# ANALYSIS OF ANTHOCIANIN AND POLYPHENOL CONTENT OF BUTTERFLY PEA FLOWER (*Clitoria ternatea* L.) EXTRACT WITH DIFFERENT SOLVENTS

**Siti Samiyarsih[1*], Juwarno[1], Wiwik Herawati[1], Nur Fitrianto[2]**
[1]Faculty of Biology, Universitas Jenderal Soedirman, Purwokerto, Indonesia
[2]Research Center for Food Technology and Processing, National Research and Innovation Agency, Yogyakarta, Indonesia

*Email: siti.samiyarsih@unsoed.ac.id

**Abstract.** Butterfly pea flower (*Clitoria ternatea* L.) is a plant with economic value, containing anthocyanin and polyphenol compounds which have the potential to act as antioxidants. Extraction of butterfly pea flower anthocyanins can be done by maceration using water and ethanol, and phenolic extraction of butterfly pea flowers with ethanol. This research aims to determine the effect of different solvents on the anthocyanin and polyphenol content of butterfly pea flowers. The methods used surveys and selected random sampling with a total of 25 plants. Anthocyanin analysis on butterfly pea flowers was performed using the differential pH method, and polyphenol analysis was conducted using the Folin-Ciocalteu method. The results showed that different solvents had a significant effect on the total anthocyanin and polyphenol content in butterfly pea flowers. Based on the BNJ analysis, extraction using ethanol yielded the highest anthocyanin content of 24,08 ± 3,8 mg/100g, followed by aquades at 15,76 ± 1,55 mg/100g, and acetone at 9,01 ± 3,24 mg/100g. The BNJ analysis showed that extraction using acetone resulted in the highest total polyphenol content of 319,6 ± 21,1 mgGAE/g, followed by aquadest at 83,2 ± 12,9 mgGAE/g, and ethanol yielded the lowest total polyphenol content of 24,72 ± 12,7 mgGAE/g.

**Keywords:** anthocyanin, butterfly pea, polar solvent, polyphenol

## A. Introduction

Butterfly pea (Clitoria ternatea L.) has distinctive colored flowers such as purple, blue, pink, and white and is included in the Fabaceae family. This plant is spread in subtropical and tropical areas such as Indonesia, to America and Africa (Angriani, 2019). The butterfly pea flower is a part known for its secondary metabolite compounds which have benefits including anthocyanins and polyphenols. Secondary metabolites such as anthocyanins and polyphenols are produced through the secondary metabolite biosynthesis pathway. Butterfly pea flowers have polyphenol and anthocyanin compounds which are dyes that have antioxidant benefits (Kusbiantoro & Purwaningrum, 2018). Extraction of active compounds in plants can be done by solvent extraction. The selection of the type of solvent used for extraction is adjusted to the polarity of the desired compound. According to the principle of like dissolves like, a solvent will tend to dissolve a compound that has the same level of polarity (Mariana et al., 2018). Studies related to the use of various types of solvents for the extraction of anthocyanins and polyphenols in butterfly pea flowers have not been widely conducted, so research is needed on the use of various types of solvents to determine the type of solvent that is optimal in the extraction of anthocyanins and polyphenols in butterfly pea plants. This research aims to

determine the effect of different solvents on the anthocyanin and polyphenol content of butterfly pea flowers.

## B. Methods

This research used a survey method and a randomly selected sampling technique of leaves and flowers of butterfly pea plants (purposive random sampling). The butterfly pea flower samples were dried until they reached a constant weight. Butterfly pea flower extraction was carried out with ethanol, acetone, and aquadest with a ratio of simplicia and solvent of 1:10. A total of 100 g of butterfly pea flower simplicia was extracted with 1L of each solvent. The sample was macerated in a tightly closed glass container for 3 days then filtered to separate the filtrate and residue. The resulting extract was evaporated using a Rotary Vaccum Evaporator until a thick extract was obtained (Handayani et al., 2020).

1. Total Anthocyanin Analysis

A total of 1 g of butterfly pea flower extract from each solvent (water, ethanol, and acetone) was dissolved in 100 mL of the same solvent. A total of 5 mL of each extract was mixed with KCl buffer pH 1.0, left for 30-60 minutes, then the absorbance was measured using a UV-Vis spectrophotometer at wavelengths of 510 nm and 700 nm. For pH 4.5, a total of 5 mL of extract was mixed with sodium acetate buffer pH 4.5, left for 30-60 minutes, and the absorbance was measured using a UV-Vis spectrophotometer. The absorbance of the sample was determined and the total anthocyanin content was calculated according to the formula: $A = (A510 - A700)_{pH\ 1,0} - (A510 - A700)_{pH\ 4,5}$ Antosianin Total (mg/100 g) = $\frac{A \times MW \times DF \times V \times 1000}{\varepsilon \times L \times W}$

2. Total Polyphenol Content Measurement

Butterfly pea flower extract from each solvent as much as 10 mg was dissolved with the initial solvent until it reached 10 mL to obtain a 1000 ppm extract solution. 0.2 mL of extract solution, added 1 mL of 10% v/v Folin-Ciocalteu reagent, homogenized, and left for 8 minutes. After that, 3 mL of 7.5% $Na_2CO_3$ solution was added, homogenized, and left for 60 minutes at room temperature. The absorbance of the extract solution was measured using a UV-Vis spectrophotometer at a wavelength of 765 nm. Measurements were carried out three times for each butterfly pea flower extract solution with different solvents. The calculation of total polyphenol content was carried out using the formula: $TPC = \frac{c.\ v.\ fp}{g}$

## C. Results And Discussion

1. Anthocyanin content

The research results show that the total anthocyanin content of different solvents showed significant results. The results of anthocyanin content of butterfly pea flowers using different solvents showed significantly different results (Table 1)

Tabel 1. Test results of the effect of different types of solvents on the anthocyanin content of butterfly pea flowers

| Types of Solvents | Total anthocyanin content (mg/100 g) |
|---|---|
| Acetone | $9{,}01 \pm 3{,}25^{a}$ |
| Ethanol | $24{,}08 \pm 3{,}8^{c}$ |
| Aquades | $15{,}86 \pm 1{,}53^{b}$ |

Note: Numbers followed by different letters indicate significantly different results at the 0.05 test level

Ethanol extract of butterfly pea flowers produced the highest anthocyanin content, namely 24.08 ± 3.8 mg/100g, followed by aquadest 15.86 ± 1.53 mg/100g, and acetone extract of butterfly pea flowers produced the lowest anthocyanin, namely 9.01 ± 3.25 mg/100g (Table 3).

The results of this study are in accordance with the research of Trinovani et al. (2022), based on the average measurements and calculations carried out on the total anthocyanin content in black sticky rice tape extract samples, it shows that the extract using ethanol has the highest anthocyanin content (8.41 ± 0.22 mg/100g), compared to water solvents (3.65 ± 0.13 mg/100g) and methanol (4.11 ± 0.17 mg/100g). Meanwhile, the research of Le et al. (2019) stated that the use of 96% ethanol solvent in the extraction of Carrisa carandas fruit was able to produce a higher total anthocyanin content of 8.99 mg/g compared to water solvent which produced a total anthocyanin content of 5.65 mg/g.

Ethanol produces higher anthocyanin content in butterfly pea flower extraction compared to acetone and aquades due to differences in solvent polarity. Anthocyanin is a polar compound belonging to the flavonoid group while ethanol has a medium polarity that is very suitable for dissolving anthocyanin effectively (Siregar, 2009). Acetone is a semipolar solvent with a lower polarity than ethanol so it is less effective in dissolving anthocyanin effectively. Aquades has a very high polarity, but is often less effective because anthocyanin is less stable in water or more susceptible to degradation. Ethanol is able to penetrate cellular structures better than water, which allows for more efficient anthocyanin extraction.

2. Polyphenol content

The results of the analysis of the total polyphenol content of butterfly pea flowers against different solvents produced significant results (Table 2).

Table 2. Results test of the effect of different types of solvents on polyphenol content of butterfly pea flowers

| Type of solvent | Total polyphenol content (mg GAE/g) |
|---|---|
| Acetone | 319,6 ± 21,1[a] |
| Ethanol | 24,7 ± 12,7[b] |
| Aquadess | 83,2 ± 12,9[c] |

Note: Numbers followed by different letters indicate significantly different results at the 0.05 test level

The results of the BNJ test showed that the polyphenol content of butterfly pea flowers using different solvents showed significantly different results. The acetone extract of butterfly pea flowers produced a content of 319.6 ± 21.1 mgGAE/g, followed by the aqueous extract of butterfly pea flowers 83.2 ± 12.9 mgGAE/g, and the ethanol extract of butterfly pea flowers 24.7 ± 12.7 mgGAE/g (Table 2).

The results of this study are in line with the research conducted by Rifai et al. (2018), which explains that the total polyphenol content of avocado seeds using three different types of solvents, namely ethanol, acetone, and methanol, gives different results. The use of acetone in their study resulted in the highest total polyphenol content of 803.46 mg/100 g, while the lowest total polyphenol was found in the methanol solvent treatment with a result of 31.2 mg/100g. Research by Nursamsiar et al. (2023), also explains that the use of acetone as a solvent in phenolic analysis in kesambi leaves resulted in the highest phenolic content of 355.3 ± 3.74 mgGAE/g. Suryanto et al. (2011), added that the acetone extract of goroho banana produced the highest phenolic content, namely 181.87 ± 0.03 mgGAE/g, and the use of ethanol extract of goroho banana produced the lowest total phenolic content, namely 152.14 ± 0.01 mgGAE/g.

## D. Conclusion

The difference in the type of solvent used in the extraction of butterfly pea flowers affects the content of anthocyanins and total polyphenols. Extraction with ethanol produced the highest anthocyanins of 24.08 ± 3.8 mg/100g, while extraction with acetone produced the highest total polyphenols of 319.6 ± 21.1 mgGAE/g.

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## F. References

Andriani, D. & Murtisiwi, L., 2018. Penetapan Kadar Fenolik Total Ekstrak Etanol Bunga Telang (*Clitoria ternatea* L.) dengan Spektrofotometer UV Vis. *Cendekia Journal of Pharmacy*, 2(1), pp. 32-38.

Angriani, L., 2019. Potensi Ekstrak Bunga Telang (*Clitoria ternatea*) Sebagai Pewarna Alami Lokal Pada Berbagai Industri Pangan. *Canrea Journal*, 2(1), pp. 32-37.

Bishoyi, A. K. & Geetha, K. A., 2012. Polymorphism in Flower Colour and Petal Type in Aparajita (*Clitoria ternatea*). *Journal of Medicinal and Aromatic Plants*, 3(2), pp. 12-14.

Chauhan, N., Rajvaidhya, S. & Dubey, B. K., 2012. Pharmacognostical, Phytochemical and Pharmacological Review on *Clitoria ternatea* for Antiasthmatic Activity. *International Journal of Pharmaceutical Sciences and Research* , 3(2), pp. 398-404.

Christi, I. V. E, & Senthamari, R., 2015. Qualitative and Quantitative Pharmacognostical Studies on *Scoparia dulcis* Linn Leaf. *Indonesian Journal of Public Policy Review*, (3)1, pp. 57-74.

Dewantara, L. A. R., Ananto, A. D. & Andayani, Y., 2021. Penetapan Kadar Fenolik dan Flavonoid Total Ekstrak Kacang Panjang (*Vigna unguiculata*) dengan Metode Spektrofotometer UV-Visible. *Jurnal Ilmu Kefarmasian*, 2(1), pp. 13-19.

Evitasari, D. & Susanti, E., 2021. Kadar Polifenol Total Teh Hijau (*Camellia sinensis*) Hasil Maserasi dengan Perbandingan Pelarut Etanol - Air. *Pharmademica : Jurnal Kefarnasian dan Gizi*, 1(1), pp. 16-23.

Handayani, H., Sriherfyna, F. H. & Yunianta, 2016. Ekstraksi Antioksidan Daun Sirsak Metode Ultrasonic Bath (Kajian Rasio Bahan : Pelarut dan Lama Ekstraksi). *Jurnal Pangan dan Agroindustri*, 4(1), pp. 262-272.

Hanura, T. A., Fauziyah, A., Nasrullah, N. & Wahyuningsih, U., 2021. Pengaruh Penambahan Ekstrak Bunga Telang Terhadap Kadar Antosianin, Kalium, dan Sifat Organoleptik Jeli Buah Naga Merah. *Ghidza : Jurnal Gizi dan Kesehatan*, 5(2), pp. 187-196.

Hariadi, H., Sunyoto, M., Nurhadi, B. & Karuniawan, A., 2018. Comparison of phytochemical characteristics pigmen extract (Antosianin) sweet purple potatoes powder (*Ipomoea batatas* L) and clitoria flower (*Clitoria ternatea*) as natural dye powder. *Journal of Pharmacognosy and Phytochemistry*, 7(4), pp. 3420-3429.

Karim, H., Azis. A. A., Nursyahida, A. & Saparudin, 2021. Pengaruh Penerapan Metode Pembelajaran Inkuiri Dipadu Keterampilan Proses Sains terhadap Hasil Belajar Biologi Materi Struktur dan Fungsi Jaringan Tumbuhan. J*urnal Pendidikan Biologi*, 6(2), pp. 124-138.

Kurniawati, A., 2019. Pengaruh Jenis Pelarut pada Proses Ekstraksi Bunga Mawar dengan Metode Maserasi sebagai Aroma Parfum. *Journal of Creativity Student*, 2(2), pp. 74-83.

Kusbiantoro, D. & Purwaningrum, Y., 2018. Pemanfaatan Kandungan Metabolit Sekunder pada Tanaman Kunyit dalam Mendukung Peningkatan Pendapatan Masyarakat. *Jurnal Kultivasi*, 17(1), pp. 544-549.

Kushargina, R., Kusumaningati, W. & Yunianto, A. E., 2022. Pengaruh Bentuk, Suhu, dan Lama Penyeduhan Terhadap Sifat Organoleptik dan Aktivitas Antioksidan Teh Herbal Bunga Telang (*Clitoria ternatea* L.). *Journal of The Indonesian Nutrition Association*, 45(1), pp. 11-22.

Le, Xuan T., Huynh, M. T., Pham, T. N., Than, V. T., Toan, T. Q., Bach, L. G. & Trung, N. Q., 2019. Optimization of Total Anthocyanin Content, Stability and Antioxidant Evaluation of the Anthocyanin Extract from Vietnamese *Carrissa carandas* L. Fruits. *Processes*, 7(468), pp. 1-15.

Listiawati, M. D. A., Nastiti, K. & Audina, M., 2022. Pengaruh Perbedaan Jenis Pelarut Terhadap Kadar Fenolik Ekstrak Daun Sirsak (*Annona muricata* L.). *Journal of Pharmaceutical Care and Sciences*, 3(1), pp. 110-120.

Mariana, E., Cahyono, E., Rahayu, E. F. & Nurcahyo, B., 2018. Validasi Metode Penetapan Kuantitatif Metanol dalam Urin Menggunakan Gas Chromatography-Flame Ionization Detector. *Indonesian Journal of Chemical Science*, 7(3), pp. 277-284.

Nursamsiar, Fadri, A., Marwati, S., Fitriyani, J., Ismail, N. S. H. R., Kasmawati, H., 2023. Pengaruh Jenis Pelarut Terhadap Kadae Fenolik dan Flavonoid Total Daun Kesambi (*Schleichera oleosa* L.) Asal Kabupaten Gowa. *Jurnal Mandala Pharmacon Indonesia*, 9(2), pp. 253-261.

Rifai, G., Widarta, I. W. R. & Nocianitri, K. A., 2018. Pengaruh Jenis Pelarut dan Rasio Bahan dengan Pelarut Terhadap Kandungan Senyawa Fenolik dan Aktivitas Antioksidan Ekstrak Biji Alpukat (*Persea americana* Mill.). *Jurnal ITEPA*, 7(2), pp. 22-32.

Senet, M. R. M., Raharja, I. G. M. A. P., Darma, I. K. T., Prastakarini, K. T., Dewi, N. M. A., Parwata, I. M. O. A., 2018. Penentuan Kandungan Total Flavonoid dan Total Fenol dari Akar Kersen (*Mutingia calabura*) serta Aktivitasnya Sebagai Antioksidan. *Jurnal Kimia*, 12(1), pp. 13-18.

Suryanto, E., Momuat, L. I., Taroreh, M. & Whhantouw, F., 2011. Potensi Senyawa Polifenol Antioksidan dari Pisang Goroho (*Musa sapien* sp.). *Agritech*, 31(4), pp. 289-296.

Trinovani, E., Kusmiyati, M., Sudaryat, Y. & Rhamadianto, M. I., 2022. Penetapan Kadar Antosianin Total dan Aktivitas Antioksidan Ekstrak Air, Metanol, Etanol Tape 70% Ketan Hitam. *Jurnal Ilmiah Kefarmasian*, 7(4), pp. 983-992.

Nursamsiar, Fadri, A., Marwati, S., Fitriyani, J., Ismail, N. S. H. R., Kasmawati, H., 2023. Pengaruh Jenis Pelarut Terhadap Kadae Fenolik dan Flavonoid Total Daun Kesambi (*Schleichera oleosa* L.) Asal Kabupaten Gowa. *Jurnal Mandala Pharmacon Indonesia*, 9(2), pp. 253-261.

Paluvi, N., M. & Linda, R., 2015. Struktur Anatomi Daun, Kantung dan Sulur Nepenthes gracilis Korth. yang Tumbuh di Area Intensitas Cahaya. *Protobiont*, 4(1), pp. 103-107.

Pham, T. N., Lam, T. D., Nguyen, M. T., Xuan, T. L., Dai, V. N., Toan, Q. T., Thanh, S. V., 2018. Effect of Various Factors on Extraction Efficiency of Total Anthocyanins from Butterfly Pea (*Clitoria ternatea* L. Flowers) in Southern Vietnam. *IOP Conference Series: Materials Science and Engineering*, 544(201), pp. 1-5.

Prayoga, D. G. E., Nocianitri , K. A. & Puspawati, N. N., 2019. Identifikasi Senyawa Fitokimia dan Aktivitas Antioksidan Ekstrak Kasar Daun Pepe (*Gymnema reticulum* Br.) pada Berbagai Jenis Pelarut. *Jurnal Ilmu dan Teknologi Pangan*, 8(2), pp. 111-121.

Purwaniati, Arif, A. R. & Yuliantini, A., 2020. Analisis Kadar Antosianin Total Pada Sediaan Bunga Telang (*Clitoria ternatea*) Dengan Metode pH Diferensial Menggunakan Spektrofotometer Visible. *Jurnal Farmagazine,* 7(1), pp. 18-23.

Rifai, G., Widarta, I. W. R. & Nocianitri, K. A., 2018. Pengaruh Jenis Pelarut dan Rasio Bahan dengan Pelarut Terhadap Kandungan Senyawa Fenolik dan Aktivitas Antioksidan Ekstrak Biji Alpukat (*Persea americana* Mill.). *Jurnal ITEPA*, 7(2), pp. 22-32.

Samin, A. A., Bialangi, N. & Salimi, Y. K., 2014. Penentuan Kandungan Fenolik Total dan Aktivitas Antioksidan Dari Rambut Jagung (*Zea mays* L.) yang Tumbuh di Daerah Gorontalo. *Jurnal Saintek,* 7(3), pp. 21-26.

Savitri, I., Suhendra, L. & Wartini, N. M., 2017. Pengaruh Jenis Pelarut pada Metode Maserasi Terhadap Karakteristik Ekstrak *Sargassum polycystum. Jurnal Rekayasa dan Manajemen Agroindustri,* 5(3), pp. 93-101.

Senet, M. R. M., Raharja, I. G. M. A. P., Darma, I. K. T., Prastakarini, K. T., Dewi, N. M. A., Parwata, I. M. O. A., 2018. Penentuan Kandungan Total Flavonoid dan Total Fenol dari Akar Kersen (*Mutingia calabura*) serta Aktivitasnya Sebagai Antioksidan. *Jurnal Kimia*, 12(1), pp. 13-18.

Siregar, S. F., 2009. Uji Aktvitas Antibakteri Ekstrak Etanol dan Air Rebusan Kulit Batang Ingul (*Toona sinensis M. Roem)* terhadap Beberapa Bakteri, Medan: Fakultas Farmasi USU.

Suryanto, E., Momuat, L. I., Taroreh, M. & Whhantouw, F., 2011. Potensi Senyawa Polifenol Antioksidan dari Pisang Goroho (*Musa sapien* sp.). *Agritech*, 31(4), pp. 289-296.

Trinovani, E., Kusmiyati, M., Sudaryat, Y. & Rhamadianto, M. I., 2022. Penetapan Kadar Antosianin Total dan Aktivitas Antioksidan Ekstrak Air, Metanol, Etanol Tape 70% Ketan Hitam. *Jurnal Ilmiah Kefarmasian,* 7(4), pp. 983-992.

Utami, Y. P., Umar, A. H. & Ernawati, 2016. Analysis of Total Anthocyanin Content on Ethanol Extract of Purple Sweet Potato (*Ipomoea batatas* L.) and Purple Yam (Dioscoreaalata L.). *Journal of Pharmaceutical and Medicinal Sciences* , 1(2), pp. 44-47.

Widyasanti, A., Nurlaily, N. & Wulandari, E., 2008. Karakteristik Fisikokimia Antosianin Ekstrak Kulit Buah Naga Merah Menggunakan Metode UAE. *Jurnal Ilmiah Rekayasa Pertanian*, 6(1), pp. 27-38.