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Determination of some chemical compounds of bignay (*Antidesma bunius*) fruit juice

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Abstract

Bignay fruits are produced from wild trees of *Antidesma bunius* Spreng which can be found in India, Ceylon, and South East Asia countries. Each mature tree can produce hundreds of kilograms of fruits per year, so this fruit has the potential to be used as a raw material in juice and beverage industries. In addition, recent trends in consumer demand indicates that consumers are more inclined to demand products which can provide health benefits. In order to assess the potential of bignay fruit as a raw material for juice production, it is important to determine the health-promoting bioactive compounds contained in fresh bignay fruit juice. Therefore, the objective of this study was to determine the concentration of bioactive compounds in fresh bignay fruit juice. Results obtained from this study showed that bignay fruit juice contained 1202.5 mg GAE/100 mL of total phenolic, 436.602 mg/100 mL of anthocyanin, 48.931 mg/100 mL of ascorbic acid, and 3.78 mg/100 mL of flavonoid. The scavenging activities obtained from DPPH and ABTS methods were 0.110 mg/mL and 0.126 mg/mL respectively. The above results indicate that bignay fruits contain health-promoting chemical compounds and can be used as a natural source of antioxidants.

Keywords: *Antidesma bunius*; color; phenolic compound; ascorbic acid; antioxidant activity.

Practical Application: Bignay fruit was found to contain active compounds with high antioxidant activity so it can provide health benefits. The utilization of this fruit as a raw material for juice industries is viable, especially in its countries of origin.

1 Introduction

The utilization of exotic fruits as a raw material in the food industry has become an emerging trend due to their health-promoting functional compounds such as antioxidants. In addition, there is a trend in food industries where synthetic antioxidants were replaced with antioxidants from natural sources (Caleja et al., 2017; Kumar et al., 2015). Among the organic compounds found in fruit that have shown health benefits for humans are vitamins and phenolic compounds such as flavonoids. These compounds have high antioxidant activities and other biological functions. As a source of natural polyphenols and other health-promoting compounds, exotic fruits contain an abundant amount of phenolic acids and flavonoids such as anthocyanin. These compounds have distinct properties such as strong antioxidant activity, anti-inflammatory, and antiproliferative activities which could inhibit cell cancer growth and prevent cardiovascular diseases (Swami et al., 2012; Timmers et al., 2015).

Antidesma bunius is an indigenous species found mainly in the South East Asia region such as Thailand, Philippine, and Indonesia but it can also be found in India and Ceylon. This fruit has different names such as "Maoberry" or "Mao-Luang" in Thailand, "Bignay" in Philippine, and "Buni" in Indonesia. In some countries, this fruit has been consumed in the form of juice, jam, and wine (Chaikham, 2015; Chaikham et al., 2016; Jorjong et al., 2015; Samappito & Butkhup, 2008).

Studies on bioactive compounds for this fruit have been conducted mainly for bignay from trees grown in northern Thailand. The major flavonoid contents found in those studies were (-)-epicatechin and (+)-catechin (flavan-3-ols), while the major contents of anthocyanin were cyanidin, followed by malvidin, pelargonidin, and delphinidin. Phenolic acids identified in bignay consist of two hydroxybenzoic acids i.e. gallic acid and vanillic acid and five hydroxycinnamic acids i.e. caffeic acid, p-coumaric acid, ferulic acid, sinapinic acid, and cinnamic acid (Butkhup & Samappito, 2008; Jorjong et al., 2015). There has been no such study conducted for bignay fruits from trees found in Indonesia.

Despite its potential beneficial impacts on health, the intake of bignay fruits in the producing countries such as Indonesia is still very low due to its stone, tart taste, and purple stains when eaten as fresh fruit. Therefore, to increase consumption of this fruit, processing into processed products such as juice and jams need to be done. The increase in consumption of juice and other processed products from fruits with high bioactive compounds can help meet the recommended daily intake for fruits and provide additional health benefits. For example, Asgary et al. (2014) reported that consumption of high-flavonoid sweetie juice provided a higher reduction of Diastolic Blood Pressure (BDP) compared to the consumption of low-flavonoid sweetie juice. The objectives of this study were to determine total phenolic, total monomeric anthocyanin, total flavonoid, and vitamin C

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contents of bignay juice and to determine the antioxidant activity of fresh bignay fruit juice. Besides, the color and pH of fresh bignay fruit juice were also measured. This is important since the color of a fruit can be used as an indicator to predict the chemical compounds contained in the fruit. Several chemical compounds that have been proven to provide a beneficial impact on health also serve as a color pigment. Anthocyanin is used as a source for the blue-purple pigment, lycopene for red pigment, and β-carotene for orange pigment (Jamal et al., 2017).

2 Materials and methods

2.1 Sample preparation

Bignay fruits were bought from a local market in Makassar, South Sulawesi, Indonesia. The fruits were washed and sorted based on the maturity level. The ripe fruits (purple blackish color) were chosen and processed into juice using a commercial juicer (Philips HR1832). The juice obtained was filtered through cheesecloth to separate the pulp and the fresh juice obtained was poured into a 500 mL PTFE bottle and stored in a freezer maintained at -18 °C until used.

2.2 Determination of pH, Total Soluble Solids (TSS), and color

The pH measurement was carried out using a pH meter (LAQUAtwin pH-11, Horiba Scientific, Japan) calibrated with buffer solutions of pH 7.0 and 4.0. Total soluble solid (TSS, expressed in °Brix) was measured using a digital refractometer (HI 96801, Hanna Instruments, Rhode Island, USA) calibrated with distilled water. The color of the juice was determined using a colorimeter (CS-10 Colorimeter, Hangzhou CHN Spec Technology, China). Color parameters of the samples were determined based on CIE L* a* b* parameters, where L* represents the lightness with value ranged from 0-100 (black-white axis), a* represents the green-red color with value ranged from -60 to +60 (green-red axis), and b* represents the blue-yellow color with ranged of -60 to +60 (blue-yellow axis).

2.3 Total phenolic content

Total phenolic measurement was conducted according to the Folin Ciocalteu method described in Tezcan et al. (2009) with some modification. The stock solution was made by diluting 0.1 mL of bignay fruit juice in 10 mL of methanol:water (6:4 v/v) solvent. The determination of phenolic compounds was performed as follows. 0.5 mL of stock solution was mixed with 1.5 mL of Folin Ciocalteu reagent (7.5%) and 1.2 mL of Na_2CO_3 (7.5%) and then topped with distilled water to reach a total volume of 5 mL. The mixture was kept for 90 minutes at room temperature before the measurement of absorbance was conducted at 760 nm using UV-Vis Spectrophotometer (UV-1800, Shimadzu, Japan). The results were expressed as gallic acid equivalent (mg GAE/100 mL juice) using calibration curved of gallic acid from 2-10 ppm.

2.4 Total anthocyanin content

The total monomeric anthocyanin assay was determined using the pH differential method as described in the literature (Jiang et al., 2013). Briefly, two dilutions of samples were prepared by adding 50 µL of samples with 4950 µL of potassium chloride (0.025 M) buffer pH 1.0 and the other with sodium acetate (0.4 M) buffer pH 4.5. The absorbance of each sample was measured after 20 minutes at wavelengths of 520 and 700 nm. Total anthocyanin content was determined based on the absorbance value obtained from Equation 1.

$$A = \left(A_{520} - A_{700}\right)_{pH\,1,0} - \left(A_{520} - A_{700}\right)_{pH\,4,5} \quad (1)$$

where A_{520} is the absorbance at the 520 nm wavelength, and A_{700} is the absorbance at the wavelength of 700 nm. Total anthocyanin was represented as cyanidin-3-glucoside and calculated using Equation 2:

$$Anthocyanin\left(\frac{mg}{L}\right) = \frac{A \times MW \times DF \times 10^3}{\varepsilon \times l} \quad (2)$$

where A is the absorbance value, MW is the molecular weight of standard (445.2 g/mol), DF is the dilution factor, ε is the molar absorption capacity (29,600 l/mol.cm), and l is the cuvette length (1 cm).

2.5 Ascorbic acid content

The ascorbic acid content was measured using Ultra-Fast Liquid Chromatography (UFLC) based on the method represented in literature (Mercali et al., 2014) with some modifications. The preparation of the stock sample was carried out by adding 1.5 mL of sample into 7.5 mL of meta-phosphoric acid solution (4.5%). The mixture was filtered through a syringe filter of 0.45 µm, and 40 µL was injected into the UFLC system (Prominence UFLC, Shimadzu, Japan).

The UFLC conditions were described as follows: The column used was a reverse C_{18} (Shimadzu, Japan) and the separation was carried out under isocratic condition using a mobile phase of KH_2PO_4 (5%). The eluent flow rate was 0.7 mL/min and the column temperature was set at 25 °C. The absorbance was measured at 254 nm and pure ascorbic acid was used as standard.

2.6 Total flavonoid content

Total flavonoid content was measured using Thin Layer Chromatography (TLC) based on the method used by several researchers (Altemimi et al., 2015) with some modification. A 20 × 20 cm TLC silica gel 60 F_{254} plates (Merck, Germany) were divided into four equal pieces (5 × 20 cm) and then placed into an oven at 110 °C for 20-30 min prior to use. The separation was carried out using the mixture of ethyl acetate: formic acid: water (10:2:3 v/v) as the solvent. 10 mL of solvents were saturated inside the chromatography chamber for 30-40 min before used. For the measurement, 2 µL of samples and standards were dotted on the TLC plate with a 1 cm gap between each dotted sample. The plate was placed inside the chamber until the separation process was completed. The determination of flavonoid content was performed by densitometer (Camag TLC scanner 3, Switzerland) at a wavelength of 254 nm and the concentration was expressed as mg rutin/100 mL of juice.

2.7 Antioxidant activity

2,2 Diphenyl-1-picrylhydrazyl (DPPH) assay

The scavenging activity of DPPH radical was measured based on the method described in Kenny et al. (2013) with modifications. DPPH solution was made by diluting 8 mg of DPPH (Sigma-Aldrich) with 50 mL of methanol. A stock solution of the sample was prepared by dilution of 5 mg juice in 5 mL of 100% methanol. Five different concentrations (75-375 ppm) of each sample were performed by pipetting 15, 30, 45, 60, and 75 µL of the stock sample across the 96-well plate. A methanol blank was also used as a negative control. The DPPH solution (75 µL) was added to the plate and then topped with methanol to the final volume of 200 µL. The mixture was stored in the dark for 30 minutes at room temperature before its absorbance at 515 nm was measured using ELISA plate reader (ELx808 BioTek, Vermont, USA). The results were expressed in IC_{50} scavenging activity.

2,20-azino-bis (3-ethylbenzthiazonline-6-sulfonic acid) (ABTS) assay

The determination of ABTS scavenging was carried out according to the method described in (Jorjong et al., 2015) with some modification. The stock solution of the ABTS reagent was prepared by mixing 7.4mM ABTS solution and 2.6mM potassium persulfate solution with the ratio of 1:1 (v/v). The ABTS solution was made by diluting 0.018 g of ABTS with 5 mL of methanol, while the potassium persulfate solution was prepared by diluting 3.6 mg of potassium persulfate with 5 mL of methanol. The mixture of ABTS and potassium persulfate solutions was topped with methanol to reach the total volume of 25 mL. The solution was then incubated in the dark for 12-16 hours.

The stock solution of the sample that had been prepared for the measurement of scavenging activity using the DPPH method was also used for this ABTS scavenging test. The varying volume of the stock solution was pipetted into a well plate to form five different concentrations. Methanol was used as the negative blank. ABTS stock solution was added to the plate with a volume of 125 µL and topped with methanol to the final volume of 200 µL. The mixture was left in the dark for 30 minutes at room temperature before absorbance measurement was conducted at 650 nm using ELISA plate reader.

All the measurements were conducted in triplicates. The IC50 values and statistical analysis were determined using GraphPad Prism 8.0. All data except antioxidant activity will be express in mean ± standard deviation value.

3 Results and discussions

3.1 Physical characteristics of bignay juice

The physical characteristics measured were color parameters of fresh juice, pH, and total soluble solid content, and the results are given in Table 1. From the color parameter data, bignay fruit juice provided a dark-colored liquid as shown by the low Lightness and Chroma value. Dark-colored fruits are generally known as a potential source for anthocyanin (Wu et al., 2012). The pH value obtained indicates that bignay fruit juice can be categorized as a high acid food (pH < 3.6). The pH of foods and beverages is an important factor that affects their shelf life and processing condition. The lower pH level of the bignay fruit juice may permit short pasteurization time at relatively moderate temperatures since pathogenic bacteria do not grow at low pH (Achir et al., 2016; Branco et al., 2016; Park & Kang, 2013). In addition, pH also influences the stability of compounds in the food product. Anthocyanin has been reported to be susceptible toward pH, where acidic condition provides higher stability of this compound (Fredes et al., 2018; Jiang et al., 2015). The pH value obtained in this study (3.45) was equivalent to the pH level of Phuchong cultivars as reported in Butkhup & Samappito (2008). The pH of bignay fruit and its products that have been reported in literature ranged from 3.40 to 3.50 (Butkhup & Samappito, 2008; Chaikham, 2015; Chaikham et al., 2016; Sripakdee et al., 2015).

The total soluble solid (TSS) of fresh bignay juice obtained in this study (14.67 °Bx) was comparable with those reported in Butkhup & Samappito (2008) for Sangkrow No.2 cultivar (14.50 °Bx) and Phuchong cultivar (14.80 °Bx). Higher TSS value (16.50 °Bx) was reported by Sripakdee et al. (2015) and Butkhup & Samappito (2008) for Sangkrow No.5 cultivar, while lower values (12.77 and 12.35 °Bx) were respectively reported in Chaikham et al. (2016) and Butkhup & Samappito (2008) for Sangkrow No.4 cultivar. In terms of pH value and total soluble solid, bignay juice originated from Indonesia has similar characteristics to the Phuchong cultivars grown in Thailand.

3.2 Chemical characteristics of bignay juice

Results of chemical analysis of bignay juice were presented in Table 2. Total phenolic (TP) content of bignay juice used in this study was much higher (1202.5 mg/100 mL) than the value reported (274.65 mg GAE/100 mL) in Chaikham et al. (2016). A lower amount of TP was also reported in several studies (Chaikham, 2015; Jorjong et al., 2015; Sripakdee et al., 2015). These authors reported TP contents of 390.67 mg/100 mL, 345.68 mg GAE/100 g dry weight (DW), and 337.52 mg GAE/100 mL respectively. On the other hand, higher TP content (1978.38 mg/100 g DW) was reported in the crude extract by Barcelo et al. (2016). It is important to note that the TP content found in this study was within the range reported by Butkhup & Samappito (2008) who reported the TP values

Table 1. Physical characteristics of bignay juice.

Parameter	Value
	Mean ± SD
Color:	
L*	25.885 ± 0.035
a*	-0.470 ± 0.010
b*	1.460 ± 0.370
Chroma (C*)	1.537 ± 0.354
Hue angle (°h)	-1.243 ± 0.071
pH	3.453 ± 0.031
Total soluble solids (°Bx)	14.667 ± 0.094

of 8.37-13.56 mg GAE/g DW (837-1356 mg GAE/100 mL). The addition of water during the extraction process might become a contributing factor that caused the TP content of bignay juice was lower in several studies (Chaikham, 2015; Jorjong et al., 2015). It is important to note that in our study, the extraction of juice from bignay fruits was accomplished without the addition of water.

The total phenolic content of bignay juice found in this study is higher than those of other juices such as pomegranate juice (230.86 mg GAE/100 mL) (Yildiz et al., 2009), grape juice (2135-2647 mg/L) (Padilha et al., 2017), and mulberry juice (266.8 mg GAE/100 g DW) (Kamiloglu et al., 2013a). A high amount of TP found in bignay juice can be explained by the major phenolic acid in this fruit, which is gallic acid and vanillic acid (Butkhup & Samappito, 2008; Jorjong et al., 2015). In comparison, the major phenolic acid reported in mulberry fruit was chlorogenic (Kamiloglu et al., 2013b).

As mentioned before, the dark-colored fruit potentially contains high anthocyanin content. This was in agreement with the result obtained in this study, where the anthocyanin content of bignay juice was 426.602 mg/100 mL of juice. The anthocyanin content found was significantly higher than those reported previously by Chaikham (2015) (59.46 mg/100 mL) and Chaikham et al. (2016) (44.32 mg/100 mL) for bignay juice, Barcelo et al. (2016) (131.42 mg/100 g DW for extract), and Butkhup & Samappito (2008) (141.94 mg/100 g DW for fruit). The varying level of anthocyanin can be due to the difference in maturity or ripeness during harvest, considering that at the earlier stage of maturity bignay fruits display bright red-color, while at the later stage the color shifts to dark-purple.

Vitamin C content of bignay juice is represented in ascorbic acid equivalent. The result obtained in this study (48.93 mg/100 mL) is higher than the ascorbic acid content of bignay juice reported by Chaikham (2015) and Chaikham et al. (2016). In these two studies, the vitamin C contents reported were 19.05 mg/100 mL and 26.14 mg/100 mL respectively. The vitamin C content of bignay juice found in this study was comparable to the ascorbic acid content of orange juice (ranged from 249.81-612.75 mg/L) (Sánchez-Moreno et al., 2003). Therefore, this result indicates that bignay juice could become a potential source of vitamin C for the human diet and can be used as a substitute for orange juice which can be expensive in certain regions.

In contrary to other compounds reported previously, the flavonoid content of bignay juice found in this study (3.78 mg/100 mL) was significantly lower than those reported by others. A study conducted by Butkhup & Samappito (2008) reported that the amount of rutin in bignay fruit ranged from 16.61-25.54 mg/100 g fruit weight (FW) and the total flavonoid content was in the range of 120.39-397.90 mg/100 g FW. A study by Jorjong et al. (2015) on bignay cultivars from Thailand reported total flavonoid content ranged from 138.60-289.60 mg catechin (CE)/100 g DW. The significant difference in the total flavonoid content found in this study and those reported by others might be attributed to the difference in the type of varieties and growing conditions. Another contributing factor might be the analytical method, mainly the use of different types of compounds as the equivalent standard.

The antioxidant activity of bignay juice (Figure 1) was measured by DPPH and ABTS method. The principal of both methods was based on the reaction between hydrogen donor antioxidants with free radicals that would result in a discoloration of those radical substances (Pisoschi & Negulescu, 2011). The antioxidant activity of bignay juice was expressed by the half-maximum inhibitory concentration (IC50) of a sample that measures the concentration needed by a sample to inhibit 50% of the free radical reaction. The IC50 found in this study for bignay juice was 0.11 mg/mL for the DPPH method and 0.126 mg/mL for the ABTS method. The value of IC50 has a negative correlation with the antioxidant activity, which means that a smaller IC50 value corresponded to higher antioxidant activity. Previous research evaluating the antioxidant activity of bignay fruit grown in Manipur, India, exhibited lower antioxidant activity with an IC50 value of 1717 µg/mL (1.717 mg/mL) (Khomdram & Devi, 2010). However, relatively similar value have been reported with other types of fruit such as *Litchi chinensis* fruit pulp extract (0.102 mg/mL) (Prakash et al., 2011), *Radix Angelicae Sinensis* extracted by ethyl acetate (0.13 mg/mL) (Li et al., 2009), *Angelicae Sinensis* extract (0.093 mg/mL by water extraction and 0.15 mg/mL by 95% ethanol extraction). The antioxidant activity of bignay juice found in this study is relatively similar to the antioxidant activity of *Radix Angelicae Sinensis* extract which many consider

Table 2. Bioactive compounds in bignay juice.

Compounds	Value
	Mean ± SD
Total Phenolic (mg/100 mL)[a]	1202.500 ± 0.001
Total Monomeric Anthocyanin (mg/100 mL)[b]	426.602 ± 3.723
Vitamin C (mg/100 mL)[c]	48.931 ± 0.001
Total Flavonoid (mg/100 mL)[d]	3.78 ± 2.114
Antioxidant activity:	
DPPH method-IC50 (mg/mL)	0.110
ABTS method-IC50 (mg/mL)	0.126

[a]gallic acid equivalent; [b]cyanidin-3-glucoside equivalent; [c]ascorbic acid equivalent; [d]rutin equivalent.

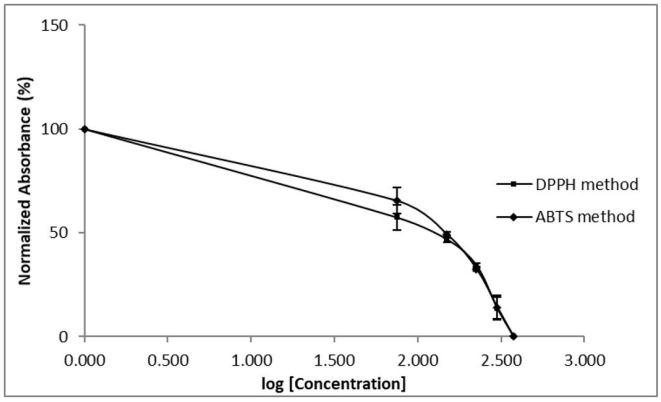

Figure 1. Radical scavenging activity of bignay fruit with DPPH and ABTS methods.

as a superior traditional Chinese medicine with various health benefits such as promoting the blood circulations, regulating menstruations, and relieving pain (Li et al., 2009).

4 Conclusions

Bignay juice was found to contain high amounts of total phenolic, anthocyanin, and vitamin C and has relatively high antioxidant activity. Therefore, bignay fruit can be used as a natural source of antioxidant and as a potential source of raw material for juice processing industries.

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