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Determination of phytochemicals and vitamin content of underutilized *Baccaurea angulata* fruit

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Abstract

The purpose of this study is to determine the phytochemicals and vitamin content of *Baccaurea angulata* fruit. *Baccaurea angulata* or locally known as 'belimbing dayak' or 'belimbing hutan' is an underutilized fruit of family Euphorbiaceae which widely distributed in Borneo and several other region of Indonesia. The fruit were divided into three different portion which is skin, berry and whole fruit and the phytochemical and vitamin content were evaluated. The results indicated that whole fruit part contains flavonoid and condensed tannin. While the berry showed positive screening for tannin and the skin containing strong flavonoid. However screening for alkaloid, saponin, triterpine and steroid test showed negative result in every part of the fruit. Result for vitamin content analysis indicated berry contain the highest amount of vitamin A compared to whole fruit and skin. While vitamin C recorded in all part of the fruit. Vitamin E is not found in any part of the *Baccaurea angulata* fruit. The finding of high amount of phytochemicals type flavonoid and tannin with high vitamin A and Vitamin C in the *B. angulata* suggested that the fruit can be useful for treating many chronic disease.

Keywords: Phytochemicals, Vitamin content, *Baccaurea angulata*, flavonoid, tannin.

Introduction

Presence of many therapeutically useful bioactive compounds and micronutrients within fruits and vegetables has been extensively studied and proven for its health effects. Reactive oxygen species (ROS) which arise naturally from endogenous and exogenous source will lead to oxidative damage which underlies many chronic diseases such as cardiovascular diseases. ROS can be removed by antioxidant. Fruits and vegetables that are usually being consumed contain high Carotenoids, vitamin C, polyphenols and other beneficial micronutrient. Recognition of the benefits of natural products such as fruit juice is increasing among worldwide population and becoming a part of regular diet and more preferable than pills [1, 10]. Fruits and vegetables that are usually being consumed contain high Carotenoids, vitamin C, polyphenols and other beneficial micronutrient. These have become an integral part of daily human diets and provide readily accessible antioxidants. Many Malaysia's underutilized fruits own 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 a native fruit of Borneo Island including Sabah and Sarawak. *B. angulata* belongs to the Euphorbiaceae family. It can be eaten fresh, used in cooking and as traditional medical practice. This underutilized fruit is found to possess essential nutrient and substantial antioxidant activity that may provide protection against degenerative disease such as cardiovascular disease (CVD) and good in promoting human health [8]. Previously Jauhari *et al* (2013) [9] have reported a study indicate on this fruit was rich in antioxidant with significant correlation with phenolic and flavonoid especially the skin fraction. However the proposed study has never been documented and very limited information on this fruits ever published. This study will be commenced with the screening of phytochemicals present in the fruit consisted of flavonoid, saponin, alkaloid and tannin by using chemical analysis method for qualitative analysis and vitamin content for quantitative analysis.

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 Kulliyah of Allied Health Science, International Islamic University, Kuantan, Pahang, Malaysia.

The fruits were immediately preserved and stored at $-30\text{ }^{\circ}\text{C}$ (Angelantoni Industrie, model KRYOLAB500H) prior to sample preparation. Fruit samples' identity was also authenticated at Forest Research Institute of Malaysia for identification and authentication. Upon arrival, the fruit were washed and rinsed with distilled water to remove any apparent dirt. The fruit were manually sliced and separated into different fraction - whole fruit, skin and berries. Samples were dried in the in an oven (Memmert Inc. Germany) at $50\text{ }^{\circ}\text{C}$. Air drying was carried out using horizontal air flow over samples that was placed in a single layer of aluminum trays. All fraction 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 $4\text{ }^{\circ}\text{C}$ for further analysis.

B. Sample Extraction

The sample extraction of all three fractions of *B. angulata* dried powders were carried out according to the adopted method used by Ibrahim *et al.* (2010) [8]. Samples (200mg) were extracted with 2mL 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 phytochemicals screening and vitamin content analysis.

C. Phytochemical Screening Test

Phytochemical screening tests was performed on a sample based on Fasihuddin and Hasmah (1993) [6] method with some modification. The goal is to obtain a rough idea of the type of chemical component groups that exist in this *Baccaurea angulata* fruit. Among the phytochemical constituent tested were from the group of compound tannin, triterpene/steroids, flavonoid, saponin and alkaloid [3].

1. Test for Alkaloids

Ammoniacal chloroform was prepared by adding 1 L of chloroform with 3.6 ml of 25% ammonia. Anhydrous sodium sulphate was added for drying. Then, the solution was filled in amber bottle or aluminum wrapped bottle and keep in refrigerator prior to use. Meyer's reagent (Kalium mercuric iodide) was prepared by adding 3.3 g of kalium iodide and 1.36 g of mercury chloride. Then, 20 ml concentrated acetic acid was dissolved and 64 ml distilled water was added. Two grams of sample was weighed into a test tube. 1 ml chloroform and 10 ml ammoniacal chloroform was added. 10 drops of 10% sulphuric acid was added and shake. Two layers were appeared. The upper layer was transferred into a test tube by using a dropper pipette with cotton covering its opening. This is done to avoid any sample from the lower layer from entering the dropper pipette. The solution was tested with 3 drops of Mayer's reagent. The result was observed and recorded. Formation of white precipitates indicates the presence of alkaloids.

2. Test for Flavonoid

Five grams of sample was weighed into a 100 ml conical flask. 60 ml chloroform was added and cover its mouth with aluminum foil to avoid evaporation. Leave in fume hood overnight. Filter in 100 ml round flask through a funnel and

filter paper. The filtrate was dried by using rotary evaporator at $45\text{ }^{\circ}\text{C}$, 60 rpm. 2 ml ether and 2 ml of 10% ammonia solution were added in a test tube and shake well to dissolve the sample. Solution was separated into 2 layers. Upper part is ether and lower part is ammonium. The formation of yellow color in the ammonium layer indicates the presence of flavonoid. While purple color for kuinon/ juglon.

3. Test for Tannin

1% ferric chloride solution was prepared by adding 1.0 g ferric chloride in 100 ml distilled water in 100 ml volumetric flask. Five grams of sample was weighed into a 100 ml conical flask. 50 ml methanol was added and cover its opening with aluminum foil to avoid evaporation. Leave in fume hood overnight. Filter in 100 ml conical flask through a funnel and filter paper. 2 ml of filtrate was poured into a test tube. 3-4 drops of 1% ferric chloride solution was added and color change was observed. Formation of blue-black color indicates the presence of hydrolysable tannins. While brownish-green color indicates condensed tannins.

4. Test for Saponin

Five grams of sample was weighed into a 100 ml conical flask. 50 ml methanol was added and cover its mouth with aluminum foil to avoid evaporation. Leave in fume hood overnight. Filter in 100 ml conical flask through a funnel and filter paper. 1 ml of filtrate was poured into a test tube (0.5" x 0.5"). 5 ml of distilled water was added by using stopper. The test tube was shake vigorously for 30 seconds. Allow standing and directly measure the high of froth exists with a ruler. The formation of a stable froth indicates the presence of saponin. The amount of saponin was measured as follow,

3+ = froth greater than 3 cm

2+ = 2-3 cm froth

1+ = 1-2 cm froth.

5. Test for Steroid and Triterpine

20 ml acetic anhydride (100%) was added into 100 ml reagent bottle and was kept in refrigerator at $-10\text{ }^{\circ}\text{C}$ for 10 minutes. Then, 1 ml of concentrated sulphuric acid was added into reagent bottle and cooled in refrigerator for 9 minutes. The reagent bottle was taken out and place it in a beaker filled with ice and 10 ml of acetic acid glacial (100%) was added. The reagent bottle was warmed to room temperature. This reagent was prepared in fresh to avoid any oxidation process if prolong.

Five grams of sample was weighed into a 100 ml conical flask. 60 ml chloroform was added and cover its mouth with aluminum foil to avoid evaporation. Leave in fume hood overnight. Filter in 100 ml conical flask through a funnel and filter paper. 2 ml of filtrate was poured into a test tube. The filtrate was tested using Liebermann-Buchard reagent by adding 1 ml of the reagent. Any color changes was observed within 1 hour. Formation of reddish-brown color (upper layer) indicates the presence saponin triterpenoids while the greenish color (lower layer) for saponin steroids. The amount of tannins is measured as follow,

3+ = strong color

2+ = medium color

1+ = weak color

D. Vitamin A (β -carotene), vitamin C (Ascorbic Acid) and vitamin E estimation using high performance liquid chromatography (HPLC) method.

Vitamin A (β -carotene)

Analysis of Vitamin A was determined based on method described by the Association of Analytical Chemist (AOAC 1990) [2] method with slight modifications. Prior to determination, the samples were hydrolyzed using alkaline hydrolysis followed by extraction. 10.0 g to 20.0 g of each samples were weighted into 250ml boiling flask respectively by using analytical balance (Sartorius, Germany). 95% ethanol with about 4 times volume weight of the sample was added into the boiling flask. Then, 10.0-20.0 ml of 20% potassium hydroxide (KOH) and few boiling chips were added. The boiling flask was attached to the water cool refluxing and temperature was adjusted to give a reflux rate of about 2 drops per second. The sample was heated by refluxing for 30 minutes and then cooled at room temperature.

Extraction

The hydrolysate is extracted 3 times with 50 ml of hexane and then water was added until solution was neutral to phenolphthalein. The extract was filter washed through anhydrous sodium sulphate. Volume of extract was reduced by using rotary evaporator. Then mobile phase was added to the mark up point. Sample was filtered through 0.45 μ membrane filter. The supernatant was underwent analyses by using High Performance Liquid Chromatography. The HPLC condition was summarized as follow an aliquot of 2ml of concentrated extracts were evaporated under running nitrogen, re-dissolved in 2 ml acetone, passed through 0.45 μ m Milipore membrane and 20 μ L aliquot were injected into the HPLC system. The methanol, ethyl acetate and acetonitrile (88:10:2 v/v/v) was used as mobile phase at flow rate 1.3 ml/min and detection was carried out at the wavelength of 450 nm.

Vitamin C (Ascorbic Acid).

Analysis of Vitamin A was determined based on method described by the Association of Analytical Chemist (AOAC 1990) [2] method with slight modifications. 10.0 g of grinded sample was weighted in 250 ml conical flask. Then, metaphosphoric acid-acetic acid solution was added and make up to volume of 200 ml. The solution was homogenized by using magnetic stirrer and filtered in 250 ml conical flask with a funnel and filter paper. 10.0 ml of sample was removed and filled in 100 ml of conical flask. Three replicates was prepared of each samples and was determined using titration of filtrate until pink color was formed in the solution.

Vitamin E

Analysis of Vitamin E was determined based on method described by Association of Analytical Chemist (AOAC 1990) [2] method with slight modifications. 2.0 - 10.0 g of each samples (S, P, and WF) were weighted into 250 ml conical flask respectively by using analytical balance (Sartorius, Germany). 50ml of the absolute Ethanol was added followed by 50ml of potassium hydroxide and 0.25 g of ascorbic acid. Then the conical flask was heated for 30 minutes at 40 °C by refluxing process. The solution was cooled at room temperature and transferred into separating funnel.

Extraction

25.0 ml of petroleum ether was added and vigorously shake. After 2 separation layer was formed, upper solution (petroleum ether extract) was collected and lower solution was removed in a beaker throw waste pipe. The lower solution was added into separating funnel and 25.0ml of ether was added 2 times to repeat the extraction step. Ether extracts was combined and washed with water to neutralize it become phenolphthalein. Then, the ether extract was filtered through anhydrous sodium sulphate and evaporated to dryness under N_2 . 10.0ml of Methanol was added for dilution and the sample was filtered through 0.45 μ membrane filter. The supernatant was underwent analyses by using High Performance Liquid Chromatography. The chromatographic condition used were as follow: A Ultrasphere octadecylsilyl (ODS) Hypersil (C18; 5 μ m, reversed-phase column, 4.6 x 150mm). Methanol and deionised water (95:5) at pH 2.2 was used as mobile phase with a flow rate of 1.0 ml/min and detection was carried out at 238nm.

Statistical Analysis

Statistical analysis were performed using SPSS. Results are expressed as means \pm S.D. Significant different was set at $p < 0.05$.

Results and Discussion

Table 1.1: Phytochemical test of whole fruit, skin and berry of *Baccaurea angulata*.

Phytochemical type	Whole fruit	Skin	Pulp
Alkaloid	–	–	–
Flavonoid	+	–	++
Tannin	++	+	–
Saponin	–	–	–
Triterpine and steroid	–	–	–

(-): Negative test (absence of turbidity, flocculation and precipitation)

(+): Weak positive test (weak precipitate or weak color)

(++): Positive test (obvious precipitate or mild color)

(+++): Test strongly positive (strong precipitate or strong color)

According to the phytochemical screening of *Baccaurea angulata* (Table 1.1) revealed that whole fruit part contains flavonoid and condensed tannin. While the skin showed positive screening for tannin and berry containing strong flavonoid. However screening for alkaloid, saponin, triterpine and steroid test showed negative result in every part of the fruit. Further analysis to recognize type of flavonoid and tannin will be conducted in order to identify major bioactive compound present in the *Baccaurea angulata*.

Table 1.2: Comparison amount of Vitamin Whole Fruits, Skin, and Pulp of BA Fruits

	Whole Fruit	Skin	Pulp
Vitamin A (μ g/100g)	25.2 \pm 2.83	0.00 \pm 0.00	28.3 \pm 12.45
Vitamin C (mg/100g)	2.55 \pm 0.00	1.29 \pm 0.00 ^{a,c}	2.57 \pm 0.00
Vitamin E (μ g/100g)	0.00 \pm 0.00	7.79 \pm 0.28 ^{a,c}	0.00 \pm 0.00

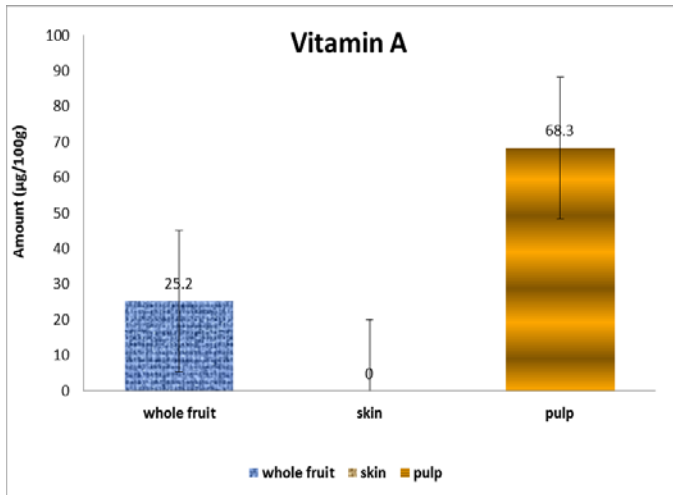
Values were the means \pm standard deviations of three replicates analysis

^a significant difference ($p < 0.05$) between whole fruit and skin

^b significant difference ($p < 0.05$) between whole fruit and pulp

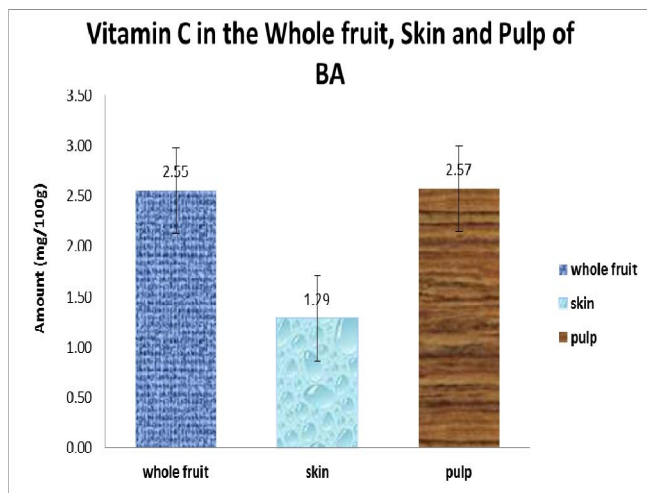
^c significant difference ($p < 0.05$) between skin and pulp

Vitamin A



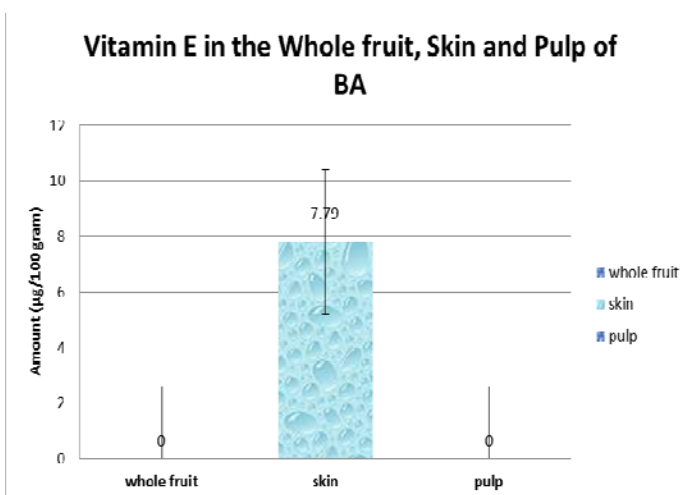
Graph 4.1: Amount of Vitamin A in Whole fruit, Skin and Pulp of BA

Vitamin C



Graph 4.2: Amount of Vitamin C in Whole fruit, Skin and Pulp of BA

Vitamin E



Graph 4.3: Amount of Vitamin E in Whole fruit, Skin and Pulp of BA

Table 1.2 indicate the data of mean and standard deviation of Vitamin A, C and E in the whole fruit, Skin and Pulp of *Baccaurea angulata* (BA). Statistical Package for Social Sciences (SPSS 21.0) was used to perform a statistical analysis, One-way ANOVA was run to compare the concentration of vitamin in three parts of BA fruit which are whole fruit (wf), skin (s) and pulp (p). Data was expressed as mean \pm standard deviation. Significance was determined at ($p < 0.05$).

The amount of Vitamin A in whole fruit, skin and pulp were recorded in the unit of microgram per 100 gram ($\mu\text{g}/100\text{ g}$) as 25.2 ± 2.83 , 0.00 ± 0.00 and 28.3 ± 12.45 respectively. Pulp recorded the highest reading as compared to whole fruit and skin. Amount of Vitamin C in whole fruit, skin pulp were recorded in the unit of miligram for each 100 gram ($\text{mg}/100\text{g}$) as 2.55 ± 0.00 , 1.29 ± 0.00 and 2.57 ± 0.00 respectively. Amount of Vitamin C indicate of significant difference at ($p < 0.05$) between whole fruit and pulp compared to skin. Another fruit under the same family with *Baccaurea angulata* also have the same average reading of vitamin C, which is 5 g of vitamin C for every 100 gram of fruit.

Vitamin E analysis shows there is no reading in the part of whole fruit and pulp compared with skin that recorded average amount of 7.79 ± 0.28 in the unit of microgram per 100 gram ($\mu\text{g}/100\text{ g}$). Analysis by One-way ANOVA indicate there is significant different at ($p < 0.05$) between skin compared to whole fruit and pulp.

Conclusion

This study provide supporting evidence on nutritional facts of *B. angulata*. The results indicated that whole fruit part contains flavonoid and condensed tannin. While the pulp showed positive screening for tannin and skin containing strong flavonoid. However screening for alkaloid, saponin, triterpine and steroid test showed negative result in every part of the fruit. Result for vitamin content analysis indicate pulp contain the highest amount of vitamin A compared to whole fruit and skin. While vitamin C recorded in all part of the fruit. Vitamin E is not found in any part of the *Baccaurea angulata* fruit except the skin fraction. The finding of high amount of phytochemicals type flavonoid and tannin with high vitamin A and Vitamin C in the *B. angulata* suggested that the fruit can be used for treating many chronic disease. Further analysis to recognize type of flavonoid and tannin is suggested to be conducted in order to identify major bioactive compound present in the *Baccaurea angulata*.

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References

1. Apak R, Guclu K, Demirata B, Ozyurek M, Celik SE, Bektasoglu B *et al.* Comparative evaluation of various total antioxidant capacity assays applied to phenolic compounds with the CUPRAC assay. *Molecules*, 2007; 12:1496-1547.

2. Association of Official Analytical Chemist AOAC. Official methods of analysis of the Association of Official Analytical Chemists. 15th edition. Washington, DC, Association of Official Analytical Chemists, 1990.
3. Ciriaco POP. Phytochemical, Microbiological and Pharmacological Screening of Medicinal Plants: A supplement of the ACTA MANILANA. Philippines: GMS Pub. Corporation, 1978.
4. Du J, Cullen JJ, Buettner GR. Ascorbic acid Chemistry, biology and the treatment of cancer. *Biochimica ET Biophysical Acta* 1826, 2012; 443-457.
5. DVries JW, Silvera KR. Determination of Vitamin A (retinol) and E (α -tocopherol) in foods by liquid chromatography: collaborative study, *J AOAC Int.* 2002; 85(2):424-434.
6. Fasihuddin Ahmad, Hasmah Raji. *Kimia Hasil Semulajadi dan Tumbuhan Ubatan*. Kuala Lumpur: Dewan Bahasa dan Pustaka, 1993.
7. Giorgi MG, Howland K, Martin C, Bonner AB. A novel HPLC method for the concurrent analysis and quantitation of seven water-soluble vitamins in biological fluids (plasma urine): A validation study and application. *The Scientific World Journal.* 2002.
8. Ibrahim D, Hazali N, Jauhari N, Yahya MNA, Ahmed IA, Mikail MA. Sub-chronic toxicological evaluation of the *Baccaurea angulata* (belimbing Dayak) fruit juice in rats, *International Journal of Applied Research in Natural Products.* 2013; 6(4):23-32
9. Jauhari NK, Ibrahim D, Ibrahim M, Yahya MNA, Nor NM, Isa KAM *et al.* Proximate Composition and Antioxidant Activity of Dried Belimbing Dayak (*Baccaurea angulata*) Fruits, *Sains Malaysiana* 2013; 42(2):129-123.
10. Sin HN, Yusof S, Sheikh Abdul Hamid N, Abdul Rahman R. Optimization of hot water extraction for sapodilla juice using response surface methodology, *Journal of Food Engineering.* 2006; 74:352-358.