

Proximate Composition and Antioxidant Activity of Dried *Belimbing Dayak* (*Baccaurea angulata*) Fruits

(Komposisi Proksimat dan Aktiviti Antioksidan Buah Belimbing Dayak (*Baccaurea angulata*))

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ABSTRACT

Baccaurea angulata or locally known as 'belimbing dayak' or 'belimbing hutan' is an underutilized fruit indigenous to Borneo with its proximate analysis and antioxidant values are yet to be explored. Proximate analysis and antioxidative properties of oven-dried *B. angulata* fruits of three fractions; whole fruit, skins and berries were evaluated. From the analysis conducted, whole fruit, berries and skins fraction of *B. angulata* contained 2.83%, 5.15% and 0.28% of total fat; 3.11%, 3.43% and 3.89% of protein; 16.66%, 19.09% and 11.37% of moisture; 4.57%, 3.68% and 7.28% of total ash and water activity (A_w) of 0.41, 0.44 and 0.44, respectively. Evaluation of antioxidant activities using ferric reducing ability of plasma (FRAP), 1,1-diphenyl-2-picrylhydrazyl (DPPH) and Trolox/ABTS equivalent antioxidant capacity (TEAC) revealed that the skins fraction exhibits highest antioxidant activities ($p < 0.05$) followed by whole fruit and berries fractions. The antioxidant activities were significantly correlated ($p < 0.05$) with total phenolic and total flavonoid content but not to anthocyanins. Considering the nutritional values it contained, *B. angulata* is another good source of natural antioxidants with significant health benefits and high value for commercialization.

Keywords: Antioxidants; *Baccaurea angulata*; nutritional compositions; phytochemicals; underutilized fruits

ABSTRAK

Baccaurea angulata atau lebih dikenali sebagai belimbing dayak atau belimbing hutan oleh masyarakat tempatan merupakan sejenis buah yang tergolong dalam buah-buahan nadir dan berasal dari Borneo. Makronutrien dan bahan antioksidan yang terkandung di dalam buah ini masih belum dikaji. Analisis proksimat dan ciri-ciri bahan antioksidan yang terkandung di dalam tiga pecahan buah *B. angulata*, iaitu keseluruhan bahagian buah, bahagian kulit dan bahagian isi buah telah dijalankan. Ketiga-tiga pecahan tersebut terlebih dahulu dikeringkan dengan menggunakan relau. Hasil analisis menunjukkan bahawa keseluruhan bahagian buah, bahagian kulit dan bahagian isi buah *B. angulata* masing-masing mengandungi 2.83%, 5.15% dan 0.28% jumlah lemak; 3.11%, 3.43% dan 3.89% protein; 16.66%, 19.09% dan 11.37% kelembapan; 4.57%, 3.68% dan 7.28% jumlah abu dan 0.41, 0.44 dan 0.44 aktiviti air (A_w). Penilaian aktiviti antioksidan menggunakan ujian kebolehan penurunan ferum pada plasma (FRAP), ujian 1,1-difenil-2-pikrilhidrazil (DPPH) dan ujian kapasiti antioksidan setara Trolox/ABTS (TEAC) menunjukkan aktiviti antioksidan yang paling tinggi ($p < 0.05$) pada bahagian kulit diikuti dengan bahagian keseluruhan buah dan bahagian isi buah. Aktiviti antioksidan tersebut secara signifikan ($p < 0.05$) berkorelasi dengan kandungan jumlah fenol dan flavonoid, tetapi tidak dengan antosianin. Justeru, dengan nilai nutrisi yang terkandung dalam *B. angulata* tersebut, ia boleh dianggap sebagai antara sumber bahan antioksidan semula jadi yang baik untuk kesihatan dan mempunyai nilai komersial yang tinggi.

Kata kunci: Antioksidan; *Baccaurea angulata*; buah-buahan nadir; fitokimia; komposisi nutrisi

INTRODUCTION

Therapeutically useful bioactive compounds and micronutrients are richly available within our food source such as fruits and vegetables. Dietary constituents of fruits and vegetables for instance, carotenoids, vitamins, and polyphenols provide readily accessible source of antioxidants (Mahattanatawee et al. 2006), creating a natural defense mechanism against development of many chronic degenerative diseases such as cancer and cardiovascular diseases (Mayne 2003). The wide range of antioxidants can be benefitted at preventing unfavorable

cellular oxidative damage, inhibiting cholesterol synthesis and improving endothelial functions which underlies etiology of many chronic diseases (Vita 2005).

Many Malaysia's underutilized fruits have high nutritional value but unfamiliar to the nation due to restriction in term of geographical dispersion. *Baccaurea angulata* or locally known as belimbing dayak or 'belimbing hutan' is widely distributed in Borneo and several other regions of Indonesia. The trees grow in wild and may reach up to 10 meters tall and profusely branched. The fruits are red to purple in color and available in loads during fruits

season. The fruits are grouped in Euphorbiaceae together with 'rambai', 'tampoi', and 'jentik-jentik' (Rukayah 2002). The white flesh can be eaten fresh while the sourish skins are used in cooking by the rural communities. Studies conducted on fruits of the same genus, *B. motleyana* (rambai) and *B. polyneura* (jentik-jentik) contain high phenolic contents and antioxidant activity (Ikram et al. 2009). The color of the fruits suggesting a presence of specific flavonols (Mohd Adzim Khalili et al. 2009) hence its antioxidant capacity may exert beneficial effect against numerous biological markers of chronic diseases.

Due to the forever increasing morbidity and mortality status of chronic diseases with modern pharmacological approach (WHO 2009), a large scale research is now directed at finding as many natural antioxidants sources as possible and emphasizing on the essence of preserving the antioxidant and other nutrient contents to be consumed as an integral part of daily human diets. Despite the endemic species distribution, high nutritional values including antioxidants properties contained in underutilized fruits are undeniable (Abu Bakar et al. 2009; Ikram et al. 2009). To date, there is very limited scientific information on *B. angulata*. Thus, the objectives of this study were to evaluate the micronutrient and chemical compositions of different parts of dried *B. angulata* together with its possible antioxidant capacity.

MATERIALS AND METHODS

PLANT MATERIALS AND SAMPLE PREPARATION

Fresh fruit samples of *B. angulata* were collected from Bau, Sarawak, Malaysia at their commercial ripening stage. The fruits were then wrapped with papers, placed in boxes and transported via airmail to the Department of Nutrition Sciences, Kulliyah of Allied Health Sciences, International Islamic University Malaysia, Kuantan, Pahang, Malaysia. Upon arrival, the fruits were washed and rinsed with distilled water to remove any apparent dirt. The fruits were manually sliced and separated into different fractions- whole fruits, skins and berries. Samples were dried in an oven (Memmert Inc. Germany) at 50°C. Air-drying was carried out using horizontal air flow over samples that was placed in a single layer of aluminum trays. All fractions of *B. angulata* were dried until a constant weight was achieved. The samples were then ground into fine powder and passed through 1.0 mm sieve before stored in air-tight containers at 4°C for further analysis.

SAMPLE EXTRACTION

The sample extractions of all three fractions of *B. angulata* dried powders were carried out according to the adopted method used by Ibrahim et al. (2010). Samples (200 mg) were extracted with 2 mL of 80% methanol and 1% hydrochloric acid for 2 h at room temperature with continuous stirring. The mixture was centrifuged at 1000 g for 15 min and the supernatant was collected and used for

determination of total phenolic content, total flavonoids, total anthocyanins and antioxidant capacity (FRAP, DPPH and ABTS assays) analyses.

CHEMICAL COMPOSITION DETERMINATION OF *B. angulata*

Two g sample of each fraction were used in every analysis including moisture, total ash, protein, crude fiber, dietary fiber and water activity. Methods of analysis were from the Association of Analytical Chemist (AOAC 2003) method with slight modifications. Percent of moisture content was obtained by drying the samples in oven (Memmert, Germany) at 110°C for 3 h. The ash content was determined via muffle furnace (ELF11/6B, Carbolite, UK) ignition method at 550°C. The fat was determined by solvent extraction method using petroleum ether 30-60°C and Soxtec Automatic System (FOSS, Sweden) while crude fiber analysis was conducted by Fibertherm FT12 (Gerhardt, Germany) according to C. Gerhardt FiberBag method. Semi-micro Kjeldahl method was adopted in crude protein determination using selenium catalyst and distillate was titrated against 0.1 N of HCl with presence of methyl red-methylene blue as pH indicator. Total dietary fiber was obtained by summation of soluble dietary fiber (SDF) and insoluble dietary fiber (IDF) determined using Fibertec 1023 System E (FOSS, Denmark) based on enzymatic-gravimetric approach. Sample was treated with MES-TRIS buffer solution of pH 8.2 before incubated with alpha amylase at 100°C, protease at 60°C and amyloglucosidase enzymes at 60°C, each for 30 min before filtered. The residue of enzyme digest was rinsed twice with 95% ethanol and acetone before dried at 105°C in an oven for IDF determination. While in SDF determination, the filtrate was treated with 95% ethanol preheated to 60°C and let to precipitate for 1 h at room temperature. It was then rinsed twice with 78% ethanol, 95% ethanol and acetone before dried at 105°C in the oven. Water activity was measured using AquaLab CX-2 (Decagon Device Inc., USA) water activity equipment. Carbohydrate and energy was calculated using formula as follows:

$$\begin{aligned} \text{Carbohydrate (\%)} &= 100 - (\% \text{ moisture} + \% \text{ crude protein} + \% \text{ crude fat} + \% \text{ crude fiber} + \% \text{ ash}) \\ \text{Energy (kcal/100 g)} &= (\% \text{ carbohydrate} \times 4) + (\% \text{ crude fat} \times 9) + (\% \text{ crude protein} \times 4). \end{aligned}$$

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 FeCl₃·6H₂O in a 10:1:1 ratio and warmed to 37°C in water bath prior to use. Three 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 µL of sample extract and 300 µL of distilled water were added into the cuvette and second reading was obtained 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 and 0.2 mM Trolox ($r^2 = 0.993$). The results are expressed in micromoles trolox equivalent per gram of dried sample (mM TE/g dried sample).

DPPH FREE RADICAL SCAVENGING ASSAY

The radical scavenging activity was estimated according to the method described by Molyneux (2004) with a slight modification. About 1 mL of sample was mixed with 2 mL of 0.1 mM 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. Scavenging activity was calculated according to the equation:

$$Q = 100 (A_0 - A_c)/A_0,$$

where, A_0 is the absorbance of control and A_c is the absorbance of sample after 30 min incubation. A linear standard calibration curve was constructed using ascorbic acid range from 0.001 to 0.007 mg/mL ($r^2 = 0.996$). The results were expressed as mg ascorbic acid equivalent antioxidant capacity per 100 g sample (mg AEAC/100 g).

TEAC/ABTS ASSAY

The improved method adopted for ABTS radical scavenging activity was described by Re et al. (1999). The stock solution was prepared which includes 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 to 16 h. Then, about 1 mL from the working solution was diluted with 60 mL methanol to obtain an absorbance of ± 0.70 at 734 nm using spectrophotometer. For every assay, fresh ABTS^{•+} solution was prepared. About 150 μ L of sample methanol extract was mixed with 2850 μ L of ABTS^{•+} and left to react for 6 min. The scavenging activity of sample was compared with trolox standard and the capacity was calculated by using this equation:

$$Q = 100 (A_0 - A_c)/A_0,$$

where, A_0 is the absorbance of control and A_c is the absorbance of ABTS^{•+} and sample after 6 min incubation. A linear standard calibration curve was constructed using trolox range from 0.5 to 18 μ M/mL ($r^2 = 0.998$). The results were expressed as mg trolox equivalents per 100 g sample (mg TE/100 g).

DETERMINATION OF TOTAL PHENOLIC CONTENTS

The total phenolic content was determined approximately using Folin-Ciocalteu reagents as described by Lim et al. (2006) with slight modifications. The extract (1 mL) was mixed with 5 mL of Folin-Ciocalteu reagent (diluted 10-fold with distilled water) and allowed to stand at room

temperature for 5 min. Four milliliter of 7.5% sodium carbonate solution was then added into the mixture. The solution was vortexed and incubated in dark for 40 min. The absorbance was recorded by UV/Vis-spectrophotometer (Lambda 35, PerkinElmer, USA) at 765 nm. A calibration curve was constructed using different concentration of gallic acid range between 0.001 and 0.02 mg/mL. The results were expressed as mg gallic acid equivalent in 1 g of dried sample (mg GAE/g).

DETERMINATION OF TOTAL FLAVONOID CONTENT

The total flavonoid content was determined by colorimetric method as described by Abu Bakar et al. (2009). The extract (0.5 mL) was mixed with 2.25 mL of distilled water and 0.15 mL of 5% NaNO₂ solution. The mixture was allowed to stand for 6 min before 0.3 mL of 10% AlCl₃·H₂O solution was added and incubated for another 5 min. NaOH (1.0 mL) was then added into the mixture, vortex and the absorbance was immediately measured using UV-Vis spectrophotometer (Lambda 35, Perkin Elmer, USA) at 510 nm. A linear standard calibration curve was constructed using quercetin range from 0.01 to 0.1 mg/mL. The results were expressed as mg quercetin equivalents per gram of dried sample (mg QE/g).

DETERMINATION OF TOTAL ANTHOCYANIN CONTENTS

The determination of total anthocyanin content was based on pH differential method according to the modified method of Lee (2005) and Abu Bakar et al. (2009). Three and a half milliliter of 0.025 M potassium chloride buffer pH 1.0 was added to 0.5 mL of 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 using UV/Vis-spectrophotometer (Lambda 35, Perkin Elmer, USA). The extract was then treated similarly with 0.4 M sodium acetate buffer pH 4.5 and the absorbance was measured of 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):

$$\frac{A \times MW \times DF \times 10^3}{\epsilon \times l},$$

where $A = (A_{515\text{nm}} - A_{700\text{nm}})_{\text{pH}1.0} - (A_{515\text{nm}} - A_{700\text{nm}})_{\text{pH}4.5}$, MW = 449.2 g/mol for cyanidin-3-glucoside, DF is the dilution factor of samples, l is the pathlength in cm, $\epsilon = 26,900$ molar extinction coefficient, in $\text{L} \times \text{mol}^{-1} \times \text{cm}^{-1}$ for cyd-3-glu and $10^3 =$ factor for conversion from g to mg.

STATISTICAL ANALYSIS

Every test was conducted in triplicate from the same extract in order to determine their reproducibility. Data were expressed as mean \pm standard deviation. Analysis of variance (ANOVA) was used to test the mean difference between fractions of *B. angulata*. Correlations among data obtained

were calculated using Pearson's correlation coefficient (r) and $p < 0.05$ was considered significantly different.

RESULTS AND DISCUSSION

NUTRITIONAL AND CHEMICAL COMPOSITIONS OF *B. angulata*

B. angulata is grouped as highly seasonal fruits which limit its potential for commercialization without proper preservation technique. In this study, oven dried method was applied to all fractions of *B. angulata*. Nutritional and chemical values supplied by the dried samples were evaluated. The method applied was based on ability of dry air to absorb the moisture released from the samples due to high oven temperature and further carried away by the circulation (Jaafar et al. 2009). Generally, oven air drying provides extra benefits in terms of cost and time compared with other commercially available drying techniques used for food products such as sun drying and freeze drying.

Among all fractions, percentage yield for berries was the highest with 20.63% followed by whole fruit with 16.79% and finally skins with 6.01%. Table 1 shows the comparison of proximate analysis data for all three fractions. Higher parameters for berries fraction are moisture (19.09%), total fat (5.15%) and crude fiber (18.78) while higher parameters for skins fraction are ash content (7.28%) and dietary fiber (43%). Dried whole fruit of *B. angulata* on the other hand carries intermediate values in many parameters. With small different in value among fractions, dried fruit of *B. angulata* can be a good source of carbohydrate, energy and dietary fiber. Other than protein, the fruits contained sum of minerals as indicated by the presence of ash (Ibrahim 2010). The results also demonstrated a good shelf life for all fractions using this drying method. This is proven by the low water activity (A_w) value where higher value of A_w signifies poor storage quality and shorter shelf life due to microbial activity (Jaafar et al. 2009).

ANTIOXIDANT ACTIVITIES

The results for antioxidant activities and constituents are summarized in Table 2. Antioxidant activities were evaluated via DPPH, FRAP and TEAC assays. DPPH implied radical scavenging capability using DPPH radical which will be reduced by antioxidants. The disappearance of DPPH radical chromogens was stoichiometric to the number of the paired electron (Pisochi et al. 2009). FRAP assay employed antioxidants as reductants in a redox-linked colorimetric method where, at low pH, reduction of ferric tripyridyl triazine (Fe^{3+}) to ferrous ion and caused an intense blue colored ferrous tripyridyl triazine (Fe^{2+}) complex to form (Benzie & Strain 1996). TEAC assay was based on the ability of tested sample to inhibit ABTS radical ($ABTS^{\bullet+}$) resulting in decolorization of the intense-colored radical (Phipps et al. 2007). Regardless of mechanism of actions, range of antioxidant value for all assays was highest in skins, followed by whole fruit and berries. This study found that the skin exhibited the highest values ($p < 0.05$) than other fractions in DPPH, FRAP and TEAC assay. Values of whole fruit were significantly higher than berries ($p < 0.05$) only in DPPH and FRAP assays. Ersus and Cam (2007) reported high content of phenolic and flavonoids contents in sour-taste fruit peel and several studies have also shown high antioxidative properties in pigmented skin fruits (Kubola & Siriamornpun 2011; Palanisamy et al. 2008).

TOTAL PHENOLIC, TOTAL FLAVONOIDS AND TOTAL ANTHOCYANIN CONTENT

The determination of antioxidants constituents was conducted for total phenolic, total flavonoids and total anthocyanin contents. Folin-Ciocalteu colorimetry method was based on metal oxides reduction by phenolates/polyphenolic antioxidants which will result in production of blue colored complex that exhibits broad light absorption with a maximum at 765 nm. The total phenolic content was strongly correlated with antioxidant activities evaluated

TABLE 1. Proximate composition and water activity of different parts of dried *B. angulata*

Nutrient composition	Whole fruit	Berries	Skins
Percentage of yield (%)	16.79±0.14	20.63±0.34	6.01±0.08
Moisture (%)	16.66±0.32	19.09±0.55	11.37±0.26
Ash (%)	4.57±0.03	3.68±0.08	7.28±0.11
Protein (%)	3.11±0.18	3.43±0.22	3.89±0.81
Total fat (%)	2.83±0.13	5.15±1.70	0.28±0.10
Carbohydrate (%) [#]	59.37	56.02	58.44
Crude fiber (%)	13.45±1.00	18.78±0.29	12.63±1.36
Gross energy (kcal/100 g) [#]	275.42	284.12	251.71
Total dietary fiber (% _{w/w})	30.20±0.00	22.60±0.00	43.00±0.00
Water activity (A_w)	0.41±0.00	0.44±0.00	0.44±0.00

*Other than #, all value represents mean ± standard deviation of three replicates analyses of powder sample

* #, results gained from calculation using data of other proximate analysis

TABLE 2. Antioxidant assays, total phenolic, total flavonoid and total anthocyanin content of dried *B. angulata*

Samples	FRAP (mM TE/g)	DPPH (mg AA/100g)	TEAC (mg TE/100g)	Phenolic (mg GAE/g)	Flavonoids (mg QE/g)	Anthocyanin (mg c-3-g/100 g)
Whole fruit	29.17 ± 1.17 ^a	53.68 ± 1.51 ^a	303.04 ± 50.02 ^{a,b}	6.85 ± 0.00 ^a	12.91 ± 0.49 ^a	0.33 ± 0.20 ^a
Berries	13.31 ± 0.74 ^b	46.23 ± 0.58 ^b	192.38 ± 8.80 ^a	3.48 ± 0.01 ^b	7.93 ± 0.15 ^b	1.20 ± 0.60 ^a
Skins	50.86 ± 4.24 ^{c, a}	78.54 ± 2.08 ^c	492.79 ± 53.77 ^b	8.62 ± 0.01 ^c	19.12 ± 0.11 ^c	0.96 ± 0.19 ^a

* Value represents mean ± standard deviation ($n=3$) which with different letters are significantly different at $p<0.05$

using DPPH ($r^2=0.950$, $p<0.05$), FRAP ($r^2=0.992$, $p<0.05$) and TEAC ($r^2=0.935$, $p<0.05$). The total flavonoid was determined based on detection of colored flavonoids-aluminum complex. Aluminum chloride formed acid stable compound at keto or hydroxyl groups of the flavonoids which lead to formation of the colored flavonoids-aluminium complexes (Chang et al. 2002). The flavonoids contents also show strong correlation in antioxidant activities as determined in DPPH ($r^2=0.966$, $p<0.05$), FRAP ($r^2=0.977$, $p<0.05$) and TEAC ($r^2=0.908$, $p<0.05$). The abundance of phenolic and flavonoids contents available in fruits and vegetable has been reported with potential effects in managing chronic and infectious diseases (Greig & Maxwell 2001; Vatterm et al. 2005).

The total anthocyanin determination was conducted based on pH differential method. Anthocyanin pigments undergo reversible structural transformations with a change in pH whereby the colored monomeric oxonium form predominates at pH1.0 and the colorless hemiketal-degraded form at pH4.5. This allowed accurate and rapid measurement of the available anthocyanins even in the presence of polymerized degraded pigments and other interfering compound (Waterhouse 2001). The low content of anthocyanins, however, proved no relation towards any of antioxidant assays conducted. Orange to red color fruits usually signifies the presence of high anthocyanins (Garzon & Wrolstad 2009) however, anthocyanins could be easily degraded under high temperature and humidity (Tonon et al. 2010).

CONCLUSION

This study provided supporting evidence on nutritional facts of *B. angulata*. This study indicated that the fruit, especially the skin fraction was rich in antioxidants with significant correlation with its phenolic and flavonoid content. Further study is being conducted to determine other potential antioxidant compounds present in this fruit to be applied in various health food products or applications. Other than gaining higher commercial value for local underutilized fruits, the data is beneficial in promoting this locally distributed fruit to be grown at a larger scale.

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