



Research Article

Extraction and Stability Test of Anthocyanin from Buni Fruits (*Antidesma Bunius* L) as an Alternative Natural and Safe Food Colorants

Fera Amelia^{1,2}, Galih Nur Afnani^{1,2*}, Arini Musfiroh^{1,2}, Alia Nur Fikriyani^{1,2}, Sisca Ucche^{1,2} and Mimiek Murrukmihadi¹

¹Faculty of Pharmacy, Gadjah Mada University, Yogyakarta 55281, Indonesia

²Prophetic Study Club, Faculty of Pharmacy, Gadjah Mada University, Indonesia

ARTICLE INFO

Received 30/06/2012

Received in revised form 05/07/2012

Accepted 07/07/2012

Available online 08/07/2012

ABSTRACT

Synthetic dyes have widely been used in food products. However, the safety issues regarding the use of synthetic dyes arise. Buni (*Antidesma bunius*) which contains anthocyanins can be an alternative of synthetic dyes. Anthocyanins are red, blue, or violet natural colorants from many types of plants. The aim of the study was to determine the best extraction process and to evaluate the stability of anthocyanins extracted from the Buni fruits. Different combinations of the solvents namely aquadest, ethanol, hydrochloride acid, and citric acid were used to extract the anthocyanins. The most appropriate combination of solvent was determined by the analysis of total anthocyanins content and color intensity using UV-Visible spectrophotometer. The stability of anthocyanins was evaluated using different pH values and temperature. Ethanol at 70% and citric acid at 3% was the most appropriate combination for the extraction with the highest total concentration of anthocyanin (0.58%) and color intensity (0.324). The stability of the anthocyanins from Buni was affected by the value of pH, which the most stable condition was set at pH values of 4-5.

Keywords: *Antidesma bunius*, anthocyanins, natural food colorants, extraction, stability

1. Introduction

People cannot be separated from the use of food additives in their everyday lives, including the food colorants. The appearance of the food, especially its color is important factor that could affect the taste. Hence, the food colorants become so important in the process of food making. Synthetic colorants have been widely used in the food industry. According to Jenie *et al.* (1994), the use of synthetic colorants for the food or beverages can cause toxic and carcinogenic. Due to those negative effects of the use of synthetic dyes, it becomes important to replace synthetic colorants with natural colorants derived from plants.

Buni fruits (*Antidesma bunius* L.) (Figure 1) are wild plants belonging to the *Euphorbiaceae* family. In Indonesia, the use of buni fruits as several medicinal plant extracts has been practiced long before the recorded history (Eun-Mi and Jae-Kwan, 2005). Buni



Fig. 1. Buni fruits (*Antidesma bunius* L.)

fruits are rich in nutritional components such as carbohydrates, sugars, organic acids, proteins, minerals,

* Corresponding author email: galih.nur.a@mail.ugm.ac.id

vitamins, anthocyanins, flavonoids and phenolic acids (Butkhuip and Samappito, 2008). Anthocyanins which belong to the widespread class of phenolic compounds, i.e flavonoids are natural pigments which cause red, orange, purple and blue flowers and abundant in fruits (Hidayat & Elfi, 2006). Anthocyanins have found considerable potential in the food industry as safe and effective food colorants (Strack and Wray, 1994). Anthocyanins have many health benefits including the enhancement of sight acuteness (Lamer-Zarawska & Oszmiański, 1994), anticarcinogenic activity (Katsube et al., 2003), antioxidant capacity (Gabrielska et al., 1999; Kähkönen et al., 2003; Kong et al., 2003; Lamer-Zarawska & Oszmiański, 1994), antiulcer activity (Cristoni & Magistretti, 1987), and the maintenance of normal vascular permeability (vitamin P) (Lamer-Zarawska & Oszmiański, 1994). Haripyaree et al. (2010) have reported that methanolic extract of Buni fruits which are grown in Manipur, India, showed higher antioxidant activity than the other fruits, with an average IC₅₀ value of 100.08 µg/ml. Hence, the use of Buni fruits (*Antidesma bunius* L) as natural food colorants will be very beneficial because the fruits do not only possess a beautiful appearance but also have benefits on health.

However, the use of anthocyanins as colorants has been limited because of their lack of stability. This study determined the best extraction process and evaluated the stability of the anthocyanins extracted from Buni fruits so it can be used as alternative food colorants that is safer than the synthetic dyes.

2. Materials and Methods

2.1. Materials

Fresh Buni fruits with red color were obtained from the district of Kulon Progo, Yogyakarta. The fruits were peeled and mashed. All reagents and solvents used were of analytical grade. The experiment was carried out in triplicate.

2.2. Extraction of anthocyanins

A modified method from the methodology of Lees and Francis (1972) was used. Aquadest and ethanol 70% in water were used as solvents, acidified with 1% hydrochloric acid (HCl), or with 3% citric acid to obtain four kinds of solvent. Fifty grams (50 g) of mashed fruits were transferred to the Beaker glass and added to 500 ml of each solvent, separately. Samples were extracted at room temperature for overnight. Extracts were filtered under vacuum filter and concentrated to dryness using a vacuum rotary evaporator. The dried samples were stored in sealed dark bottles at refrigerator temperature (10 ± 2 °C) until being used for analysis. Extracts obtained were weighed and compared with initial weight of fresh Buni fruit (Tensiska et al., 2006).

$$\text{Yield (\% w/w)} = \frac{\text{weight of anthocyanin extract (g)}}{\text{weight of fresh Buni fruit (50 g)}} \times 100\%$$

2.3. Qualitative determination of anthocyanins

Qualitative analysis of anthocyanins were performed using thin layer chromatography (TLC). Analysis was carried out using silica gel F 254 with the solvents of ethyl acetate-glacial acetic acid-formic acid-water (100:11:11:26 v/v/v/v) to determine the existence of anthocyanin in extract.

2.4. Determination of total anthocyanins content

The total anthocyanins content was determined according to pH differential method by Wrolstad and Giusti (2001). Each sample was dissolved in potassium chloride-hydrochloric acid buffer solution pH 1.0 and sodium acetate trihydrate (CH₃COONa.3H₂O) buffer solution pH 4.5. The absorbance was determined at 510 nm in a UV-Visible spectrophotometer. The dilution factor for each sample was firstly determined by dissolving the sample in KCl buffer pH 1.0 until its absorbance at 510 nm obtained less than 1.2 versus the KCl buffer pH 1.0 as blank. Sample was then dissolved in KCl buffer pH 1.0 (allowed to stand for another 15 min.) and CH₃COONa.3H₂O buffer pH 4.5 (allowed to stand for another 5 min.) based on the dilution factor. The absorbance for each sample was then read versus the buffer solution pH 1.0 and buffer solution pH 4.5 as the blank at λ = 510 nm (for the cyanidin 3-glucoside) and λ = 700 nm (for correction factor). The final absorbance (A) was calculated using the following formula:

$$A = (A_{510} - A_{700})_{\text{pH } 1.0} - (A_{510} - A_{700})_{\text{pH } 4.5}$$

Total anthocyanins concentration was then calculated using the formula:

$$\text{TAC} = \frac{A}{\epsilon \times L} \times \text{MW} \times \text{DF} \times \frac{V}{W_t} \times 100\%$$

where TAC is total anthocyanins content (mg. 100 g⁻¹ of sample); ε is molar absorption coefficient of cyanidin 3-glucoside (226,900 L (mol.cm)⁻¹); L is width of cuvette (1 cm); MW is molecular weight of cyanidin 3-glucoside (449.2 g. mol⁻¹); DF is dilution factor; V represents final volume or sample volume after dilution (L); and W_t is weight of original extract (g).

2.5. Determination of color intensity

A method from FAO (1984) was used to determine the color intensity of anthocyanins from Buni fruits. Citric acid-dibasic sodium phosphate buffer solution pH value of 3 was used for dissolving the samples. Firstly, the maximum wavelength was measured by diluting 20 mg sample in 25 ml volumetric flask using citric acid - dibasic sodium phosphate buffer solution pH 3. Furthermore, the absorbance was measured such a way that the absorbance value is in the range of 0.2 to 0.7. Citric acid - dibasic sodium phosphate pH 3 buffer solution was used as a blank. Absorbance (A) of the other samples were prepared with the same method and measured at the same maximum wavelength, as determined before.

Stability test of anthocyanins

The stability of the anthocyanins extracted from Buni fruits was evaluated using different pH values. A 2.0 ml aliquot of each sample was dissolved in 100 ml of citric acid buffer solution with 3 different values of pH (pH 4.0; 5.0; and 7.0). The absorbance of each sample was subsequently measured at wavelength of 510-550 nm.

3. Result and Discussion

3.1. Extraction of anthocyanins

The anthocyanins were extracted with various combinations of aquadest and ethanol 70% in water, acidified with 1% hydrochloric acid (HCl) and 3% citric acid. Aquadest and ethanol were used because of the good solubility of anthocyanins in polar solvents, according to the principle of "like dissolve like". The acids were added to enhance its stability. The greatest yield of anthocyanins extraction from Buni fruits was obtained from ethanol 70% acidified with citric acid solvent (Table 1). Extraction with solvent containing HCl resulted in a lower yield value than that using citric acid combination. This can be explained by the use of HCl, which may cause pigment degradation during concentration, especially the occurrence of acid hydrolysis of labile acyl and sugar residues (Strack and Wray, 1989). This degradation was reduced with the use of weaker organic acids such as citric acid.

Table 1. The yield percentage (%) of anthocyanin extracted from Buni fruits (*Antidesma bunius* L.) with various combinations of solvents

Solvent	Extract (g)	Yield (% w/w)
Distilled water + HCl 1%	0.21	1.85
Distilled water + citric acid 3%	6.66	3.3
Ethanol 70% + citric acid 3%	8.36	41.8
Ethanol 70% + HCl 1%	0.57	2.85

The qualitative test using thin-layer chromatography (TLC) was carried out in order to ensure the existence of anthocyanin in extracts. TLC results showed that Buni fruits extract contained anthocyanins. This was proven by the appearance of red spots on the TLC plate under visible light. This result was in agreement with that reported by Wagner and Bladt (1978), stating that anthocyanin shows red to blue-violet under visible light.

3.2. Determination of total anthocyanins contents and color intensity

The total anthocyanins content in Buni fruits extract could be determined using visible spectrophotometry. Figure 2 showed that total anthocyanin contents varied from 0.08 % to 0.23 % w/w of sample using distilled water as extracting solvent, and from 0.51 % to 0.58 % w/w of sample using ethanol 70%. These results are close to those reported by Butkhup and Samappito (2011), reporting that the level of

anthocyanins in Buni fruits extracted using aqueous methanol containing 1,2 M HCl was 141,94 mg/100 g (0,14% w/w). The slight difference between both results can be explained by the use of different cultivar, crop time, maturation stage, as well as the other factors such as solvent and extraction method.

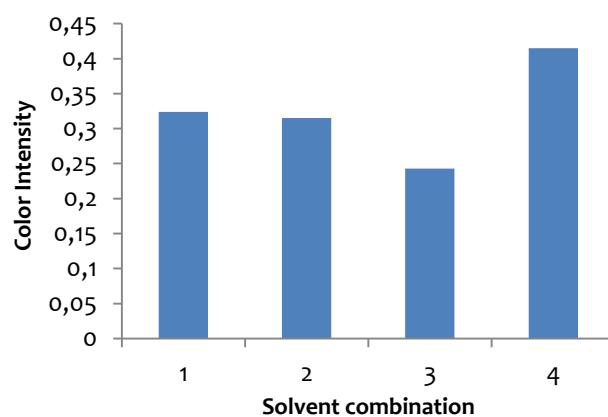
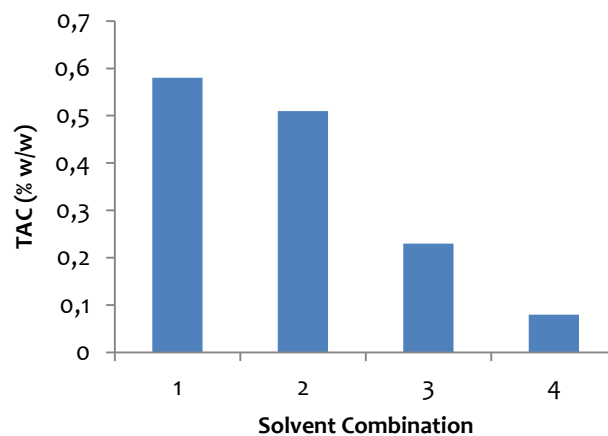


Fig. 2. Total Anthocyanins Content (TAC) and color intensity of anthocyanin extracts: (1) ethanol 70%-citric acid extract (2) aquadest-citric acid extract (3) ethanol 70%-HCl extract, and (4) aquadest-HCl extract. The optimum solvent for anthocyanin extraction from Buni fruit based on TAC and color intensity was ethanol 70% acidified with citric acid 3%.

Ethanol 70% gave the greater total anthocyanin contents than distilled water. Ethanol is a good solvent for polyphenol extraction (Shil *et al.*, 2005). In preparing anthocyanin containing phenolic groups, an acidified organic solvent is used. Metivier *et al.* (1980) compared the efficiency of extraction with three different solvents (methanol, ethanol, and water) and found that ethanol was more effective than water when used for anthocyanin extraction from grape pomace.

Anthocyanins in the form of the flavylium cation show strong absorbance in the visible region between the wavelengths of 465 and 530 nm. The color intensity was determined at the maximum wavelength of 515 nm. The results of color intensity showed that the color intensity of Buni fruits extract was 0.243 to 0.415. The highest intensity was found in anthocyanin extracted with ethanol 70% acidified with HCl.

Solvent acidified with citric acid showed the higher total anthocyanin contents than solvent acidified with HCl. This difference could be explained by the occurrence of pigment degradation due to the use of solvent containing HCl. HCl could cause the hydrolysis of labile acyl groups, co-pigment, or metal complexes that are part of the native form of the anthocyanin contributing for its stability. The use of citric acid, as weaker organic acids, may minimize the degradation of pigments. Besides, citric acid also less corrosive than HCl. The extraction of anthocyanin using ethanol acidified with citric acid (0,01 %) instead of HCl was also reported by Main *et al.* (1978).

It could be concluded that the most optimum solvent in extraction of anthocyanins from Buni fruits was ethanol 70% acidified with citric acid 3%. Total anthocyanins content obtained of 0.58% w / w with the color intensity of 0.324. In fact, the highest of color intensity was showed from anthocyanin extracted by solvent ethanol 70% and HCl. However, the main goal in the extraction process was the highest total anthocyanin content. Accordingly, ethanol 70% acidified with citric acid 3% might be the optimum solvent compared with the others.

3.3. Stability of Anthocyanin

Based on stability studies, anthocyanins from Buni fruit were stable under acidic media at low pH values (pH 4 and 5) (Figure 3).

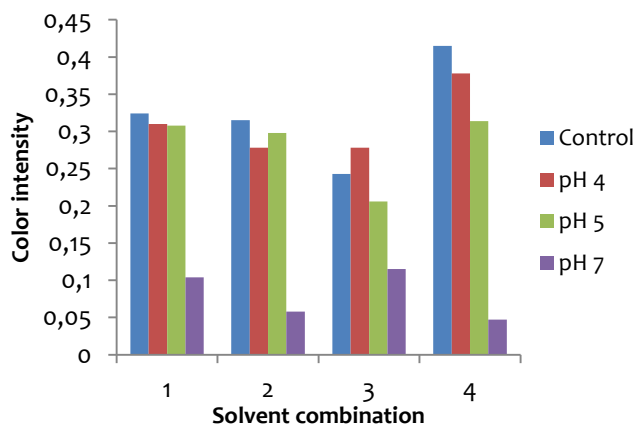


Fig. 3. Stability of anthocyanins toward pH and temperature: (1) ethanol 70%-citric acid extract, (2) aquadest-citric acid extract, (3) ethanol 70%-HCl extract, and (4) aquadest-HCl extract. Anthocyanin was stable at pH 4 and 5 (acidic pH).

Anthocyanins are easily susceptible to pH changes due to the ionic nature of anthocyanin. Anthocyanins exist as four equilibrium forms (figure 4), namely the quinonoidal base, the flavylium cation, the carbinol (pseudobase) and the chalcone. Under low pH, the anthocyanin exists primarily in the the form of flavylium cation in red. As the pH is raised (>5), a rapid loss of proton occurred to form quinoidal base that tend to become blue or violet. In addition, the increasing of pH causes the hydration of the flavylium cation to form a carbinol (pseudobase) or chalcone which are colorless (Cavalcanti *et al.*, 2011).

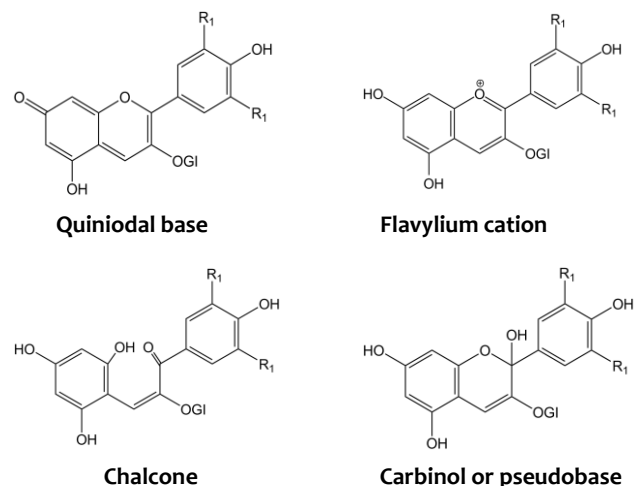


Fig. 4. Four equilibrium forms of anthocyanin

Color intensity of anthocyanin extracts decreased when dissolved in buffer pH 4 and 5 except ethanol-HCl extract. At pH 4-6, the red flavylium cation hydrated to yield the colourless carbinol, which could be due to decreasing of color intensity. However, color intensity at pH 4 and 5 had no significantly difference with control solution. Whereas at pH 7, color intensity of anthocyanin extracts decreased significantly than intensity of control solution. On the other hand, these anthocyanins are stable at acidic pH (≤ 5) and unstable at pH 7. Laleh *et al.* (2006) have reported that the increasing of pH or temperature is able to decrease the stability of anthocyanin in four *Berberis* species.

Anthocyanin extracts from Buni fruits have been applied for making jelly. The colorless jelly turned red after being added with anthocyanin extracts. It showed that anthocyanin extract from Buni fruits was a potential natural dyes.

4. Conclusions

Among the four extraction solvents, ethanol 70% acidified with citric acid 3% was the most optimal solvent in the extraction of anthocyanins from Buni fruits. Anthocyanins showed the greatest stability in acidic pH (4 and 5).

5. Acknowledgement

The authors are grateful to DP2M DIKTI for the financial support.

References

- Butkhup, L., and Samappito S. An analysis on flavonoids contents in Mao Luang fruits of fifteen cultivars (*Antidesma bunius*), grown in Northeast Thailand. *Pakis. J. Biol. Sci.* **2008**, 11, 996-1002.
- Cavalcanti, N.R., Diego T. Santos, and Maria-Angela A. M. Non-thermal stabilization mechanisms of anthocyanins in model and food systems—An overview. *Food Research International.* **2011**, 44, 499-509.

- Cristoni A. and Magistretti M.J. Antiulcer and healing activity of *Vaccinum padi* folium anthocyanosides. *Farmaco.Ed. Prat.* **1987.** 42, 29–43.
- Eun-Mi C. and Jae-Kwan H. Screening of Indonesian medicinal plants for inhibitor activity on nitric oxide production of RAW264.7 cells and antioxidant activity. *Fitoterapia.* **2005.** 76, 194-203.
- FAO. *Specifications for Identity and Purity of Food Colours.* Rome: FAO of The United Nations. **1984.**
- Francis, F.J. Analysis of Anthocyanins In: *Anthocyanins as Food Colors.* New York: Academic Press Inc. **1982.**
- Gabrielska J., Oszmiański J., Komorowska M., and Langner M. Anthocyanin extracts with antioxidant and radical scavenging effect. *Z. Naturforsch.* **1999.** 54, 319–324
- Harborne J.B. and Grayer R.J. The anthocyanins. In: *The flavonoids: advances in research since 1980* (Harborne JB. ed.). London: Chapman and Hall. **1988.** 1–20.
- Haripyaee A., Guneshwor K., and Damayanti M. Evaluation of antioxidant properties of some wild edible fruit extracts by cell free assays. *Ejeafche.* **2010.**9: 345-350.
- Hidayat, Nur and Elfi Anis. *Membuat Warna Alami.* Surabaya : Trubus Agrisarana. **2006.**
- Jeenphakdee, M and Samappito S. Optimization of an Analytical Method for Determining Flavonoid Content of Ma-mao juice. *J Sci Technol MSU.* **2008.** Vol. 28 No. 2
- Jenie, B.S.L., Helianti, and S. Fardiaz. Pemanfaatan Ampas Tahu, Onggok dan Dedak untuk Produksi Pigmen Merah oleh *Monascus purpureus.* *Buletin Teknologi dan Industri Pangan.* **1994.**5 (2): 22-29
- Kähkönen M.P., Heinämäki J., Ollilainen V., and Heinonen M. Berry anthocyanins: isolation, identification and antioxidant activities. *J. Sci. Food Agric.* **2003.** 83,1403–1411.
- Katsube N., Iwashita K., Tsushuda T., Yamaki K., and Kobori M. Induction of cancer cells by bilberry (*Vaccinium myrtillus*) and the anthocyanins. *J. Agric. Food Chem.* **2003.** 51, 68–75.
- Kong J.M., Chia L.S., Goh N.K., Chia T.F., and Brouillard R. Analysis and biological activities of anthocyanins. *Phytochem.* **2003.** 64, 923–933.
- Laleh, G.H., Frydoonfar H., Heidary R., Jameei R., and Zare S. The Effect of Light, Temperature, pH and Species on Stability of Anthocyanin Pigments in Four *Berberis* Species. *Pakistan Journal of Nutrition.* **2006.** 5 (1), 90-92
- Lamer-Zarawska E. and Oszmiański J. New investigations of flavonoids bioactivity. *Wiad. Ziel.* **1994.** 3, 11–13.
- Lees, D. H. And Francis, F. G. Standardization of pigment analysis in cranberries. *Hortscience.* Alexandria. **1972.** 7 (1), 83-84.
- Main, J. H., Clydesdale, F. M., and Francis, F. J. Spray Drying Anthocyanin Concentrates for Use as Food Colorants. *J. Food. Sci.* **1978.** 43, 1693-1697.
- Metivier, R.P., Francis, F.J., and Clydesdale, F.M. Solvent extraction of anthocyanins from wine pomace. *J. Food Sci.* **1980.** 45, 1099-1100.
- Saija. Anthocyanin as Natural Food colours – Selected Aspects. In: *Food Chemistry* (Bridle, P. and Timberlake, C.F. eds.). **1994.** 58 (1-2), 103 – 109.
- Shil, J., Nawaz, H., Pohorly, J., Mittal, G., Kakuda, Y and Jiang, Y. Extraction of polyphenolics from plant material for functional foods-engineering and technology. *Food Rev. Int.* **2005.** 21, 139-166.
- Strack, D. and Wray, V. Anthocyanins. In: *Methods in Plant Biochemistry*, Vol. 1, Plant Phenolics (P.M. Dey and J.B. Harborne. eds.). Academic Press, San Diego. **1989.**
- Strack D. and Wray V. The anthocyanins. In: *The Flavonoids: Advances in Research Since 1986.* (J.B. Harborne, ed.). Chapman and Hall. **1994.**
- Syah D., Utama, S., and Mahrus, Z. *Manfaat dan Bahaya Bahan Tambahan Pangan.* Bogor: Himpunan Alumni Fakultas Teknologi Pertanian IPB. **2005.**
- Tensiska, Sukarminah, E. and Natalia, D. *Ekstraksi pewarna alami dari buah arben (Rubus idaeus (linn.)) dan aplikasinya pada sistem pangan.* Bandung: Alumni Program Studi Teknologi Pangan, Fakultas Pertanian UNPAD. **2006.**
- vonElbe, J.H. and Schwartz, S. J. Colorants. In: *Food Chemistry* third edition (O.R. Fennema, ed.). New York: Marcell Dekker. **1996.**
- Wagner, H. and S. Bladt. *Plant Drug Analysis.* New York: Springer. **1978.**
- Wrolstad, R.E. and Giusti, M.M. Characterization and measurement of anthocyanins by UV-visible spectroscopy. In: *Food Analytical Chemistry* (Wrolstad, R. E. ed.). New York: John Wiley & Sons, Inc., **2001.** Unit F1.2.