In vitro Antioxidant Properties of Underutilized *Baccaurea angulata* Fruit

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***Abstract*—**Six different crude extracts were obtained from the skin, pulp and whole fruit freeze-dried samples of *Baccaurea angulata*, an underutilized tropical fruit from Borneo Island of Malaysia. The effect of solvents on; total phenolic contents, total flavonoid contents, total carotene contents, free radical scavenging activities, phosphatidylcholine peroxidation inhibition activities and qualitative–quantitative compositions of ascorbic acid, carnosic, catechin, cinnamic and myricetin using using ultra high performance liquid chromatography were determined using their respective assays. The results indicated that methanol extracts contain higher TPC, TFC and TCC than PBS extracts for the various fruits' parts. Methanol extracts also showed remarkable antiradical activity. A strong correlation was also found between pulp and whole fruit. Overall, the variations in the UHPLC results among the various extracts were consistent with the results obtained for TPC, TFC, TCC, DPPH radical scavenging activity and lipid peroxidation inhibition activities of the various crude extracts, especially, methanol extracts.

***Keywords*—**Antioxidants, *Baccaurea angulata*, Lipid peroxidation, Underutilized.

I. Introduction

THE toxicity concern of synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT), which are being widely used as food additives to prevent oxidative deterioration of food products [1] has been generating endless controversies and debates among nutrionists and dieticians. Medicinal and aromatic plants, as alternatives, are known to produce a variety of antioxidants, which can militate against cellular oxidative damage by removing reactive oxygen species (ROS) and reactive nitrogen species (RNS), both in food and human body, through the delay of the oxidation process as well as inhibition of the polymerization chain reaction initiated by free radicals [2-5]. Several epidemiologic studies have also established the fact that high polyphenol intake from fruits and vegetables are associated with decreased risk for cardiovascular disease and other developing degenerative diseases [6, 7].

In addition, fruits and vegetables contain antioxidant compounds, such as flavonoids, carotenoids, and polyphenols, which have protective effects to human body [8]. These compounds help our body to balance out the ratio with free radical in many ways, such as; by scavenging free radicals, decomposing peroxides, and making complex of redox-catalytic metal ions [7].

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Baccaurea angulata, which belongs to the family Euphorbiaceae (now, Phyllanthaceae) is an underutilized tropical fruits from Borneo Island of Malaysia. Though, it is commonly consumed by the locals, the nutritional and health benefits of the fruit are yet to be thoroughly explored by researchers. Data on its nutritional benefit are not readily available because very limited studies have been conducted on this fruit, thus, In view of this, the determination of antioxidant content and antioxidant activities of this fruit are deemed necessary to fill these hiatuses. The main objectives of this study were: (i) to study the effect of extraction solvents on polyphenols and antioxidants from freeze-dried powdered fruit samples; (ii) to determine possible correlation between extracts' antioxidant activity, total phenolic, total flavonoid and total carotene contents; and (iii) to quantify ascorbic acid and polyphenols present in the various crude extracts using ultra high performance liquid chromatography (UHPLC).

II. Materials And Methods

A. Plant Materials

Fresh and ripe *Baccaurea angulata* fruits were obtained from Bau, Sarawak, Malaysia. The fruits were supplied in black perforated plastic bag, packed in boxes and sent to the Kulliyyah of Allied Health Science, International Islamic University, Kuantan, Pahang, Malaysia. The fruits were immediately preserved and stored at –30°C prior to sample preparation. Fruit samples' identity was also authenticated at Forest Research Institute of Malaysia for identification and authentication.

B. Sample Preparation

Different masses (1.5, 2.0 and 2.5 kg) of healthy fruits, with individual fruits 'weight ranging between 25–40 g, were carefully selected after being defrozen under running tap water to remove dirt. Fruits were then rinsed with distilled water, and later manually separated into whole fruit, skin, and pulps & seeds for further sample preparation procedures. The weights of the skins and pulps (with the seeds) obtained after separation were determined. The individual weights of the pulp and seed, however, were not determined because the whole pulp could not be properly separated from the seeds. The samples were then freeze-dried for a period of two weeks. The freeze-dried samples were then carefully grinded into fine powder using a laboratory blender and then kept in air tight UV-resistant amber stained Schott Duran glass bottles and stored at -80°C prior to extraction.

C. Chemicals and Solvents

All chemicals and solvents were from Sigma–Aldrich (Chemie, Steinheim, Germany), Merck (Darmstadt,

Germany) or Nacalai-Tesque (Kyoto, Japan), while all solvents were either analytical or chromatographic grade.

D. Sample Extraction

For sample extraction, earlier reported [9, 10] were employed with some modifications. In brief, three different fruits' portions; whole fruit (WF), skin (SK), and pulp (berries or edible portion) (BR) were extracted separately. Ten g equivalence of each sample was suspended in either 100 mL of 100% cold methanol or PBS (1:10) and left for 24 h in the dark inside an oven shaker set at 250 rpm and 37°C; this was followed by centrifugation at 9,000 rpm for 15 min at 4 °C. The supernatant was kept and the precipitate was then re-extracted using 100 mL of each extraction solvent and centrifuged again. The two supernatants were combined and then filtered through a Whatman No. 4 filter by vacuum suction and concentrated using a rotary evaporator (BUCHI vacuum controller V-800, rotavapor R-200, heating bath B-490) under reduced pressure at 60°C and 337 mbar for methanol extract.

The various crude extracts were either dissolved in PBS or methanol, and then kept in amber bottle and stored at −80 °C for further analysis. The various crude extracts obtained afterwards were: skin methanol extract (SKM); pulp methanol extract (BRM); whole fruit methanol extract (WFM); skin PBS extract (SKP); pulp PBS extract (BRP); and whole fruit PBS extract (WFP).

E. Estimation of Total Polyphenol Content (TPC)

The Folin–Ciocalteu reagent was used to evaluate the total phenolic content of the various extracts and standards [5]. Catechin, and quercetin were used as standards. 50 µL diluted Folin–Ciocalteu reagent (1:4 reagent: water) was placed in each well of a 96-well plate, then, 10 µL sample or standards (31.25-1000 µg/mL) was added and incubated at room temperature for 5 min. This was followed with addition of 50 µL sodium carbonate (2:3 saturated sodium carbonate: water) and a further incubation of 2 h at room temperature. The absorbance was then read at λ=725 nm against blank (solvents used for extraction) using Multiskan EX microplate reader (Thermo Fisher Scientific, Finland).

The total phenolic (TPC) was determined using various standards' calibration curves. Y=mX+c; where, Y=absorbance value at 725 nm, m=slope of the standard calibration curve, X=concentration of standard (µg/mL), c=y intercept of the curve. Results are expressed as µg standard equivalence per gram crude extracts sample.

F. Estimation of Total Flavonoid Content (TFC)

The method reported by [11] was modified to determine the total flavonoid content of the various BA fruit extracts. Briefly, 1 mL of 2% aluminium trichloride ($AlCl_3$) in methanol was mixed with the same volume (1 mL) of known concentrations of extracts. Absorption readings at 415 nm were taken after 10 min against blank samples consisting of 1 mL of the various crude extracts with 1 mL methanol only (without $AlCl_3$). All the blank values were subtracted from their respective crude extracts. The total flavonoid content (TFC) was determined using a standard calibration curve with quercetin (0.0098–5 mg/mL) as the standard. Y=mX+c; where, Y=absorbance value at 415 nm, m=slope of the quercetin standard calibration curve, X=concentration of quercetin (mg/mL), c=y intercept of the curve (r^2=0.994). Results are expressed as mg of quercetin equivalence (QE)/ g of various fruit parts crude extracts.

G. Estimation of Total Carotene Content (TCC)

The extraction of carotenoids was carried out according to a reported method [12], with slight modifications. Briefly, 10 ml of 100% methanol was added to 1 g of each sample. The mixture was stirred for 30 min inside a shaking incubator and then filtered through a Whatman 1 filter paper. One millilitre of hexane was added to equal volume of the filtered crude supernatant to extract the lipophilic components. This step was repeated a number of times until lipid compounds were completely extracted. The combined hexane layer was then washed with distilled water. Then, anhydrous sodium sulphate was added to the pooled hexane extract, after the aqueous layer was discarded. Then, a Büchi rotary evaporator was used to evaporate hexane from the extract. The remaining lipid layer was dissolved in hexane for total carotenoid contents. The absorbance for both β-carotene (BC) (0.1953-100 µg/mL) and the various extracts were initially scanned from 400 nm to 550 nm, and the λ_{max} was confirmed at 450 nm using a UV–Vis spectrophotometer.

The TCC in all the samples were estimated using BC standard calibration curve: Y=mX+c; where, Y=absorbance value at 450 nm, m=slope of the BC standard calibration curve, X=concentration of BC (µg/mL), c=y intercept of the curve (r^2=0.990). All tests were conducted in triplicate. The mean and error mean (SEM) of the three readings were used and expressed as µg of β-carotene equivalence (BCE)/ 100g of various fruit parts' crude extracts.

H. Determination of DPPH Radical Scavenging Activity (DPPH-RSA)

The assays were modified from the method of [5, 13] using 96 wells microplates. In brief, samples were analysed with DPPH as follows: 150 µL of DPPH (100 µM, final concentration) in methanol was put in the microtiter plate, then 50 µL of standards or serially diluted different concentrations of extracts (7.81–500 µg/mL) were added, while for blank, only 50 µL of extraction solvent was added to the DPPH solution. The plate was immediately placed in a spectrophotometer and their absorbance was read at 517 nm after 30 min. Catechin and quercetin were used as reference standards. The decreased absorbance of the DPPH solution at λ=517 nm indicates an increase of the DPPH radical-scavenging activity. The percentage of DPPH discolouration was used to calculate DPPH scavenging effect, using the equation:

$$\text{DPPH-RSA activity } (\%) = \left[\frac{A_{BLANK} - A_{SAMPLE}}{A_{BLANK}} \right] \times 100 \qquad (1)$$

Where A_{BLANK} is the absorbance of control reaction (containing all reagents except the extract/standard) and A_{SAMPLE} is the absorbance of solution with the sample extract or standard. The amount of antioxidant necessary to decrease the initial DPPH absorbance by 50% (EC50) was used to define the antiradical activity of various extracts. The EC50 value for each extract was graphically determined by plotting the DPPH scavenging percentage as a function of extract concentration.

I. Lipid Peroxidation Assay

The assay was modified from the method of [14-16]. Simply, a solution of soybean phosphatidylcholine (200 mg)

and cholesterol (30 mg) in chloroform (20 mL) was dried under vacuum in a rotary evaporator (<50°C) to yield a thin, homogenous film, which was placed in a desiccator for 24 h. The film was then dispersed in phosphate buffered saline (PBS) solution (pH 7.4, 20 mL) in a water bath (37°C). The mixture was sonicated to obtain a homogeneous suspension of liposome. Lipid peroxidation (LP) was initiated by adding 60 µL of AAPH (2, 2'-azobis (2-amidinopropane) hydrochloride) to 600 µL liposome and 500 µL of the various extracts, with concentrations ranging from 15.63–1000 ppm (µg/mL) and incubated at 37°C. PBS and methanol were used to substitute the BA extracts in the blank samples. The reaction mixture was incubated at 37°C for 24 h. After incubation, 250 µL of thiobarbituric acid (0.6% w/v), 100 µL of Triton X-100 (3% v/v) and 500 µL of BHT (0.4% w/v) were added to terminate the reaction. The samples was then heated at 90°C for 30 min, and then allowed to cool. The mixture was centrifuged and the absorbance of the supernatant was measured at 540 nm. The results were compared with those of blue pulp (BB), straw pulp (SB), ascorbic acid, catechin, quercetin and tocopherol standards. All tests were conducted in triplicate. The percentage inhibition of phosphatidylcholine peroxidation was defined as:

$$\text{LP inhibition (\%)} = \left[\frac{A_{BLANK} - A_{SAMPLE}}{A_{BLANK}} \right] \times 100 \qquad (2)$$

J. UHPLC–MS Analysis of Ascorbic Acid and Phenolic Compounds

The chromatographic separation was carried out using ultra high performance liquid chromatography (UHPLC), owing to its several advantages such as; shorter run time, better resolution, higher sensitivity, and ability to be coupled to MS for routine analysis, thus allowing a rapid detection of a high number of compounds [17]. In brief, AB Sciex 3200QTrap Liquid Chromatography tandem Mass Spectrometer (LCMS/MS) (AB Sciex, Toronto, Canada) coupled to Perkin Elmer Flexar FX15 ultra high performance liquid chromatography (UHPLC) system (Massachusetts, USA), operated using electrospray (ESI) ionisation in negative ion mode, with AB Sciex Analyst software for data acquisition and equipped with a binary pump, with column oven and an auto sampler with a 20 µL loop was used to analyse various methanol extracts. The standards (ascorbic acid, carnosoic, catechin, cinnamic and myricetin) and samples were separated on a reverse-phase Zorbax C18, 150mm X 2.1mm, 5µm (Agilent, USA) with A (water with 0.1% formic acid and 5mM ammonium formate) and B (acetonitrile with 0.1% formic acid and 5mM ammonium formate), flow rate interchanging from 800uL/min to 1mL/min, temperature was set at 40 °C, rapid screening at 15min run time, linear gradients from 10% B to 90% B from 0.01min to 8min, hold 90% B for 2min were applied, followed by a return to the initial conditions (10% B) in 5 min and re-equilibration of the column. The identification of ascorbic acid and phenolic compounds in the various crude extracts was obtained by using the full mass spectrum and its unique mass fragmentation spectrum (a unique fingerprint for a particular compound). Reference standards were also used and compared to the retention times of the extracts for further confirmation and identification.

III. Statistical Analysis

Statistical Package for Social Sciences (SPSS, version 20.0, IBM) for Windows software was used for the statistical analysis. Multiple comparisons between the various groups were performed using one-way analysis of variance (ANOVA) with Tukey's honestly significant different (HSD) post hoc test. Significance was accepted at ($p<0.05$ and $p<0.01$). The same statistical package was also used to carry out the regression analysis between various dependent variables using Pearson correlation. All the results were expressed as mean values ± standard error mean (SEM) of the means.

IV. Results And Discussion

The earliest and most relevant step in optimizing the recovery of antioxidant compounds from a sample is the selection of an appropriate solvent system [13]. Fresh and ripe samples of BA fruits were used in this study for accurate quantification and to retain their original edible quality. The skin makes up the largest percentage of the fruit (69.28±0.95%) (Fig.1).

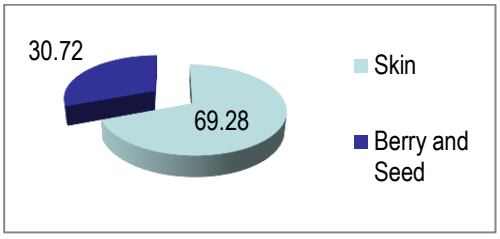

Fig. 1 Composition of *Baccaurea angulata* fruit. Values are means of triplicate analyses

Total phenolic, total flavonoid and total carotene contents

Polyphenols are important constituents of human diet. They are widely distributed in plants as a large heterogeneous group of secondary plant metabolites [18]. The list of the total phenolic content (TPC), total flavonoid content (TFC) and total carotene content (TCC) of the different crude extracts from the various morphological parts of BA fruit is shown in Table I.

TPC ranged from 87.82±1.57 (for WFP) to 11308.59±12.54 (for BRM) µg CAT/ g crude extracts sample, using the standard curve of catechin (r^2=0.997). While the phenolic content of all the methanolic extracts were significantly higher than those of PBS extracts by 50-129 folds, significant differences were also found between BRM phenolic contents and those of SKM and WFM. The values of TPC in increasing order were: WFM (4407.46±125.51) <SKM (4444.23±133.66) <BRM (11308.59±12.54) µg CAT / g crude extracts sample, with BRM having 2.5 to 2.6 folds TPC than SKM and WFM respectively.

Similarly, for TFC, all the methanolic extracts were significantly higher than those of PBS extracts, though by 3.2–6.4 folds, there were no significant differences in the TFC values for all the PBS extracts, albeit significant differences found among the methanolic extracts, SKM (18.36±0.72) <WFM (28.23±0.84) <BRM (37.32±0.55) mg QE/ g crude extracts sample. Furthermore, TFC values for all the crude extracts ranging from 5.82±0.18 (for BRP) to 37.32±0.55 (for BRM) mg QE/ g crude extracts sample were found to be higher than TPC, showing that flavonoids are

more largely distributed in the matrix composition of the various fruit's parts in comparison with the overall phenolics.

For TCC estimation, like TPC and TFC, the results of the total carotenoid contents also varied widely. TCC of all the methanol extracts were significantly higher than those of PBS extracts. While there were significant differences ($p<0.01$) in the mean values of TCC among the methanolic extracts, SKM (545.65±19.91) <WFM (1175.35±13.84) <BRM (6571.43±185.86) µgBC/100g crude extracts sample.

Interestingly, however, TCC of all sample were found to be less than TPC and TFC. This means that carotenoids are more restricted in their distribution and composition in the various fruit's parts in comparison with the overall phenolics. In general, methanolic extracts were significantly ($p<0.01$) found to contain higher TPC, TFC and TCC than PBS extracts for all the fruits parts. Thus, methanol is considered as the most efficient solvent system for extracting polyphenols and carotenoids from the fresh matrixes of these highly nutritive fruit. The strong variation of TPC, TFC and TCC in the two different extracts of each of the morphological parts showed the effect of extraction solvents in changing the analytical estimation of polyphenols and carotenoids from the same fruit sample matrix [13]. There were very strong correlations between TPC and TFC (R^2=0.937**), TCC (R^2=0.957**), DPPH radical scavenging activity (R^2=0.723**), as well as percentage linoleic acid peroxidation inhibition (R^2=0.768**)

DPPH radical scavenging activity

The 2,2-diphenyl-1-picrylhydrazyl stable radical (DPPH*) has its maximum absorption at λ=517 nm, which decreases when the stable radical is reduced to the hydrazine derivative by an antioxidant via either electron or hydrogen atom transfer reactions[19]. The statistical analysis of DPPH radical scavenging activity obtained from the antioxidant activity of different extracts' concentrations (7.81-125 µg/mL) and reference standards (catechin and quercetin) are shown in Table II. Similarly, the EC50 values were obtained for each of the samples and standards. The DPPH radical scavenging activity of SKM (96.80±0.53%) was the highest, while BRP (8.90±0.12%) has the lowest radical scavenging activity at125 µg/mL. Additionally, all the methanol extracts showed better and more significant radical scavenging activities ($p<0.01$) than PBS extracts, with weak activities. Similarly, BRM radical scavenging activity (82.00±1.36%) was found to be comparable to that of quercetin (81.30±2.17%) reference standard at 125 µg/mL, while WFM, with the least radical scavenging activity (78.80±2.19%), at 125 µg/mL, among all the methanol extracts, gave higher radical scavenging activity than catechin (68.50±0.35%) reference standard. The results also showed that SKM and BRP, with the highest and lowest radical scavenging activities, have the lowest (49.52±0.29 µg/mL) and highest (960.22±4.84 µg/mL) EC50 values, respectively. This is in line with the fact that a lower value of EC50 indicates a higher antioxidant activity and vice versa [18]. The remarkable antiradical activity of all the methanol extracts could be attributed to the efficiency of methanol in extracting phenolic compounds and carotenoids, which in turn, are known to be responsible for exerting a profound effect on antiradical activity through their potent hydrogen-donor ability [18, 20]. The results also indicated a positive correlation between DPPH radical scavenging activity and TPC (R^2=0.723, $p<0.01$), TFC (R^2=0.825, $p<0.01$) and TCC (R^2=0.495, $p<0.05$).

Lipid (phosphatidylcholine) peroxidation assay

The role of lipid peroxidation in food deterioration has led to the growing interest in the study of antioxidants. Thus, antioxidants can be added to foods and food products, basically, to extend their shelf lives, minimize rancidity, retard the formation of toxic oxidation products, halt the production of off-odors and off-tastes and maintain nutritional quality [21, 22]. Therefore, the assessment of dietary components as potential antioxidants, using phospholipids, is a genuine and useful model substrate [19]. The result of lipid peroxidation inhibition is shown in Table III. For each of the crude extracts' samples and controls, the IC50 values for inhibition of peroxidation of phosphatidylcholine, estimated as thiobarbituric acid reactive substances were also obtained. At 250 µg/mL, BB has the highest lipid peroxidation inhibition activity (79.69±0.65%), which was comparable to those of the methanol extracts; WFM (78.48±0.85%), BRM (77.85±0.80%) and SKM (77.84±0.80). All the PBS extracts only showed weak lipid peroxidation inhibition activities, with BRP (29.48±0.80) having the lowest lipid peroxidation inhibition activity. WFM has the lowest IC50 value (19.60±0.34 µg/mL), this is significantly different ($p<0.01$) from those of BRM (33.67±0.86 µg/mL) and SKM (61.51±1.01 µg/mL), SKM (29.23 µg/mL), while BRP (579.19±3.44 µg/mL) has the highest and highly significant ($p<0.01$) IC50 value, in spite of its lowest lipid peroxidation inhibition activity. A strong correlation was also found between lipid peroxidation inhibition activities of the extracts and TPC (R^2=0.811 at $p<0.01$) as well as between lipid peroxidation inhibition activities and TFC (R^2=0.943 at $p<0.01$), while the correlation between lipid peroxidation inhibition activities and TCC (R^2=0.632 at $p<0.01$) was also moderate.

UHPLC–MS analysis of ascorbic acid and phenolic compounds

The multiple reactions monitoring (MRM) is a highly selective and sensitive LCMS/MS analytical mode for specific compound qualification and quantification. Parent compounds were targeted and then fragmented to its unique fragments in the instrument, while further confirmation was based on retention time as compared with reference standards. Ascorbic acid, carnosic, catechin, cinnamic and myricetin were all investigated and quantitatively identified in all the morphological BA fruit parts. Table IV shows the results of the quantitative analysis (mg) of each identified phenolic compound per gram of various fruit parts' crude extracts. The amount of identified phenolic compounds varied widely in different crude extracts, with catechin being the most abundant (2280.20±4.82, 4792.82±4.84 and 5163.14±8.11 mg/g) and myricetin, the least abundant (210.04±3.21, 497.39±3.87 and 273.40±2.54 mg/g) in skin (SK), pulp (BR) and whole fruit (WF) respectively. The results also showed that WF contains the least concentrations of carnosic and cinnamic, and the highest concentrations of both ascorbic acid and catechin. The concentration of cinnamic was, however, observed to sharply reduce in WF (143.31±3.75 mg/g), despite their high concentrations in both SK (426.81±2.13 mg/g) and BR

(745.30±2.77 mg/g), a negative (interference) or antagonistic effect between SK and BR could have been responsible for this [23].

TABLE I
TOTAL PHENOLIC, TOTAL FLAVONOID AND TOTAL CAROTENE CONTENTS OF THE VARIOUS EXTRACTS

Extracts	TPC (µgCAT/g)	TPC (µgQE/g)	TFC (mgQE/g)	TCC(µgBC/100g)
SKM	4444.23±133.66^{B}	4089.52±53.48^{B}	18.36±0.72^{B}	545.65±19.91^{A}
BRM	11308.59±12.54^{C}	12852.80±38.25^{C}	37.32±0.55^{D}	6571.43±185.86^{C}
WFM	4407.46±125.51^{B}	4097.13±102.30^{B}	28.23±0.84^{C}	1175.35±13.84^{B}
SKP	96.47±7.07^{A}	54.25±2.36^{A}	7.24±0.18^{A}	230.73±1.95^{A}
BRP	94.99±3.06^{A}	55.10±2.18^{A}	5.82±0.18^{A}	183.54±4.70^{A}
WFP	87.82±1.57^{A}	71.85±3.90^{A}	6.37±0.30^{A}	207.18±1.51^{A}

Values are mean ±standard error mean (SEM) of mean of triplicate analyses. The results of all the crude extracts were analysed using one-way ANOVA. Values of the various crude extracts with the same letter (A, B, C, etc.) in the same column are not significantly different (both at $p < 0.05$ and $p<0.01$), as measured by Tukey's HSD Test. **Correlation is significant at the 0.01 level (2-tailed)

TABLE II
DPPH RADICAL SCAVENGING ACTIVITY OF VARIOUS EXTRACTS AND STANDARDS

Extracts	% Inhibition at 125 µg/mL	% Inhibition at 62.5 µg/mL	% Inhibition at 31.25 µg/mL	% Inhibition at 15.63 µg/mL	% Inhibition at 7.81 µg/mL	EC 50 (µg/mL)
SKM	96.80±0.53^{D}	63.30±0.40^{G}	45.20±0.23^{F}	29.60±0.36^{D}	11.50±0.29^{B}	49.52±0.29^{A*}
BRM	82.00±1.36^{C}	55.87±0.58^{F}	38.60±0.35^{E}	20.80±0.40^{BC*}	12.10±0.87^{B}	62.56±0.68^{B*}
WFM	78.80±2.19^{C}	45.10±0.81^{DE}	38.20±0.46^{E}	22.40±1.46^{C*}	10.50±0.55^{B}	69.04±0.61^{BC}
SKP	14.30±0.29^{A}	12.70±0.29^{C}	9.30±0.17^{B*}	6.30±0.17^{A}	5.10±0.12^{A}	573.20±1.91^{D}
BRP	8.90±0.12^{A}	5.10±0.12^{A}	4.60±0.06^{A*}	3.40±0.06^{A}	2.80±0.06^{A}	960.22±4.84^{F}
WFP	11.90±0.40^{A}	9.20±0.52^{B}	7.50±0.35^{AB*}	6.40±0.21^{A}	4.30±0.23^{A}	775.02±2.69^{E}
CAT	68.50±0.35^{B}	43.90±0.69^{D}	33.80±2.19^{D}	21.40±1.04^{BC*}	10.60±0.87^{B}	79.89±3.07^{C}
QUE	81.30±2.17^{C}	47.50±0.35^{E}	28.10±0.87^{C}	18.50±0.35^{B*}	10.10±1.10^{B}	69.33±3.41^{BC}

Values are mean ±standard error mean (SEM) of mean of triplicate analyses. The results of all the crude extracts and standards were analysed using one-way ANOVA. Values of the various samples with the same letter (A, B, C, etc.) in the same column are not significantly different (both at $p < 0.05$ and $p<0.01$), while values with different letters and (*), in the same column are not significantly different ($p<0.01$) as measured by as measured by Tukey's HSD Test.

TABLE III
PHOSPHATIDYLCHOLINE PEROXIDATION INHIBITION ACTIVITY OF DIFFERENT EXTRACTS AND STANDARDS

Extracts	% Inhibition at 250 µg/mL	% Inhibition at 125 µg/mL	% Inhibition at 62.5 µg/mL	% Inhibition at 31.25 µg/mL	% Inhibition at 15.63 µg/mL	IC 50 (µg/mL)
BB	79.69±0.65^{D*}	68.17±0.79^{FG*}	65.71±0.69^{D}	52.08±0.57^{C*}	41.41±0.98^{C}	15.24±0.62^{A}
SB	75.08±1.17^{C}	65.71±0.69^{FG*}	59.71±1.78^{BCD}	47.72±0.80^{B}	39.68±0.24^{C}	41.09±1.16^{BC*}
SKM	77.84±0.80^{CD*}	54.37±0.50^{C}	52.89±1.36^{B*}	49.74±0.27^{BC*}	40.10±1.27^{C}	61.51±1.01^{E*}
BRM	77.85±0.80^{CD*}	64.66±0.38^{EF*}	61.11±0.09^{CD}	49.92±0.18^{BC*}	40.55±0.89^{C}	33.67±0.86^{B}
WFM	78.48±0.85^{CD*}	65.30±1.08^{FG*}	59.69±1.15^{BC}	56.39±0.28^{D}	40.93±0.36^{C}	19.60±0.34^{A}
SKP	34.77±0.19^{B}	27.74±1.01^{A}	25.13±1.02^{A}	20.99±0.91^{A}	19.02±0.62^{B}	480.39±6.19^{G}
BRP	29.48±0.80^{A}	26.27±0.92^{A}	24.10±0.58^{A}	20.31±0.74^{A}	12.61±0.28^{A}	579.19±3.44^{H}
WFP	36.28±0.53^{B}	32.71±0.22^{B}	28.51±0.34^{A}	20.87±0.71^{A}	19.19±0.53^{B}	415.47±2.12^{F}
ASC	75.70±0.69^{C*}	61.30±0.69^{DE}	58.13±0.12^{BC*}	47.20±0.21^{B}	40.15±0.84^{C}	49.47±0.74^{CD*}
CAT	79.50±0.35^{D*}	68.50±0.64^{G*}	53.90±0.21^{B*}	48.80±1.10^{B*}	38.70±0.82^{C}	48.36±1.06^{CD*}
QUE	76.90±0.12^{CD*}	61.30±0.69^{DE}	57.50±0.52^{B*}	48.10±0.12^{B}	40.01±0.75^{C}	48.89±0.99^{CD*}
TOC	76.65±0.27^{CD*}	60.67±0.48^{D}	51.58±0.66^{B}	49.84±0.44^{BC*}	39.57±0.77^{C}	57.21±1.69^{DE*}

Values are mean ±standard error mean (SEM) of mean of triplicate analyses. The results of all the crude extracts and reference standards were analysed using one-way ANOVA. Values of the various samples with the same letter (A, B, C, etc.) in the same column are not significantly different (both at $p < 0.05$ and $p<0.01$), while values with different letters and (*), in the same column are not significantly different ($p<0.01$) as measured by Tukey's HSD Test.

TABLE IV
QUANTITATIVE ANALYSIS OF PHENOLIC CONTENT (MG/G CRUDE EXTRACT) OF SKIN (SK), PULP (BR) AND WHOLE FRUIT (WF) OF *BACCAUREA ANGULATA*

Standards	Retention Time (mins)	MRM (Da)	SK (mg/g)	BR (mg/g)	WF (mg/g)
Ascorbic	1.87	175.0/87.0	370.66± 2.39^{B}	3806.71±6.03^{D}	4491.82±4.79^{D}
Carnosic	10.05	331.0/287.0	443.66±3.14^{D*}	718.32±4.10^{B}	388.81±3.08^{C}
Catechin	3.66	288.9/109.1	2280.20±4.82^{E}	4792.82±4.84^{E}	5163.14±8.11^{E}
Cinnamic	6.32	147.0/103.0	426.81±2.13^{C*}	876.54±4.41^{C}	143.31±3.75^{A}
Myricetin	5.21	317.0/179.0	210.04±3.21^{A}	497.39±3.87^{A}	273.40±2.54^{B}

Values are mean ±standard error mean (SEM) of mean of triplicate analyses. The results of all the crude extracts and reference standards were analysed using one-way ANOVA. Values of the various samples with the same letter (A, B, C, etc.) in the same column are not significantly different (both at $p < 0.05$ and $p<0.01$), while values with different letter and (*), in the same column are not significantly different ($p<0.01$) as measured by Tukey's HSD Test.

There was a strong correlation between BR and WF (R^2=0.989 at p<0.01), and a moderate correlation between SK and WF (R^2=0.697 at p<0.01), as well as SK and BR (R^2=0.735 at p<0.01). In general, the variations in the UHPLC results among and within the various extracts were consistent with the results obtained for TPC, TFC, TCC, DPPH radical scavenging activity and lipid peroxidation inhibition activities of the various crude extracts. The results are also in line with the earlier and preliminary reports on the fruit [24-27].

V. Conclusions

The differences between methanol and PBS in extracting phenolic compounds and other antioxidants from the skin, pulp and whole fruit samples of the plant were investigated. Overall, methanol is considered as a better alternative and a more efficient solvent system than PBS. There were also very strong correlations between TPC and TFC, TCC, DPPH radical scavenging activity, as well as percentage lipid peroxidation inhibition. There was a strong correlation between lipid peroxidation inhibition activities of the extracts and TPC, as well as between lipid peroxidation inhibition activities and TFC. Ascorbic acid, carnosic, catechin, cinnamic and myricetin were all quantitatively identified in all the morphological BA fruit parts. The results also showed that WF contains the least concentrations of carnosic and cinnamic, and the highest concentrations of both ascorbic acid and catechin. A strong correlation was also found between pulp and whole fruit, while a moderate correlation exists between skin and whole fruit, as well as skin and pulp. Thus, the variations in the UHPLC results among the various extracts were consistent with the results obtained for TPC, TFC, TCC, DPPH radical scavenging and lipid peroxidation inhibition activities; this is also in line with earlier reports and thus suggests different kinds of positive and negative interactions between various fruits 'parts.

Acknowledgment

This work was supported by Ministry of Science, Technology and Innovation (MOSTI), under e-Science, Project No. 06-01-08-SF0111 & Ministry of Science, Technology and Innovation (e-Science), Project No. 06-01-08-SF0112. We would also like to appreciate the technical supports provided by the Kulliyyah of Allied Health Sciences and Integrated Centre for Research Animal Care and Use, IIUM.

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