

## Full Length Research Paper

## Physicochemical and antioxidant characteristics of *Baccaurea angulata* fruit juice extract

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The purpose of this study was to explore the physicochemical and antioxidant characteristics of *Baccaurea angulata* fruit juice extract. Freeze-dried whole fruit (FDWF), freeze-dried berry (FDB), and freeze-dried skin (FDS) of *B. angulata* were analyzed for total phenolic, total flavonoid, total anthocyanin, and antioxidant activities. FDS recorded the highest moisture and ash content, protein, total fat, and water activity, compared to FDWF and FDB. FDS also contained the highest total phenolic, total flavonoid, and total anthocyanin, while FDWF recorded the highest in scavenging xanthine oxidase (35.9%) and ferric reducing activity (44.9  $\mu\text{M TE/g}$ ). FDS, however, showed the highest DPPH (102.66 mg AA/100 g) and TEAC (847.46 mg TE/100 g) values. DPPH, TEAC and FRAP were strongly correlated with total phenol ( $r = 0.979; 0.948; 0.997$ ) and total flavonoid ( $r = 0.987; 0.960; 0.992$ ). Total anthocyanin had no correlation with DPPH and TEAC, but moderately with FRAP ( $r = 0.734$ ). Physicochemical and antioxidant characteristics of *B. angulata* may indicate that this fruit may impart health benefits when consumed and can be suggested as a good source for nutraceutical beverages.

**Key words:** Antioxidant properties, *Baccaurea angulata*, freeze-dried fruit, nutritional composition, physicochemical.

### INTRODUCTION

Reactive oxygen species (ROS) is as important as antioxidant to human health. However, due to imbalance of free radicals and antioxidant in our body, development of chronic diseases such as hyperlipidemia, cancer, diabe-

tes, and many others may occur (Liu et al., 2010). There are many conventional therapies and pharmacological drugs available to combat these diseases. Despite this, the treatment is quite expensive; taking synthetic drugs in

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**Abbreviations:** ROS, Reactive oxygen species; XOD, xanthine oxidase dismutase; SOD, superoxide scavenging assay; NBT, 4-nitroblue tetrazolium chloride; FDWF, freeze-dried whole fruit; FDB, freeze-dried berry; FDS, freeze-dried skin; DPPH, 1,1-Diphenyl, 2-picryl hydrazyl; FRAP, ferric reducing /antioxidant power; TPC, total phenolic content; AEAC, ascorbic acid equivalent capacities.

a long run may also impose a threat to the human's health. Therefore, medications, or any products derived from natural sources such as plants and fruits have a great demand from the public (Leong and Shui, 2002).

Epidemiological studies showed that there is an inverse association between fruit and vegetable consumption and the risk of developing degenerative diseases (Oliveira et al., 2009). This is because fruits and vegetables contain antioxidant compounds, such as flavonoids, carotenoid, and polyphenols, which have protective effects to human body (Ashraf et al., 2010). These compounds help our body to balance out the ratio with free radical by scavenging free radicals, decomposing peroxides, and making complex of redox-catalytic metal ions (Zibadi et al., 2007).

Malaysia has plenty of nutritious fruits and medicinal plants due to constant exposure to 6-8 h of daily sunlight throughout the year. Therefore, tropical fruits in Malaysia are rich in antioxidants. Interestingly, there are some fruits which are commonly consumed by the locals but yet to be discovered by researchers in terms of nutritional as well as health benefits. *Baccaurea angulata*, locally known as 'belimbing dayak' or 'belimbing hutan', is one of the underutilized fruits from Borneo island of Malaysia. *B. angulata* belongs to the *Euphorbiaceae* family. The tree is about 8-10 m tall. It has thick and broad leaves, and the leaves are ellipse in shape. This fruit is seasonal, and the fruit grows on stems and branches of the tree (Rukayah, 2002). The review of literature showed that very limited studies have been conducted using this fruit in terms of physical and chemical characteristics. In view of this, the determination of antioxidant content and antioxidant activities of this fruit is carried out in this work.

## MATERIALS AND METHODS

### Sample preparation

Fresh *B. angulata* fruits were collected from Bau, Sarawak, Malaysia. These fruits were separated into three portions, whole fruit, fruit skin, and berries (edible portion). The fruits were rinsed with distilled water, sliced, and blended with ratio of 1:1 (v/v) of edible portion to water. For berries, they were blended with ratio of 1:3 (v/v) of edible portion to water. These juices were filtered and transferred into separated containers. The samples were freeze-dried at -52°C, 0.63 mbar (Freezone 18 Liter Console Freeze Dry System, Labconco, USA). Freeze-dried powder retrieved was stored in an air-tight container at 4°C for further analysis.

### Sample extraction of freeze-dried *Baccaurea angulata*

Extraction of powdered sample was carried according to a method by Velioglu et al. (1998) with slight modification. About 200 mg of sample was extracted with 2 mL of methanol at room temperature for 2 h with continuous stirring. Then, the mixture was centrifuged at 1000 g for 15 min. The supernatant was collected and kept at -20°C before the determination of total phenolic content, total flavonoid, total anthocyanin, and antioxidant capacity study.

### Physical characteristics of *B. angulata* fruits

The fruit was randomly selected for physical measurement which includes average fruit, skin, and berry weight (g), fruit and berry length (cm), fruit and berry width (cm). Fruit weight was measured using calibrated weighing scale. The length and width of the fruit were measured using vernier caliper. All the measurements were done in triplicate and the average of the reading was reported.

### Nutritional composition of *B. angulata* freeze-dried extract

Analysis of the moisture, ash, protein, total fat, crude fiber, dietary fiber, and carbohydrate contents were carried out according to the Association of Official Analytical Chemist (AOAC) (2003) method. Gross energy was calculated according to FAO/WHO/UNU (1981) method. Water activity was measured using AquaLab CX-2 water activity equipment.

### Determination total phenolic content

Total phenolic content was determined according to the method of Lim et al. (2006) with slight modifications. About 1 mL of extract was mixed with 5 mL of Folin-Ciocalteu reagent and allowed to stand at room temperature for 5 min. 4 mL of 7.5% sodium carbonate solution was then added into the mixture. The solution was incubated for 40 min. The absorbance was recorded at 765 nm. Calibration curve was constructed using different concentration of gallic acid range between 0.001 to 0.02 mg/mL. Results were expressed as mg gallic acid equivalent in 1 g of dried sample (mg GAE/g).

### Determination of total flavonoid content

Total flavonoid content was determined by colorimetric method as described by Abu Bakar et al. (2009). Briefly, 0.5 mL of extract was mixed with 2.25 mL of distilled water and 0.15 mL of 5% NaNO<sub>2</sub> solution. The mixture was allowed to stand for 6 min before 0.3 mL of 10% AlCl<sub>3</sub>.H<sub>2</sub>O solution was added and incubated for another 5 min. 1 mL of NaOH was then added into the mixture, vortexed, and the absorbance was immediately measured at 510 nm. A linear standard calibration curve was constructed using quercetin range from 0.01- 0.1 mg/mL. Results were expressed as mg quercetin equivalents per gram of dried sample (mg QE/g).

### Determination of total anthocyanin content

Determination of total anthocyanin content was based on pH differential method according to the modified method by Lee et al. (2005) and Abu Bakar et al. (2009). About 3.5 mL of 0.025 M potassium chloride buffer pH 1.0 was added to 0.5 mL of the sample extract and mixed thoroughly using vortex. The mixture was allowed to stand for 15 min before the absorbance was read at 515 and 700 nm. The extract was then treated similarly with 0.4 M sodium acetate buffer pH 4.5 and the absorbance was measured at the same wavelengths. The total anthocyanin concentration was calculated using the following equation and expressed as mg of cyanidine-3-glucoside equivalent in 100 g of dried sample (mg c-3-gE/ 100g dried sample).

$$\text{Total anthocyanin concentration} = \frac{A \times MW \times DF \times 103}{\epsilon \times 1}$$

**Table 1.** Physical characteristics of whole fruit, berries, and skin of *Baccaurea angulata*.

Physical characteristic	Parameter
<b>Whole fruit</b>	
Weight (g)	22.69±9.05
Length (cm)	4.61±0.59
Circumference (cm)	11.12±1.61
Width (cm)	3.02±0.52
pH	3.34±0.03
<b>Whole berry</b>	
Weight (g)	7.73±4.55
pH	4.05±0.53
<b>Skin</b>	
Weight (g)	15.95±7.03
pH	3.13±0.07

Values were the means standard deviations of three replicates analysis.

Where,  $A = (A_{515 \text{ nm}} - A_{700 \text{ nm}})$  pH 1.0 –  $(A_{515 \text{ nm}} - A_{700 \text{ nm}})$  pH 4.5; molecular weight (MW) = 449.2 g/mol for cyanidin-3-glucoside (cyd-3-glu); DF = dilution factor of samples;  $l$  = path length in cm;  $\epsilon = 26,900$  molar extinction coefficient, in  $L \times \text{mol}^{-1} \times \text{cm}^{-1}$  for cyd-3-glu; 103 = factor for conversion from g to mg.

#### Determination of antioxidant capacity

##### Xanthine oxidase dismutase (XOD)/superoxide scavenging assay (SOD)

Xanthine oxidase dismutase (XOD) assay was conducted according to the method of Vimala et al. (2003) with slight modification. About 4.1 mM/l 4-nitroblue tetrazolium chloride (NBT) solution was prepared by adding 3.15 g TrisHCl, 0.1 g  $\text{MgCl}_2$ , 15.0 mg 5-bromo-4-chloro-3-indolyl phosphate and 34.0 mg NBT to 100 mL of distilled water. Reaction mixture was prepared by dissolving 0.53 g of  $\text{Na}_2\text{CO}_3$  (pH10.2), 4.0 mg ethylenediaminetetra acetate (EDTA), and 5.0 mg xanthine before the addition of 10 mL of NBT solution prior to use and kept at 4°C. 5  $\mu\text{L}$  of the sample extract, 995  $\mu\text{L}$  of the reaction mixture and 0.1  $\mu\text{L}$  of XO were added into a cuvette, mixed thoroughly and the absorbance was taken at 560 nm for 120 s. Absorbance of 1000  $\mu\text{L}$  of the reaction mixture and 0.1  $\mu\text{L}$  XO of the same spectrophotometer condition was considered as control.

##### 1,1-Diphenyl, 2-picryl hydrazyl (DPPH) free radical scavenging assay

Radical scavenging activity of Freeze-dried whole fruit (FDWF), freeze-dried berry (FDB), and freeze-dried skin (FDS) was determined according to the method described by Molyneux (2004) with slight modification. About 1 mL of sample was mixed with 2 mL of DPPH solution. The mixture was shaken vigorously and left to stand in a dark room for 30 min. After incubation, the absorbance of the mixture was measured using UV/Vis spectrophotometer at 517 nm. A linear standard calibration curve was constructed using ascorbic acid range from 0.001 - 0.007 mg/mL ( $r^2 = 0.996$ ). Results were expressed as mg ascorbic acid equivalents per 100 g sample (mg

AA/100 g).

##### Trolox-equivalent antioxidant capacity (TEAC)/2,2-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid (ABTS) assay

The improved method adopted for ABTS radical scavenging activity was described by Re et al. (1999). The stock solution was prepared which include 7 mM of ABTS and 2.45 mM of potassium persulfate. To prepare the working solution, both solutions were mixed in equal quantities and left to stand in a dark room for 12-16 h. Then, about 1 mL from the working solution was diluted with 60 mL methanol to obtain an absorbance of  $\pm 0.70$  at 734 nm using spectrophotometer. For every assay, fresh  $\text{ABTS}^{\cdot+}$  solution was prepared. About 150  $\mu\text{L}$  of the methanol extract sample was mixed with 2850  $\mu\text{L}$  of  $\text{ABTS}^{\cdot+}$  and left to react for 6 min. A linear standard calibration curve was constructed using ascorbic acid range from 0.5 – 18  $\mu\text{M}/\text{mL}$  ( $r^2 = 0.998$ ). Results were expressed as mg trolox equivalents per 100 g sample (mg TE/100g).

##### Ferric reducing /antioxidant power (FRAP) assay

FRAP assay was conducted based on Benzie and Strain (1996) with slight modification. The fresh working solution of FRAP reagent was prepared by mixing 300 mM acetate buffer pH 3.6, 10 mM 2,4,6-tripyridyl-s-triazine (TPTZ), and 10 mM  $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$  in a 10:1:1 ratio and warmed to 37°C in water bath prior to use. Briefly, 3 mL of FRAP reagent was taken into a cuvette and a blank reading was measured at 593 nm and considered as blank reading. Then, 100  $\mu\text{L}$  of the sample extract and 300  $\mu\text{L}$  of distilled water were added into the cuvette and the second reading was performed after 4 min incubation in dark condition at the same wavelength. The change in absorbance was compared with a standard curve. The standard curve was linear between 0.002 to 0.2  $\mu\text{M}$  trolox ( $r^2 = 0.993$ ). Results were expressed in micromolar trolox equivalent per gram of dried sample ( $\mu\text{M TE/g}$  dried sample).

## RESULTS AND DISCUSSION

### Physical characteristics of *B. angulata*

Physical characteristics of *B. angulata* which include weight of the whole fruit, berry, and skin, the length and perimeter of whole fruit are shown in Table 1. The shape of this fruit is like star fruit (*Averrhoa carambola*), from which the name “belimbing” was derived. The ripe fruit is bright red in colour. It is round at the base of the fruit and pointed at the end. Each fruit contains 1 – 4 cloves of berry which are white in colour and sticky.

The average length and width of *B. angulata* was 4.61±0.59 cm and 3.02±0.52 cm compared to diameter of *B. ramiflora* (Burmese grape) which was 2.5 – 3 cm (Singh, 2007). About 70.3% of whole fruit weight of *B. angulata* was contributed by fruit skin. The edible portion was about 34.1% fraction of fruit weight which is lesser than *B. ramiflora* by 12% (Haque et al., 2009). Furthermore, the average weight of berry of *B. angulata* was 7.73±4.55 g which is heavier when compared to *B. ramiflora* berry (2 g) (Singh, 2007). pH of edible portion of *B. ramiflora* was more acidic (pH 2.4) than *B. angulata* (pH 4.05) (Haque et al, 2009).

**Table 2.** Nutritional and chemical composition of FDWF, FDB, FDS.

Nutrient composition	FDWF	FDB	FDS
Moisture (%)	19.63±0.04	20.71±0.54	21.04±0.20
Ash (%)	4.74±0.25	5.04±0.05	10.04±0.97
Protein (%)	1.33±0.05	1.28±0.16	2.12±0.21
Total fat (%)	0.11±0.03	1.73±1.76	2.09±1.89
Carbohydrate (%)	74.12±0.00	70.85±0.00	64.58±0.00
Crude fiber (%)	0.07±0.06	0.36±0.21	0.13±0.11
Gross energy (kcal/100g)	302.79±0.00	304.09±0.00	285.61±0.00
Total dietary fiber (% w/w)	6.3	3.6	6.3
Water activity ( $W_A$ )	0.210	0.384	0.467

Values were the means standard deviations of three replicates analysis.

**Table 3.** Antioxidant content and antioxidant capacities of FDWF, FDB, and FDS.

Sample	TPC (mgGAE/g)	TFC (mg QE/g)	TAC (mgc-3-g/100 g)	XOD (%)	DPPH (mgAA/100g)	TEAC (mg TE/100g)	FRAP ( $\mu$ M TE/g)
FDWF	7.91±0.05	12.74±0.24	0.42±0.28	35.9±1.95	60.96±4.24	412.39± 33.33	44.91±0.73
FDB	4.78 ±0.04	7.86 ±0.10	0.25±0.02	22.9± 2.90	56.31± .32	406.83± 75.93	15.54±0.49
FDS	16.58±0.05	31.05±0.52	0.72±0.13	20.6± 5.35	102.66± 2.12	847.46± 10.57	112.88±3.86

Values are presented in mean ± standard deviations of three replicates analyses of freeze-dried samples.

### Nutritional and chemical composition of *B. angulata*

Nutritional and chemical compositions of freeze-dried samples are shown in Table 2. From the results, almost 20% of freeze-dried powder from all samples contained moisture. Furthermore, water activity ( $a_w$ ) recorded for these samples ranges from 0.2 to 0.5. Moisture and water activity are essential properties as they can determine the shelf life of food (Ruzainah et al, 2009). Presence of high moisture content can facilitate microbial growth in food products. However, in food with water activity that ranges from 0.2 to 0.7  $a_w$ , microbial proliferation will not be observed (Fontana and Campbell, 2004).

Percentage of ash represents the mineral content that is present in the sample. Therefore, FDS recorded the highest ash content (10.04 %), protein content (2.12 %) and total fat (2.09%) compared to FDB (5.04%; 1.28%; 1.73%) and FDWF (4.74%; 1.33%; 0.11%), respectively. Furthermore, FDB has higher crude fiber content (0.36%) compared to FDS and FDWF with 0.13 and 0.07%, respectively. Carbohydrate contributed the major composition for FDWF, FDB and FDS which was about 65 - 75% and gross energy for all three samples were about 300 kcal/100 g. FDS and FDWF contained about 6.3% of total dietary fiber which is higher than orange juice (NDB No: 09206) and apple juice (NDB No: 09016) by 6.1% (USDA National Nutrient Database for Standard Reference, 2011). Dietary fiber further adds nutritional benefits to FDS because it is known to have many

positive effects such as lowering the risk of cardiovascular disease, obesity, and gastrointestinal diseases (Ibrahim et al., 2010; Rosamond, 2002).

### Antioxidant constituent and capacity of samples

From the results (Table 3), FDS showed the highest total phenolic content (TPC) of 16.58 mg GAE/g, compared to FDB (4.78 mg GAE/g) and FDWF (7.91 mg GAE/g). The result was expressed in gallic acid equivalent because, in plant, gallic acid is considered as one of the major phenolic compounds (Norshazila et al., 2010).

Natural phenolic compounds are largely found in teas, fruits, and vegetables. The most common and widely distributed plant phenolic compounds are flavonoids that are characterized by benzo-y-pyrone structure (Abu Bakar et al., 2009). From the results, FDS contained flavonoid compound of 31.05 mg QE/g and anthocyanin compound, which is higher compared to other samples. Skin of freeze-dried *B. angulata* was richer in total flavonoid compared to total phenol and anthocyanin. Therefore, flavonoids probably are the most attributable scavenger in it and it has high pharmacological activity as radical scavengers (Abu Bakar et al., 2009). Polyphenols are able to scavenge free radicals in human body such as superoxide anion radicals that cause oxidative stress (Ajila et al., 2007). Thus, phenolic substances in freeze-dried skin of *B. angulata* may exert an antioxidant effect,

thus, may prevent the development of atherosclerosis (Gorinstein et al., 1999).

XOD, DPPH, TEAC and FRAP assays were selected to measure the capacity of polyphenols in *B. angulata*. Xanthine oxidase dismutase assay measure the ability of sample to scavenge superoxide free radical anions. From the result, superoxide scavenging (%) by XOD was as follows: FDWF>FDB>FDS. DPPH assay measures the ability of *B. angulata* extract to donate hydrogen to the radicals (Lim et al., 2006). The result for DPPH was in the order of FDS>FDWF>FDB. Reduction capability of DPPH is determined by the degree of discoloration at 517 nm. This indicates the reaction of antioxidant with DPPH by converting it to 2,2-diphenyl-1-picrylhydrazine (Ajila et al., 2007). Ascorbic acid equivalent capacities (AEAC) for FDWF and FDB are less than 70 mg AA<sub>eq</sub>/100g (Leong and Shui, 2002). These results indicate that FDWF and FDB fall into low antioxidant capacity category. While, FDS, having more than 70 mg AA<sub>eq</sub>/100g, but less than 200 mg AA<sub>eq</sub>/100g, falls into moderate antioxidant capacity. Nevertheless, the reducing ability of FDWF, FDB and FDS is strongly correlated with total phenol ( $r = 0.979$ ,  $p < 0.01$ ) and total flavonoid ( $r = 0.987$ ,  $p < 0.01$ ), while anthocyanin constituent has no correlation with DPPH values.

ABTS scavenging assay is widely adopted to study food substances because this intensely-coloured protonated radical has maximum absorbance at 734 nm and mostly coloured food do not absorb light at 734 nm. ABTS<sup>+</sup> is a cation radical that will decrease as the protons are scavenged. From the result, trolox equivalent antioxidant capacities are as follows: FDS > FDWF > FDB. Pearson correlation shows strong correlation between trolox value in studied extract and total phenol ( $r = 0.948$ ,  $p < 0.01$ ) and total flavonoid ( $r = 0.960$ ,  $p < 0.01$ ), while there is no correlation between anthocyanin and trolox value.

The freeze-dried samples were further evaluated for antioxidant capacities through FRAP assay. This assay was adopted in order to measure the ability of *B. angulata* to reduce Fe<sup>3+</sup> complex of tripyridyl triazine Fe(TPTZ)<sup>3+</sup> to the intensely blue coloured Fe<sup>2+</sup> complex Fe(TPTZ)<sup>2+</sup> in acidic medium (Lim et al, 2006). FRAP value was highest in FDS, followed by FDWF and FDB. A strong correlation was observed between all samples with total phenol ( $r = 0.997$ ,  $p < 0.01$ ) and total flavonoid ( $r = 0.992$ ,  $p < 0.01$ ). However, correlation was found moderate with total anthocyanin ( $r = 0.734$ ,  $p < 0.05$ ). The presence of polyphenol compounds may contribute to the high antioxidant activities via hydrogen donation or electron donation (Ajila et al, 2007).

## Conclusion

The result of this investigation shows that FDWF, FDB, and FDS contain the essential nutrients including dietary fiber that are good in promoting human health. Besides,

all freeze-dried powder showed presence of essential amount of antioxidant constituents as confirmed by antioxidant activities. Comparing all the samples, it was noted that FDS has the best potential as nutrient supplement due to its higher antioxidant activity contributed by polyphenol compounds. Therefore, FDS derived from *B. angulata* has the potential to be promoted as functional food and drinks. Further study is suggested to isolate and identify phenolic compounds present in the *B. angulata*, as well as identifying other biomarker compounds such as ascorbic acid and beta-carotene.

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