectra of elephant apple proanthocyanidins. (C) UV/Vis spectra of alcoholysis reaction media of EAPs under an acidic condition.
ESI mass spectrum of EAPs was obtained on a Finnigan/MAT LCQ ion trap mass spectrometer (San Jose, Calif., U.S.A.) by using a negative ion mode. The full-scan mass range was from m/z 50 to 2000 while the heated capillary and voltage were set at 250 °C and 4.5 kV, respectively. MALDI-TOF mass spectrum of EAPs was collected from a Voyager-DE STR mass spectrometer (Applied Biosystems, Foster City, Calif., U.S.A.) equipped with delayed extraction and a N2 laser that was set at 337 nm. The measurements were carried out by applying these conditions: positive polarity, acceleration voltage of 21 kV in linear flight path, 3 ns for the length of one laser pulse and 100 pulses per spectrum (Wei and others 2014).

Oxygen radical absorbance capacity (ORAC)

The ORAC method of Huang and others (2002) was performed using a Synergy HT fluorescence microplate reader (Synergy HT, Bio-tek Instruments Inc., Winooski, Vt., U.S.A.). Briefly, AAPH (0.414 g) was dissolved in 10 mL phosphate buffer (75 mM, pH 7.4, working buffer) to produce the 153 mM AAPH solution as the inductor of peroxidation. FL was dissolved in 75 mM phosphate buffer (pH 7.4) to make 500 mM of fluorescein stock solution (4.19 × 10−3mM) which was stored in dark condition at 4 °C. The stock solution was daily diluted in the working buffer to produce the fresh fluorescein working solution (8.16 × 10−5 mM). EAPs was dissolved in methanol to make a 5 mg/mL of proanthocyanidin solution which was further diluted with the 75 mM phosphate buffer (pH 7.4) to produce the testing solutions with various concentrations. Blank, proanthocyanidin solution, or Trolox standard in working buffer (25 μL), was added to wells in a 96-well microplate. Then the fresh fluorescein solution (150 μL) was added to each well. The mixed solutions were incubated with intermittent shaking at 37 °C for 5 min before the addition of 25 μL of the 153 mM AAPH solution. The reaction lasted for 2 h and the fluorescence of each well was recorded kinetically every 2 min. The excitation wavelength and emission wavelength were set at 485 and 530 nm, respectively. The areas under the curve (AUC) of fluorescence versus time for the samples minus the AUC for the blank were calculated and compared to a standard curve (y = 2.8763x + 2.9972, R² = 0.9983) of the AUC for Trolox standards (0.625 to 10 μM, final concentration) minus the AUC for blank. ORAC values of EAPs were expressed as micromoles of Trolox equivalents per gram (μmol TE/g).

FRAP assay

FRAP assay was used to measure the antioxidant ability of proanthocyanidins by reducing the Fe(III)-2,4,6-tri-pyridyl-s-triazine (TPTZ) to the ferrous iron form (Benzie and Strain 1996). Briefly, FRAP reagent was prepared by mixing a 10 mM TPTZ solution in 40 mM HCl, 20 mM FeCl3, and 300 mM acetate buffer (pH 3.6) at a ratio of 1:1:10. Then, 50 μL of EAPs or cGSPs (0.2 mg/mL in methanol) were added to 3 mL of FRAP reagent. The solution was mixed with shaking and incubated at 37 °C for 5 min. The blue color of the reaction mixture was characterized with diode-array spectrophotometer based on the absorption (y) at 593 nm. Solutions of 0.25, 0.5, 0.75, 1, 1.25, 1.5, and 2 mM Ferrous sulfate heptahydrate solutions were used to create the calibration curve y = 0.8214x + 0.0313, R² = 0.9969, for calculation of FRAP value. FRAP values for samples were expressed as μmol of Fe(II)/ g.

Statistical analysis

All assays described above were performed in triplicate. The results were expressed as mean ± SD (standard deviation). Significance of differences was determined by using the Duncan’s multiple range test (Granato and others 2014). Differences leading to values of P < 0.05 were considered significant. Statistical analyses were carried out with SAS software (version 9.2, Cary, N.C., U.S.A.).

Results and Discussion

UV and visible spectra of EAPs

After freeze drying, the EAPs produced a light brown powder 656 mg from 2.0 kg of fresh elephant apple fruit. The moisture content of this fruit was 86.2% ± 0.5%. The EAPs after freeze-dried were amorphous powder with a final yield of 0.23% based on dry basis. The yield of EAPs is greater than the abundance of proanthocyanidins in many fruits, such as mangoes, kiwis, avocados, and vegetables, as well as some berries such as raspberries (Gu and others 2004). Proanthocyanidins can be isolated from grape seeds and cinnamon bark with higher yield (Gu and others 2004). The cost of EAPs can be lower than that of proanthocyanidins from berries and many popular fruits due to the low price of elephant apple. Chromatographic separation technique with Sephadex-LH 20 column was successfully used to purify proanthocyanidins from different plants (Fu and others 2007; Ku and Mun 2007; Wang and others 2012; Chen and others 2014). In this current study, the EAPs were purified with Sephadex-LH 20 column. The UV/vis spectrum of EAPs eluted from chromatographic column showed a catechin-like and symmetrical peak near 280 nm (Fig. 2B), which is typical spectral characteristic of proanthocyanidins (Ku and Mun 2007; Nakamura and others 2013; Chen and others 2014). When the phenolic structure exists in molecules, the addition of NaOH shows the bathochromic shift (Ku and Mun 2007). Bate-Smith alcoholysis was used to approximately estimate the basic structure of EAPs based on different maximum absorbance wavelength and extinction coefficients. Visible spectrum of EAPs after alcoholysis under the acidic condition was shown in Figure 2(C). The maximum absorption peak of EAPs appeared at 545 nm. According to the previous reports from several research groups, (epi) catechin-based proanthocyanidins produced cyanidins pigments (kmax at 540 to 550 nm) while (epi) gallolatechin-based proanthocyanidins produced delphinidins (kmax at 550 to 560 nm) pigments through the butanol–HCl reaction (Porter and others 1986; Hussein and others 1990). Thus, the current result indicated that the main extension units contained in EAPs were largely composed of (epi) catechin rather than (epi)gallolatechin.

FTIR

The FTIR spectrum of EAPs clearly showed bands at 3368, 1610, 1521, 1444, 1366, 1285, 1108, 1060, 975, 822, 782, and 676 cm−1(Figure 3). Specifically, the band at 3368 cm−1 was assigned to −OH stretch vibration associated with the phenolic structure of EAPs. Bands at 1521 and 1444 cm−1 were corresponding to the aromatic structure of EAPs. Peaks at 1521 and 782 cm−1 generally corresponded to the aromatic ring breathing structure, while the band at 1444 cm−1 was attributed to −CH deformation. Generally, proanthocyanidins containing galloyl group yield absorption band at about 1710 cm−1 because of the stretching vibration of C = O in galloyl group (Yazaki and Hillis 1977; Hussein and others 1990; Ku and Mun 2007). The absence of band at 1710 cm−1 suggested that EAPs did not contain gal-
loyl group. PC is the dominant subclass which consisted exclusively of (epi)catechin while PD is another main subclass containing subunits of (epi)gallocatechin. Bands at 767 and 730 cm⁻¹ were assigned to out-of-plane conformations of CH₃ in PC and PD respectively (Ku and Mun 2007). In the current study, the strong absorption bands were 3368, 1610, 1521, and 767 cm⁻¹, which might result from the characteristic functional groups of EAPs (Table 1) (Yazaki and Hillis 1977; Hussein and others 1990; Ku and Mun 2007; Xu and others 2012). The strong presence of 767 cm⁻¹ band and the much milder presence of 730 cm⁻¹ band indicate EAPs contained mainly PC but small amount of PD units (Ku and Mun 2007).

**NMR**

¹H NMR spectrum of proanthocyanidins shows the A-ring proton (H6 and H8) signals (5.8 to 6.5 ppm) and the H4 signals of the terminal units (2.4 to 3.0 ppm), therefore the mDP of EAPs was 3.8 according to the calculation from the integration value of the H6, H8, and H4 signal area in ¹H NMR of EAPs followed the methods of Guyot and others (1999) (Figure 4A).

Figure 4(B) shows ¹³C NMR spectrum of EAPs in methanol-d₄. It revealed that EAPs contained a dominant amount of B-type PC and a minor amount of B-type PD but no A-type interflavan linkage (Czochanska and others 1980). Particularly, C5, C7, C8α, and C4′ carbon positions of PC were at 160 to 150 ppm. Peaks at 145.5 and 145.3 ppm were associated with the C4′ and C3′ of PC units. The C4′ peak appearing at 131 ppm suggested a minor amount of PD. The peak clusters assigning to C8 and C6′ of PC, C6′ (PD) and C2′ (PD) emerged between 110 and 90 ppm. As for the stereo-chemical properties, peaks at the region from 70 to 90 ppm are sensitive to the stereochemistry properties of the C-ring (C2 to C3) (Czochanska and others 1980). The ratio of the 2,3-cis to 2,3-trans isomers was determined by measuring the distinct differences from their respective C2 chemical shifts. C2 had a resonance at 75.3 and 79 ppm corresponding to the cis and trans form, respectively. C3 occurred at ~71.4 ppm within both cis and trans isomers. The peak areas for C2 showed that the cis isomer was dominant in EAPs. The C4s of the extension units for both the cis and trans form showed at 36 ppm with an extensive peak but the terminal C4 exhibited a line at 29 ppm. There was no A-type linkage in EAPs since at 151 to 152 ppm the signals, which was due to C5 and C7 structures of the A-ring, did not appear in the spectrum. The absence of galloyl group was further confirmed by the absence of peaks appearing at 131 ppm which were assigned to the signal of C4″, C5″, and C6″ (Czochanska and others 1980). This result was consistent with the FT-IR results.

**ESI and MALDI-TOF MS**

The structural profile of EAPs was characterized by mean of ESI-MS in the negative mode. Proanthocyanidins with different degree of polymerization (DP) are shown in Figure 5(A). In compliance with the FTIR and ¹³C NMR spectra, ESI mass spectra of EAPs showed a series of abundant ions corresponding to the molecular masses of proanthocyanidins with DP 2 to 6 (m/z 577, 865, 1153, 1441, and 1729). Some PD, for example (epi) catechin-(epi)gallocatechin (m/z 595), was also observed in ESI mass spectrum although with less abundant signals. The ESI/MS spectrum also provided some information for the doubly charged species of heptameric procyanidins because it had 144 mass units greater than trimer ion (m/z 865) and 144 less than tetramer (m/z 1153). By ESI mass spectrum, proanthocyanidins with DP up to 6 were effectively detected. However, the range of quadruple analyzer in ESI-MS was lower than 200 Da and failed to precisely detect high-mass molecules.

MALDI-TOF MS can precisely detect high-mass molecules and was successfully applied to analyze profile of proanthocyanidins with high DP (Fu and others 2007; Chen and others 2014). Figure 5(B) shows the MALDI-TOF mass spectra of the EAPs.

![Figure 3–FTIR spectrum of elephant apple proanthocyanidins.](image)
recorded in the positive reflectron ion mode ([M+Na]+). Some regular mass increase was detected and recorded. A series of poly(epi) catechins extending from the trimer (m/z 889) to DP11 (m/z 3209) were observed. The observed masses agreed well with the calculated masses according to the equation 290 + 288a + 152b + 16c − 2d + 23 for proanthocyanidin molecular weights, where 290 denotes the molecular mass of the terminal (epi)catechin unit, a is the DP due to the extending (epi)catechin unit, b is the galloyl group number, c is the additional hydroxyl group number in prodelphinidins, and d is the A-type interflavan bond number while 23 is the molecular mass of sodium. Besides the homoprocyanidins mass (m/z 889, 1177, 1465, 1753, and 2041), mass signals of several peaks were 16 Da more than homoprocyanidins, suggesting the addition of one galloycatechin/epigallocatechin units. Specifically, the peak at m/z 905, 1193, 1481, 1769, 2057, 2345, 2633, 2921, and 3209 corresponded to PD trimer, tetramer, pentamer, hexamer, heptamer, octamer, nonamer, decamer, and polymer with DP11, respectively. The monomeric units, interflavan linkage and proanthocyanidin subclasses for observed molecules are listed in Table 2. Consistent with NMR and ESI MS data, the absence of A-type linkage was confirmed according to the MALDI-TOF MS signals. No obvious proanthocyanidin signal after m/z 3209 was observed, indicating that EAPs were mainly oligomeric proanthocyanidins, suggesting EAPs could be easily absorbed if in the future consumed as functional foods (Ou and Gu 2014).

Antioxidant activities

Two antioxidant assays, ORAC and FRAP, were used in the present work in order to indicate the different aspects of antioxidant efficacy of EAPs. ORAC assay aims to detect antioxidant capacity of the isolated EAPs based on HAT while FRAP assay is based on SET. The antioxidant values of EAPs were determined together with those of cGSPs, a commercial proanthocyanidins extracted from grape seed for exploring the potential commercial value of EAPs or elephant apple extracts containing EAPs.

The kinetic curves of EAPs from the ORAC assay showed a dose dependent manner with a clear lag phase compared to a Trolox standard. An excellent linear relationship was observed between the net area under the kinetic curve and the concentration of proanthocyanidins (data not shown). The ORAC value...
of EAPs was $1.06 \times 10^4 \mu\text{mol TE/g}$ and the FRAP value of EAPs was $2320 \pm 49 \mu\text{mol Fe(II)/g}$ (Table 3). cGSPs possess a FRAP value of $2312 \pm 76 \mu\text{mol Fe(II)/g}$, which was slightly lower than that of the EAPs but the difference was not significant. Besides EAPs, proanthocyanidins from many plant sources including pine barks, mangosteen pericarps, *Caryotauchlandra* Hance and sea buckthorn, are potential antioxidants (Fu and others 2007; Arimboor and Arumughan 2012; Chen and others 2014). For example, commercial pine bark proanthocyanidins gave an ORAC value of $0.75 \mu\text{mol TE/g}$ (Fu and others 2007). With a higher ORAC value than commercial proanthocyanidins from pine bark, EAPs could become a promising alternative. The present results indicated that both SET and HAT antioxidant mechanisms exist in parallel in EAPs. Therefore, it is necessary and important to take

![Figure 5](image)

Figure 5–Profiles of elephant apple proanthocyanidins. (A) Electron spray ionization (ESI) mass spectra recorded in the negative ion mode. (B) MALDI-TOF-MS spectra recorded in the [M+Na]$^+$ mode.
more than one assay into account when investigating antioxidant activities of polyphenol-type foods.

Conclusions

EAPs extracted from elephant apple fruits and purified using a Sephadex LH-20 column. The final yield of EAPs was 0.23% dry basis. The EAPs purity, structure complexity, and antioxidant activities were investigated. Bate-Smith alcoholysis, UV/vis, FT-IR, H and 13C NMR spectra demonstrated that EAPs were primarily composed of B-type PC units (catechin and epicatechin) with a mean DP of 3.8, additionally, 13C NMR spectrum indicated that the cis isomer was dominant in EAPs. Moreover, the results were substantiated by MALDI-TOF-MS and ESI-MS spectra of EAPs which also suggested the predominant presence of PC oligomers and a small amount of PD. No A-type linkage was found in EAPs. Moreover, it was found that EAPs have strong chemical antioxidant properties as evidenced by a high oxygen radical scavenging capacity of 1.06 × 10^4 μmol TE/g and ferric reducing antioxidant power of 2320 μmol Fe(II)/g. Overall, these results indicate that EAPs could be developed as a promising functional food component.

Table 2–Masses of elephant apple proanthocyanidins tested by MALDI-TOF-MS.

<table>
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<tr>
<th>m/z [M + Na]^+</th>
<th>Polymer and subclassa</th>
<th>Basic unitb</th>
<th>Interflavan bond</th>
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<tbody>
<tr>
<td>889</td>
<td>Trimer, PC</td>
<td>[(epi)cat]</td>
<td>B type</td>
</tr>
<tr>
<td>905</td>
<td>Trimer, PD</td>
<td>[(epi)cat]</td>
<td>B type</td>
</tr>
<tr>
<td>1177</td>
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<td>1481</td>
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<td>B type</td>
</tr>
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<td>Hexamer, PC</td>
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<td>B type</td>
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<td>B type</td>
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<td>2041</td>
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<td>B type</td>
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<tr>
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<td>3209</td>
<td>DP11, PD</td>
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Table 3–Antioxidant activities of elephant apple proanthocyanidins.a

<table>
<thead>
<tr>
<th>Proanthocyanidins</th>
<th>EAPs</th>
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<tr>
<td>Yield (%)</td>
<td>0.23%</td>
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<tr>
<td>mDP</td>
<td>3.8</td>
</tr>
<tr>
<td>FRAP value (μmol Fe(II)/g)</td>
<td>2320 ± 49</td>
</tr>
<tr>
<td>ORAC value (× 10^3 μmol TE/g)</td>
<td>1.06 ± 0.02</td>
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<td>Mechanism of free radical scavenging activities</td>
<td>SET, HAT</td>
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</table>

Acknowledgments

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Author Contributions

Caill Fu collected the experimental data and interpreted the results. Dongying Yang, Wan Yi Elaine Peh, and Xiao Feng collect the structural and bioactive data. Shaojuan Lai and Caill Fu drafted the manuscript. Hongshun Yang designed the study and improved the manuscript.

References


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