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Total Anthocyanins Content in Various Extract of Butterfly Pea (*Clitoria ternatea* Linn) Flower.

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ABSTRACT

The flower of butterfly pea (*Clitoria ternatea* Linn) contains anthocyanin which can be applied as an indicator of acid-base titration. This study aims to determine the effect of solvent and pH on total anthocyanins content in extracts of butterfly pea flower. Determination of total anthocyanins content was conducted by pH differential method. The highest total anthocyanins content was obtained in methanolic extract pH 1.0. Concluded that solvent and pH affect total anthocyanins content.

Keywords: Quantitative analysis, pH differential, solvent, pH

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INTRODUCTION

Butterfly pea (*Clitoria ternatea* Linn) flower contains anthocyanins, i.e. water-soluble flavonoids pigments in plants [1]. The butterfly pea flowers have been used as natural dyes, nutritious food ingredients, and medicines, traditionally. Antocyanins have activity as an antioxidant, eye tissue protection [2], antidiabetes, anti-inflammatory, protect the immune system, anti-platelet [3], analgesics and cancer prevention [4]. In Thailand, dried flowers have been sold commercially, and its extracts have been applied to various products, such as shampoo and lotion. While, people of Kerala, India and the Philippines consume fresh flowers [5].

Butterfly pea flower is classified as a flower with relative high anthocyanins content [6]. Six major anthocyanins ternatins (A1, A2, B1, B2, D1 and D2) [7] of butterfly pea flower are characterized as malonylated delphinidin 3,3',5'-triglucosides having 3',5'-side chains with alternating D-glucose and *p*-coumaric acid [8]. Butterfly pea extract which contain anthocyanins can be applied as acid-base indicator [9]. This study aims to determine the effect of solvent and pH on total anthocyanins content in extracts of butterfly pea flower.

MATERIALS AND METHODS

Materials

Butterfly pea flowers were obtained from Subang District, West Java, Indonesia. The plants were identified at Herbarium Bandungense School of Biological Sciences and Technology, Bandung Institute of Technology with No. 1692/K01.14.2/PP.2.4.2/2015. Methanol, ethanol, hydrochloride acid, sodium hydroxide, potassium chloride, and sodium acetate are analytical grade (Merck, Germany).

Extraction

Each of 10 g of butterfly pea were macerated for 24 h in 30 mL of methanol, ethanol, and aquadest with various at pH 1.0 and 2.0. All extract was filtered and centrifuged to obtain a clear extract. Color reaction of Fransworth method was conducted to phytochemical screening [10].

Determination of Total Anthocyanins Content

Total anthocyanins content was determined by the pH differential method as listed in Saptarini [11]. The dilution factor of pH 1.0 and 2.0 was 20 for methanolic and ethanolic extract, and 5 for aqueous extract.

Statistical Analysis

The mean ± standard deviation (SD) was presented for the results. Data comparisons between groups was analyzed by oneway ANOVA followed by t-Student test, with considered statistically significant at $p < 0.05$.

RESULTS AND DISCUSSION

Extraction

The extraction solvents were methanol, ethanol, and water, due to anthocyanins polarity. Acidified solvent pH 1.0 and 2.0 are used for extraction, because the acid breaks the vacuole cell walls, so anthocyanins is more easily extracted [12]. All extract color were red. This results was agreed with the literature, anthocyanins is purple to red in acidic and green to yellow in an alkaline solution [13]. The anthocyanins stability is influenced by pH, heat, light, oxygen, enzymes, sugar, the presence of other compounds, such as flavonoids, proteins, and minerals [13], pigment concentrations, copigmentation, hydroxy and methoxy group amounts [14]. Phytochemical screening showed that all extracts contain polyphenols, flavonoids, and anthocyanins. This result was agreed with the previous results [9].

Determination of Total Anthocyanins Content

The anthocyanin form is depent to pH, i.e. red oxanium form at pH 1.0 and colorless hemicetal form at pH 4.5. An increased pH reduces the color and concentration of the flavilium cation as it is hydrated by nucleophilic attack from water into a colorless carbinol form. The breaking of glycosidic bond produce labile aglikons and the opening of pyrillium ring produce a colorless carbinol and chalcone. The carbinol form lost the conjugated double bond between the A and B rings [15]. Total anthocyanins content were higher in extract pH 1.0 than pH 2.0 (Table 1). This results was appropiate to anthocyanins are most stable at pH 1.0 [15].

Table 1: Total Anthocyanins Content of Extract

Extract	pH	Total anthocyanins content (mg/L)
Methanol	1.0	145.17 ± 0.81
	2.0	118.23 ± 0.54
Ethanol	1.0	84.07 ± 0.43
	2.0	71.48 ± 0.87
Aqueous	1.0	58.06 ± 0.23
	2.0	54.55 ± 0.48

Anthocyanins dissolve in a polar solvent [1], such as water (1.00) with higher polarity compared to ethanol (0.56) and methanol (0.76) [16]. Highest total anhocyanins content was methanolic extract pH 1.0. This suggested that the anthocyanins polarity of the butterfly pea flower are similar to methanol. Most secondary metabolites are soluble in methanol [1], but have high toxic effects, such as blindness, metabolic acidosis, neurologic sequelae, and even death [17]. Ethanol is used as an alternative solvent, especially if the extract will be applied to herbal preparations or food additives, due to ethanol toxicity lower than methanol. Statistical analysis showed significant differences for the solvent variations at pH 1.0 (p value 4.84×10^{-12}) and pH 2.0 (p value 4.37×10^{-10}). Significant differences were also observed in pH variations for methanol (p value 3.19×10^{-6}), ethanol (p value 1.44×10^{-5}), and aquadest (p value 4.89×10^{-3}).

CONCLUSION

Solvent and pH affect total anthocyanins content of butterfly pea flower.

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REFERENCES

[1] Harborne JB. Phytochemical methods: A guide to modern techniques of plant analysis. Thompson Science, London. 1998. pp. 60-94.
[2] Ghosh D, Konishi T. Anthocyanins and anthocyanin-rich extracts: Role in diabetes and eye function. Asia Pac J Clin Nutr. 2007; 16(2): 200-8.
[3] Mukherjee PK., Kumar V, Kumar NS, Heinrich M. The Ayurvedic *medicine Clitoria ternatea* – From traditional use to scientific assessment. J Ethnopharm. 2008; 120(3): 291-301.
[4] Morris JB, Wang WL. Anthocyanin and potential therapeutic traits in *Clitoria, Desmodium corchorus, Catharanthus*, and *Hibiscus species*. Acta Hortic. 2007; 381-388.
[5] Lee PM, Abdullah R, Lee KH. Thermal degradation of blue anthocyanin extracts of *Clitoria ternatea* flower. 2nd International Conference on Biotechnology and Food Science IPCBEE. 2011; 7: 49-53.
[6] Vankar PS, Srivastava J. Evaluation of anthocyanin content in red and blue flowers. Int J Food Eng. 2010; 6(4): 9-18.
[7] Terahara N, Saito N, Honda T, Toki K, Osajima Y. Acylated anthocyanins of *Clitoria ternatea* flowers and their acyl moieties. Phytochemistry. 1990; 29(3): 949-53.
[8] Francis FJ. Miscellaneous colorants, in Natural Food Colorants, GAF Hendry and JD Houghton, Editors. Blackie and Son Ltd., New York.1992. pp. 242-47.

[9] Saptarini NM, Suryasaputra D, Nurmalia H. 2015. Application of butterfly pea (*Clitoria ternatea* Linn) extract as an indicator of acid-base titration. J Chem and Pharm Res. 2015; 7(2): 275-80.
[10] Fransworth N. Biological and phytochemycal screening of plants. J Pharm Sci. 1966; 1(5): 247-68.
[11] Saptarini NM, Fathi, Sofian FF. The effect of acetic acid in anthocyanins extraction from mangosteen (*Garcinia mangostana* L.) pericarp. Res J Pharm, Biol and Chem Sci. 2013; 4 (2): 213-20.
[12] Jackman RL, Smith JL. Anthocyanins and betalains, in Natural Food Colorants, GAF Hendry and JD Houghton, Editors. Blackie Academic and Professional, Great Britain, 1996.
[13] Rein M. Copigmentation reactions and color stability of berry anthocyanins. [Disertation]. Department of Applied Chemistry and Microbiology. University of Helsinki. Helsinki, Finland. 2005.
[14] Satyatama DI. Pengaruh kopigmentasi terhadap stabilitas warna antosianin buah duwet (*Syzygium cumini*). [Thesis]. Faculty of Agriculture. Bogor Agricultural University. West Java, Indonesia. 2008.
[15] Wrolstad RE, Acree TE, Decker EA, Penner MH, Reid DS, Schwartz SJ, Shoemaker CF, Smith D, Sporns P. Handbook of Food Analytical Chemistry: Pigments, Colorants, Flavors, Texture, and Bioactive Food Components. John Wiley and Sons Inc., New Jersey. 2005. pp. 156-67.
[16] Reichardt C. Solvents and solvent effects in organic chemistry. 3rd ed. Wiley-VCH Publishers. 2003.
[17] Pohanka M. Toxicology and the biological role of methanol and ethanol: Current view. *BIOMED PAPERS.* 2016; 160 (1): 54-63.