



Characteristics of natural blue dye of double-petaled butterfly pea flower (*Clitoria ternatea* L.) on different extraction time and temperature: Comparative study on fresh extract, concentrated extract, and rehydrated extract

Arif P. Santosa¹, Teguh Pribadi¹, Hamami A. Dewanto¹, Tutie Djarwaningsih², Diah Sulistiarini², Florentina I. Windadri², Siti Susiarti³, Muhammad R. Hariri², Ina Erlinawati²

¹Agrotechnology Study Program, Faculty of Agriculture and Fisheries, Muhammadiyah University of Purwokerto, Jl. Raya Dukuwaluh, Dukuwaluh, Kembaran, Kabupaten Banyumas, Jawa Tengah 53182, Indonesia

²Research Center for Biosystematics and Evolution, Indonesian Research and Innovation Agency, Jl. Raya Jakarta-Bogor Km. 46, Cibinong, 16911, Indonesia

³Research Center for Ecology and Ethnobiology, Indonesian Research and Innovation Agency, Jl. Raya Jakarta-Bogor Km. 46, Cibinong, 16911, Indonesia

Clitoria ternatea L., a tropical legume known for its vivid blue flowers, is rich in anthocyanins which function as natural pigments with strong antioxidant properties. Owing to its bioactive potential and colorant stability, this species presents a promising source of natural dyes for functional food and beverage applications. This study investigates the characteristics of natural blue dye derived from double-petaled *Clitoria ternatea* L. (butterfly pea) flowers, focusing on the effects of different extraction temperature and duration on the yield, anthocyanin content, antioxidant activity, and color properties of fresh, concentrated, and rehydrated extracts. The extraction process was conducted via aqueous maceration, followed by concentration through controlled thermal evaporation and rehydration to original volume. Results showed that higher extraction temperatures increased anthocyanin content in fresh extracts, with the highest recorded at 75°C for 6h. However, prolonged heating during concentration (70°C for 10h) reduced antioxidant activity, despite increasing anthocyanin content. Rehydrated extracts exhibited similar anthocyanin levels to fresh extracts but significantly lower antioxidant activity. Notably, the antioxidant activity of fresh extracts ($70.11 \pm 13.42\%$) exceeded values reported in previous studies. Colorimetric analysis revealed temperature- and process-dependent changes in brightness and chromatic attributes (a^* , b^*). The K2T4 treatment (50°C, 24h) produced optimal extract quality across parameters. These findings support the potential application of concentrated *C. ternatea* extract as a ready-to-use natural blue dye in food and beverage products, though further studies are recommended on storage stability and broader phytochemical profiling.

Keywords: Anthocyanin, Antioxidant, Butterfly pea flower, *Clitoria ternatea*, Extract

INTRODUCTION

Indonesia hosts an exceptionally high diversity of flora, accounting for 25% of the world's flowering

plant species—approximately 20,000 species—ranking seventh globally (Limanan et al., 2018). This biodiversity offers significant benefits to the Indonesian population, particularly in the areas

ARTICLE HISTORY

Submitted: February 19, 2025

Accepted: July 3, 2025

CORRESPONDANCE TO:

Ina Erlinawati
Research Center for Biosystematics and Evolution, Indonesian Research and Innovation Agency, Jl. Raya Jakarta-Bogor Km. 46, Cibinong, 16911, Indonesia
Email: inaerlinawati@gmail.com
Tel: +6281328003910

DOI: 10.21608/ejbo.2025.360063.3197

EDITED BY: Neveen Mahmoud Khalil

of food and traditional medicine. Additionally, several flower species play vital roles in traditional ceremonies and cultural practices.

Clitoria ternatea L., commonly known as butterfly pea (Indonesian: *bunga telang*), is a leguminous plant in the Fabaceae family. It is synonymous with *Nauclea ternatea* (L.) J.T. Descourt and *Clitoria parviflora* Raf. (POWO, 2024). This perennial, herbaceous climbing plant features elliptic, obtuse leaflets and large, solitary, axillary, papilionaceous flowers. The species is characterized by variability in flower color and petal morphology (Bishoyi & Geetha, 2012). *Clitoria ternatea* is native to Tropical America but has become naturalized across tropical regions, including warmer parts of India (Parrota, 2001). Indian populations of *C. ternatea* exhibit three distinct flower colors: blue, white, and white with a blue tinge (Bishoyi et al., 2014). Linnaeus suggested that the species' name, which references Ternate Island in the Maluku Islands of Indonesia, indicates this region as its possible origin (Tropical Forages, 2023).

Butterfly pea flowers offer a wide range of benefits, including antioxidant, antidepressant, antidiabetic, antiobesity, anticancer, antiinflammatory, antifungal, antibiotic, and hepatoprotective properties (Marpaung et al., 2020; Mittal et al., 2021; Hamzah et al., 2024). They contain both lipophilic and hydrophilic bioactive components, such as flavonol glycosides, anthocyanins, flavones, flavonols, phenolic acids, terpenoids, alkaloids, and cyclic peptides or cyclotides (Mittal et al., 2021). Additionally, butterfly pea flowers are utilized in traditional medicine, handicrafts, ornamental gardening, animal feed, and as a natural food coloring. In the health sector, they are used to treat eye infections, ear disorders, skin conditions, throat issues, and tumors (Ramaswamy et al., 2011; Gollen et al., 2018).

The blue color of butterfly pea flowers is attributed to anthocyanins, which serve as natural alternatives to synthetic dyes, avoiding potential health risks associated with synthetic blue dyes (Gollen et al., 2018; Suebkhampet & Sottibandhu, 2019). Butterfly pea flowers are widely used in food products, such as butterfly pea tea, Balinese drinks for disease prevention, ice cream, muffin dough, yogurt, and steamed bread. When anthocyanins are microencapsulated, they expand the possibilities for new food innovations (Yurisna et al., 2022).

Anthocyanin concentration influences the perceived color: low concentrations yield blue, medium concentrations produce purple, and high concentrations result in red (Khoo et al., 2017). Compared to anthocyanins from other flower extracts, those from butterfly pea flowers exhibit higher antioxidant activity (Kazuma et al., 2003). As part of the flavonoid family, anthocyanins act as bioactive compounds with antioxidant properties. These natural pigments can produce orange, red, and purple hues in plants and serve as natural food colorings (Priska et al., 2017).

The antimicrobial potential of butterfly pea flowers is notable. Methanol extracts of roots, stems, leaves, seeds, and flowers have demonstrated antibacterial and antifungal properties (Budiasih, 2017). Ethanol extracts from butterfly pea flowers and soursop leaves exhibit gram-positive antibacterial activity, particularly against *Staphylococcus aureus* and *Staphylococcus epidermidis* (Hidayah, 2015). Despite their diverse applications, the use of butterfly pea flowers as natural dyes primarily involves direct extraction through brewing. Ready-to-use preparations of butterfly pea flower dye are less common. Therefore, this study aims to develop a concentrated butterfly pea flower extract and evaluate its physicochemical and sensory characteristics.

MATERIAL AND METHODS

Plant Materials and Research Design

This research was conducted from January to June 2024, with sampling methods based on standard method (van Balgooy, 1987; Rugayah et al., 2004). The materials used included dried double-petaled blue butterfly pea flowers sourced from a garden at BRIN, distilled water, pH 1 buffer (0.2N KCl and 0.2N HCl), pH 4.5 buffer (sodium acetate and HCl), aluminum foil, technical methanol, analytical methanol, and DPPH solution. The study employed a factorial Completely Randomized Design (CRD) with two treatment factors: temperature and extraction time. The temperature variable had three levels (25°C, 50°C, and 75°C), while the extraction time had four levels (6, 12, 18, and 24h). Each treatment combination was replicated three times, resulting in a total of 12 combinations and 36 experimental units.

Anthocyanin extraction and measurement

Anthocyanin extraction from dried double-petaled butterfly pea flowers was performed through maceration, a technique involving the soaking of plant material in a solvent to dissolve bioactive

compounds. In this study, 10g of dried flower sample was immersed in 200ml of distilled water (pH 7) and subjected to extraction at controlled temperatures of 25°C, 50°C, and 75°C for durations of 6, 12, 18, and 24 hours, as described by Sari et al. (2005). To minimize photooxidation of anthocyanins, the process was conducted in a closed water bath. After extraction, the mixture was filtered and the resulting filtrate collected. The dry extract obtained was subsequently analyzed to determine the total extract yield, anthocyanin concentration, and antioxidant capacity.

The extract was concentrated following the method described by Hasim et al. (2016), wherein the fresh extract was evaporated using a water bath maintained at temperatures between 50–70°C—below the boiling point—to preserve the antioxidant compounds. The evaporation process was conducted in a dark, closed room equipped with an exhaust fan, and was continued for 10h until a concentrated extract was obtained. Rehydration of the concentrated extract was performed by diluting it with distilled water (aquades) to restore the original volume of the fresh extract.

All collected extracts—including the fresh, concentrated, and rehydrated forms—were stored in a refrigerator at 5°C. The concentrated and rehydrated extracts were stored in glass jars, while concentrated extracts were also kept in glass vials. All containers were covered with aluminum foil to protect the anthocyanins from light-induced degradation during storage. Throughout the storage period, no changes in pH were observed in any of the extracts.

The amount of distilled water added was adjusted based on the concentration of the solution prior to concentration.

$$VAq = VESA - VEP$$

where, VESA: Initial fresh extract volume (ml), VEP: Concentrated extract volume (ml), VAq: Volume of distilled water added (ml).

The yield of fresh double-petaled butterfly pea flowers extract is calculated by comparing the weight of the extract obtained to the volume of the solvent used, expressed as a percentage. The formula is as follows:

$$RES = \frac{Esh}{Vol\ liquid} \times 100\%$$

where, RES: Fresh extract yield (%), Esh:

Anthocyanin extract was obtained (ml), Vol liquid: Aquades used (ml).

The yield of the concentrated double-petaled butterfly pea flowers extract is calculated by comparing the weight of the concentrated extract to the weight of the initial fresh extract, expressed as a percentage. The formula is as follows:

$$REP = \frac{Esp}{ES_0} \times 100\%$$

where, REP: Concentrated extract yield (%), Esp: Concentrated anthocyanin extract (ml), ES_0 : Early fresh extract (ml).

The anthocyanin content of double-petaled butterfly pea flowers extract is determined using the pH differential method (Giusti et al., 1998). This method leverages the principle that anthocyanins exhibit different colors depending on pH. At pH 1, anthocyanins exist as oxonium or flavylium ions, which have high color intensity, while at pH 4.5, they are in the carbinol form, which is colorless. This method involves adding pH 1 and pH 4.5 buffers to the extract, followed by absorbance measurements at λ_{max} (512 nm) and λ_{700nm} to correct for haze. The difference in absorbance between these pH conditions is proportional to the anthocyanin content.

The monomeric anthocyanin pigment content (mg/L) as cyanidin-3-glycoside is calculated using the following formula:

$$KA\ (mg/ml) = \frac{A}{(\epsilon \times 1)} \times MW \times DF$$

where, KA: Anthocyanin content, A: Absorbance {A = [(A₅₁₂ – A₇₀₀)pH 1 – (A₅₁₂ – A₇₀₀)pH 4.5]}, MW: Molecular weight (449.2), DF: Dilution factor (10), ϵ : Molar extinction coefficient of cyanide 3-glycoside = 26.900

Antioxidant activity test

The principle of testing antioxidant activity using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging method is based on the ability of antioxidant components to donate hydrogen atoms to neutralize DPPH radicals, resulting in a color change (Molyneux, 2004). In this test, 0.1ml of each sample was mixed with 0.5ml of 0.5mM DPPH solution and 4ml of methanol. The mixture was vortexed for 1min and incubated for 60min in the dark. Absorbance was then measured at 515.3nm. The antioxidant activity of anthocyanin

from double-petaled butterfly pea flowers, both fresh and concentrated extracts, was determined using the following formula (Akl & Younos, 2024):

$$\text{DPPH} = \frac{\text{AK} - \text{AS}}{\text{AK}} \times 100\%$$

where, DPPH: Free radical scavenging (%), AK: Control absorbance, AS: Sample absorbance

Colorimetry test

The principle of colorimetry is based on the Beer-Lambert Law, which defines a linear relationship between the absorption of light by a solution and the concentration of the absorbing species (Hui, 1992). This law forms the foundation for color measurements in analytical testing. In this study, colorimetry was used to test both fresh and concentrated extracts of double-petaled butterfly pea flowers anthocyanins. Calibration of the colorimeter was performed first, and the calibration results were recorded. The receptor tip was then attached to the sample, and absorbance readings were taken, with each measurement repeated three times to ensure accuracy. Color tests were categorized into three parameters, L value (Lightness) represents the lightness or brightness of the color, ranging from black to white, a value (Green-Red axis) indicates the color spectrum between green and red, and b value (Blue-

Yellow axis) measures the color intensity along the blue-yellow spectrum. These measurements provide a comprehensive assessment of the color characteristics of the anthocyanin extracts.

Statistical test

The research data were presented as means and standard deviations. The differences between treatment groups were analyzed using analysis of variance (ANOVA) at a 5% significance level. If the ANOVA indicated significant differences, a post-hoc comparison was performed using the Tukey's Honestly Significant Difference (Tukey's HSD) test at the 5% level to further assess the differences between mean values. All statistical analyses were conducted using Statistical Package for the Social Sciences (SPSS) software version 25.

RESULT

Based on field exploration of *C. ternatea*, four distinct flower color phenotypes were identified: blue, white, white with a blue tinge, and purple. In addition, two floral morphologies were observed, consisting of single-petaled and double-petaled forms (Figure 1). This study specifically employed the double-petaled blue flower variant of *C. ternatea* due to its high visual anthocyanin intensity and previously reported elevated anthocyanin concentrations relative to other color morphs and floral structures.



Figure 1. *Clitoria ternatea* flower variation [A. Single and double-petaled blue flower (used in this study), B. Single and double-petaled white flower, C. Single and double-petaled white with bluish hue flower, and D. Single and double-petaled purple flower]

Yield and content

The results of the 5% Tukey's HSD analysis regarding the interaction between temperature and extraction time on the yield of butterfly pea flower extract are presented in Table 1. The findings indicate that temperature significantly impacts the yield of both fresh and concentrated extracts. A temperature of 75°C resulted in the lowest yield. However, extraction time did not show any significant effect. In terms of treatment combinations, distinct yield outcomes were observed for concentrated and fresh extracts. The combination of K2T1 (50°C for 6h) produced the highest yield of fresh extract at 93.00±0.02%. For concentrated extracts, there were no significant differences in yield across most treatments, except for the K3T3 and K3T4 treatments (75°C for 18

and 24h, respectively).

Table 2 demonstrates that the anthocyanin content in fresh extracts increases with rising extraction temperatures, whereas no significant differences in anthocyanin content were observed among concentrated and rehydrated extracts across the various temperature treatments. Extraction time did not significantly influence anthocyanin content in any of the extract types—fresh, concentrated, or rehydrated. Among the combinations of extraction temperature and time, the K3T1 treatment (75°C for 6 hours) resulted in the highest anthocyanin content in fresh extracts. However, this trend did not persist in the concentrated and rehydrated extracts, as no statistically significant differences were detected among treatments.

Table 1. Average results of the analysis of the effect of temperature and extraction time on the yield of double-petaled butterfly pea flowers extract

Treatment	Percentage yield (%)	
	Fresh	Concentrated
Temperature (K)		
K1 (25°C)	89±0,01 b	26±0,06 b
K2 (50°C)	91±0,02 b	26±0,04 b
K3 (75°C)	86±0,02 a	15±0,13 a
Time (T)		
T1 (6h)	90±0,03a	25±0,07a
T2 (12h)	88±0,03a	28±0,07a
T3 (18h)	89±0,02a	17±0,10a
T4 (24h)	88±0,01a	19±0,12a
Combination of temperature and time (K x T)		
K1T1	91±0,01bc	28±0,01b
K1T2	90±0,00abc	21±0,03b
K1T3	89±0,00abc	25±0,04b
K1T4	89±0,01abc	30±0,03b
K2T1	93±0,02c	27±0,05b
K2T2	91±0,00bc	30±0,02b
K2T3	90±0,02bc	22±0,03b
K2T4	89±0,00abc	25±0,02b
K3T1	86±0,01ab	20±0,04b
K3T2	84±0,01a	33±0,07b
K3T3	87±0,03abc	3±0,00a
K3T4	83±0,03abc	4±0,00a

The average number followed by the same lowercase letter in the row shows no significant difference at Tukey's HSD 5%.

Table 2. The treatment results of temperature, length time, and its combination on the anthocyanin content of double-petaled butterfly pea flowers

Treatment	Anthocyanin (ppm)		
	Fresh	Concentrated	Rehydration
Temperature (K)			
K1 (25°C)	62,81±23,99 _A	98,35±22,26 _B	51,46±17,12 _A
K2 (50°C)	63,52±18,73 _A	105,28±20,10 _B	59,39±14,67 _A
K3 (75°C)	84,94±14,28 _A	124,49±45,49 _B	62,60±36,17 _A
Time (T)			
T1 (6h)	60,06±29,80 _A	103,62±31,52 _B	52,74±32,07 _A
T2 (12h)	67,76±19,33 _A	112,05±25,34 _B	57,51±24,58 _A
T3 (18h)	82,08±20,13 _{AB}	97,55±31,27 _B	59,45±21,65 _A
T4 (24h)	71,80±8,81 _A	124,24±39,59 _B	61,56±21,15 _A
Combination of temperature and time (K x T)			
K1T1	39,80±0,62 _A	70,57±15,72 _B	28,11±16,64 _A
K1T2	56,72±6,74 _{abc}	108,37±23,59 _B	50,54±7,03 _A
K1T3	85,22±37,28 _{bcd}	113,05±9,12 _A	62,20±0,25 _A
K1T4	69,52±7,69 _{abcd}	101,41±13,89 _B	65,01±4,22 _A
K2T1	41,19±8,85 _B	99,85±5,53 _C	52,04±2,66 _A
K2T2	53,60±2,92 _{ab}	91,79±17,14 _B	69,74±5,11 _{AB}
K2T3	78,76±10,90 _{bcd}	96,79±15,84 _B	42,97±7,86 _A
K2T4	80,54±6,78 _{bcd}	132,70±9,76 _B	72,80±13,18 _A
K3T1	99,19±5,27 _d	140,44±2,25 _A	79,09±44,21 _A
K3T2	92,95±2,22 _{cd}	135,98±14,94 _B	52,27±44,75 _A
K3T3	82,27±8,99 _{bcd}	82,83±54,77 _A	73,19±33,34 _A
K3T4	65,35±4,54 _{abcd}	138,71±69,13 _A	46,86±32,65 _A

– The average number followed by the same lowercase letter in the row shows no significant difference at Tukey's HSD 5%.

– The average number followed by the same capital letter in the same column shows no significant difference at Tukey's HSD 5%.

– Notation with superscript lowercase letters are read vertically, meanwhile subscript notation with capital letters are read horizontally.

– Different letters mean statistically different values in the 5% Tukey's HSD test.

Furthermore, Table 2 indicates that the concentration process effectively enhances the anthocyanin content relative to fresh extracts. Nonetheless, rehydration of the concentrated extracts generally resulted in anthocyanin levels comparable to those of the fresh extracts. This is evidenced by the consistent letter notation assigned to both fresh and rehydrated extracts within the same treatment row, suggesting no significant difference between them.

Antioxidant activity

Table 3 shows that extraction temperature significantly affects the antioxidant activity of fresh and concentrated extracts but does not have a significant impact on the antioxidant activity of rehydrated extracts. The extraction time only significantly influenced the antioxidant activity

of rehydrated extracts. The combination of extraction time and temperature treatments did not yield significant differences in antioxidant activity for fresh extracts.

The highest average antioxidant activity in the concentrated extract was observed in the K3T3 treatment (75°C for 18h), with an antioxidant content of 68.78±2.15%. In the rehydrated extract, the lowest antioxidant activity was found in the K3T4 treatment (75°C for 24h), with an antioxidant content of 13.21±20.53%, while the highest average antioxidant activity was in the K1T1 treatment (25°C for 6h) with 52.86±4.95%. Generally, antioxidant activity in concentrated and rehydrated extracts was lower than in fresh extracts, as indicated by the difference in capital notation within the same column for each treatment.

Table 3. The treatment results of temperature, length time, and its combination on the antioxidant activity of double-petaled butterfly pea flowers

Treatment	Antioxidants (%)		
	Fresh	Concentrated	Rehydration
Temperature (K)			
K1 (25°C)	70,11±13,42 ^b _B	40,06±11,75 ^a _A	41,65±9,63 ^a _A
K2 (50°C)	38,41±20,50 ^a _A	32,15±9,55 ^a _A	31,96±13,27 ^a _A
K3 (75°C)	62,33±16,76 ^b _B	59,76±13,86 ^b _B	27,97±17,29 ^a _A
Time (T)			
T1 (6h)	52,23±21,41 ^a _A	47,41±18,23 ^a _A	42,29±10,94 ^b _A
T2 (12h)	59,76±25,74 ^a _B	40,99±17,49 ^a _A	32,45±14,43 ^{ab} _A
T3 (18h)	53,76±22,62 ^a _A	47,41±16,69 ^a _A	36,01±12,71 ^{ab} _A
T4 (24h)	62,51±17,89 ^a _B	39,79±14,48 ^a _A	24,70±16,06 ^a _A
Combination of temperature and time (K x T)			
K1T1	63,84±20,61 ^a _A	51,29±9,72 ^{abc} _A	52,86±4,95 ^b _A
K1T2	78,21±0,56 ^a _B	36,04±7,78 ^{abc} _A	46,58±0,69 ^{ab} _A
K1T3	65,54±17,96 ^a _B	31,92±3,84 ^{ab} _A	29,84±3,80 ^{ab} _A
K1T4	72,85±16,61 ^a _A	40,99±16,61 ^{abc} _B	37,33±3,00 ^{ab} _B
K2T1	38,98±13,61 ^a _A	29,18±7,17 ^{ab} _A	33,47±9,26 ^{ab} _A
K2T2	38,86±33,51 ^a _A	28,65±8,70 ^a _A	24,56±2,43 ^{ab} _A
K2T3	29,97±15,84 ^a _A	42,58±3,99 ^{abc} _A	46,25±14,73 ^{ab} _A
K2T4	45,84±23,71 ^a _A	28,21±11,88 ^a _A	23,57±12,75 ^{ab} _A
K3T1	53,88±27,48 ^a _A	61,78±18,78 ^{bc} _A	40,53±8,92 ^{ab} _A
K3T2	60,83±18,99 ^a _A	58,30±19,32 ^{abc} _A	26,21±19,38 ^{ab} _A
K3T3	65,78±14,12 ^a _B	68,78±2,15 ^c _B	31,93±13,26 ^{ab} _A
K3T4	68,84±14 ^a _B	50,17±7,50 ^{abc} _B	13,21±20,53 ^a _A

– The average number followed by the same lowercase letter in the same row shows no significant difference at Tukey's HSD 5%.

– The average number followed by the same capital letter in the same column shows no significant difference at Tukey's HSD 5%.

– Notation with superscript lowercase letters are read vertically, meanwhile subscript notation with capital letters are read horizontally.

– Different letters mean statistically different values in the 5% Tukey's HSD test.

Brightness of blue flower

Table 4 indicates that neither temperature, extraction time, nor their combination significantly affect brightness in fresh or concentrated extracts. In rehydrated extracts, extraction time did not show significant differences, but temperature treatment did. The K3 treatment (75°C) produced the highest brightness compared to the other extraction temperatures. The combination of temperature and extraction time treatments in the rehydrated extract showed significant differences, with the highest brightness observed in the K2T1 treatment (50°C for 6 hours) and the lowest in the K1T2 treatment (25°C for 12h).

The concentration process resulted in a darker color brightness compared to both fresh and rehydrated extracts. However, the color

brightness of rehydrated extracts was mostly not significantly different from that of fresh extracts, as indicated by the same capital notation between the two.

Color (a and b) of anthocyanin extract Value a (red-green color)

Table 5 presents the red-green color values of the anthocyanin extracts. Positive values indicate a shift toward red, while negative values indicate a shift toward green. No significant differences were observed in the red-green color value across long extraction times, whether for fresh, concentrated, or rehydrated extracts. However, in the extraction temperature treatment, significant differences were only found in the fresh extract. The same trend was observed for the combination of temperature and extraction time.

Table 4. The treatment results of temperature, length time, and its combination on the brightness level of double-petaled butterfly pea flowers

Treatment	Brightness test		
	Fresh	Concentrated	Rehydration
Temperature (K)			
K1 (25°C)	31,17±1,80 ^a _A	20,97±4,66 ^a _B	31,13±1,86 ^a _A
K2 (50°C)	31,79±1,64 ^a _A	21,03±4,22 ^a _B	32,71±1,92 ^a _A
K3 (75°C)	31,99±1,54 ^a _A	21,46±4,23 ^a _B	34,62±0,90 ^b _A
Time (T)			
T1 (6h)	32,08±1,10 ^a _A	20,40±4,74 ^a _B	32,54±2,04 ^a _A
T2 (12h)	31,11±2,00 ^a _A	21,39±4,61 ^a _B	31,80±1,99 ^a _A
T3 (18h)	31,81±1,69 ^a _A	21,24±3,45 ^a _B	33,25±2,42 ^a _A
T4 (24h)	31,61±1,84 ^a _A	21,59±4,75 ^a _B	33,69±1,96 ^a _A
Combination of temperature and time (K x T)			
K1T1	31,42±1,38 ^a _A	21,30±6,20 ^a _B	31,14±0,51 ^{ab} _A
K1T2	29,17±2,33 ^a _A	19,83±2,62 ^a _B	29,96±1,25 ^a _A
K1T3	32,51±0,83 ^a _A	23,42±1,97 ^a _B	31,92±3,52 ^{abcd} _A
K1T4	31,59±0,91 ^a _A	19,34±7,41 ^a _A	31,50±1,29 ^{abc} _A
K2T1	32,17±0,99 ^a _{AB}	18,69±4,59 ^a _B	31,42±1,36 ^{abc} _A
K2T2	32,25±1,34 ^a _{AB}	18,05±4,37 ^a _B	31,32±0,60 ^{ab} _A
K2T3	32,60±1,07 ^a _A	22,20±2,78 ^a _B	32,56±0,45 ^{abcd} _A
K2T4	30,15±2,33 ^a _A	25,18±0,94 ^a _C	35,54±0,65 ^d _B
K3T1	32,64±0,88 ^a _A	21,21±4,88 ^a _B	35,05±0,55 ^{bcd} _{AB}
K3T2	31,90±0,48 ^a _A	26,29±1,65 ^a _B	34,14±0,56 ^{bcd} _A
K3T3	30,32±2,15 ^a _A	18,10±3,59 ^a _B	35,29±1,10 ^{cd} _{AB}
K3T4	33,09±0,88 ^a _A	20,24±2,21 ^a _B	34,02±0,90 ^{bcdA}

– The average number followed by the same lowercase letter in the same row shows no significant difference at Tukey's HSD 5%.

– The average number followed by the same capital letter in the same column shows no significant difference at Tukey's HSD 5%.

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– Different letters mean statistically different values in the 5% Tukey's HSD test.

In the extraction temperature treatment, the K1 treatment (25°C) showed the lowest value of -2.08 ± 1.77 , while in the combination treatment, the K1T2 (25°C for 12h) treatment had the lowest value of -4.62 ± 1.32 . The concentration process increased the red-green value, with concentrated extracts showing a more positive (redder) value than the fresh extracts. Upon rehydration, the value of the extract decreased, returning to levels similar to those of the fresh extract.

Value b (yellow-blue color)

Table 6 presents the b value (yellow-blue color) of the anthocyanin extracts, where positive values indicate yellow and negative values

indicate blue. No significant differences were observed in the b value for the long extraction time treatment, whether for fresh, concentrated, or rehydrated extracts. However, in the extraction temperature treatment, significant differences were only noted in the fresh extract.

A significant difference was observed between fresh and rehydrated extracts in the combination of temperature and extraction time treatments. The concentration process resulted in a more negative b value (bluer) for concentrated extracts compared to fresh extracts. Upon rehydration, the b value increased again, returning to levels similar to those of the fresh extract.

Table 5. The treatment results of temperature, length time, and its combination on the value a (red-green color) of double-petaled butterfly pea flowers

Treatment	Color Test (a)		
	Fresh	Concentrated	Rehydration
Temperature (K)			
K1 (25°C)	-2,08±1,77 ^a _A	1,08±0,89 ^a _B	-2,25±1,15 ^a _A
K2 (50°C)	-1,03±0,78 ^b _B	0,07±0,42 ^a _C	-2,08±0,68 ^a _A
K3 (75°C)	-0,71±0,98 ^{ab} _A	0,84±1,52 ^a _B	-1,51±0,51 ^a _A
Time (T)			
T1 (6h)	-0,79±0,93 ^a _A	0,54±1,05 ^a _B	-1,87±0,88 ^a _A
T2 (12h)	-2,20±2,00 ^a _A	0,45±0,87 ^a _B	-1,84±0,84 ^a _A
T3 (18h)	-1,20±1,15 ^a _A	1,02±1,37 ^a _B	-2,19±1,11 ^a _A
T4 (24h)	-0,90±0,64 ^a _A	0,62±1,21 ^a _B	-1,88±0,67 ^a _A
Combination of temperature and time (K x T)			
K1T1	-1,260±1,50 ^b _A	1,28±1,68 ^a _A	-1,94±0,60 ^a _A
K1T2	-4,62±1,32 ^a _A	1,48±0,25 ^a _C	-1,35±1,81 ^a _B
K1T3	-0,89±0,05 ^b _B	1,25±0,49 ^a _B	-3,21±1,48 ^a _A
K1T4	-1,54±0,16 ^b _A	0,35±0,36 ^a _B	-2,49±0,77 ^a _A
K2T1	-0,81±0,48 ^b _B	0,16±0,17 ^a _B	-2,49±1,04 ^a _A
K2T2	-0,75±0,71 ^b _B	0,39±0,58 ^a _B	-2,50±0,19 ^a _A
K2T3	-0,68±1,41 ^b _A	-0,83±0,50 ^a _A	-1,56±0,31 ^a _A
K2T4	-0,59±0,79 ^b _{AB}	-0,18±0,23 ^a _B	-1,76±0,50 ^a _A
K3T1	-0,31±0,58 ^b _A	0,19±0,62 ^a _A	-1,19±0,60 ^a _A
K3T2	-1,23±0,72 ^b _A	-0,45±0,14 ^a _A	-1,66±0,59 ^a _A
K3T3	-2,02±1,34 ^{ab} _A	1,91±1,89 ^a _B	-1,81±0,54 ^a _A
K3T4	-0,57±0,27 ^b _{AB}	1,70±1,68 ^a _B	-1,40±0,27 ^a _A

– The average number followed by the same lowercase letter in the same row shows no significant difference at Tukey's HSD 5%.

– The average number followed by the same capital letter in the same column shows no significant difference at Tukey's HSD 5%.

– Notation with superscript lowercase letters are read vertically, meanwhile subscript notation with capital letters are read horizontally.

– Different letters mean statistically different values in the 5% Tukey's HSD test.

DISCUSSION

Yield and anthocyanin content

The 5% Tukey's HSD analysis results, presented in Table 1, highlight the effect of the interaction between temperature and extraction time on the yield of butterfly pea flower extract. The combination of the K2T1 treatment (50°C for 6h) produced the highest fresh yield of 93±0.02%, compared to other treatments, while the K3T2 treatment (75°C for 12h) resulted in the lowest yield at 84±0.01%.

Table 1 demonstrates that higher extraction temperatures lead to lower yields of butterfly pea flower extract. This is because elevated temperatures can damage the alginate structure and reduce water content due to evaporation during the heating process. In contrast, lower temperatures

result in less evaporation, yielding higher and more abundant extracts. The water content of the material significantly influences yield differences between high and low temperatures. Additionally, factors such as the material's initial moisture content and size also impact the heating process (Sukma et al., 2017).

The increasing extraction time enhances solvent-material interaction, which can initially increase extract yield as suggested in previous research (Prasetya et al., 2020). However, prolonged extraction times may decrease the yield as maceration exceeds the optimal time, causing a reduction in extracted compounds. The solvent will no longer extract compounds effectively, even if the extraction time continues after reaching the optimal extraction time as noted in previous research (Yulianti et al., 2014).

Table 6. The treatment results of temperature, length time, and its combination on the value b (yellow-blue color) of double-petaled butterfly pea flowers

Treatment	Color Test (b)		
	Fresh	Concentrated	Rehydration
Temperature (K)			
K1 (25°C)	-1,64±1,20 ^b _{AB}	-2,76±1,41 ^a _A	-1,32±1,38 ^a _B
K2 (50°C)	-2,71±0,84 ^a _{AB}	-3,57±1,77 ^a _A	-2,17±0,86 ^a _B
K3 (75°C)	-2,25±0,82 ^{ab} _B	-4,30±1,96 ^a _A	-2,25±0,75 ^a _B
Time (T)			
T1 (6h)	-2,03±0,82 ^a _A	-2,93±1,72 ^a _A	-1,61±0,85 ^a _A
T2 (12h)	-1,97±1,57 ^a _{AB}	-3,67±2,17 ^a _A	-1,56±1,11 ^a _B
T3 (18h)	-2,49±0,78 ^a _A	-3,30±1,26 ^a _A	-2,43±1,27 ^a _A
T4 (24h)	-2,31±0,89 ^a _B	-4,27±1,93 ^a _A	-2,04±1,03 ^a _B
Combination of temperature and time (K x T)			
K1T1	-1,43±0,61 ^{ab} _A	-2,87±1,61 ^a _A	-0,79±1,38 ^{bc} _A
K1T2	-0,37±1,47 ^b _A	-2,51±0,38 ^a _A	-0,31±3,02 ^c _A
K1T3	-2,76±0,75 ^{ab} _A	-3,24±0,91 ^a _A	-2,77±1,00 ^{ab} _A
K1T4	-2,02±0,48 ^{ab} _A	-2,43±2,61 ^a _A	-1,43±1,00 ^{abc} _A
K2T1	-2,55±0,72 ^{ab} _A	-2,93±2,27 ^a _A	-1,73±1,12 ^{abc} _A
K2T2	-3,15±0,55 ^a _A	-2,24±1,75 ^{aA}	-2,03±1,54 ^{abc} _A
K2T3	-2,88±0,53 ^{ab} _{AB}	-3,81±0,99 ^a _A	-1,62±0,82 ^{abc} _B
K2T4	-2,28±1,47 ^{ab} _B	-5,32±0,27 ^a _A	-3,30±2,48 ^a _{AB}
K3T1	-2,11±0,92 ^{ab} _A	-3,01±2,03 ^a _A	-2,32±1,34 ^{abc} _A
K3T2	-2,40±1,08 ^{ab} _B	-6,28±0,67 ^a _A	-2,34±0,64 ^{abc} _B
K3T3	-1,85±0,82 ^{ab} _A	-2,84±1,95 ^a _A	-2,92±2,31 ^{ab} _A
K3T4	-2,64±0,70 ^{ab} _B	-5,06±0,59 ^a _A	-1,43±0,89 ^{abc} _B

– The average number followed by the same lowercase letter in the same row shows no significant difference at Tukey's HSD 5%.

– The average number followed by the same capital letter in the same column shows no significant difference at Tukey's HSD 5%.

– Notation with superscript lowercase letters are read vertically, meanwhile subscript notation with capital letters are read horizontally.

– Different letters mean statistically different values in the 5% Tukey's HSD test.

The results in Table 2 show that the anthocyanin content in the fresh and concentrated extracts increased, as indicated by the capital letter notation. However, the rehydrated extract did not show a significant difference from the fresh extract. The overall average anthocyanin content in the fresh extract was higher than in the concentrated extract, but the rehydrated extract could not maintain the higher anthocyanin concentration, returning to levels like the fresh extract.

This change is attributed to the reduction in water content during the concentration of the butterfly pea flower extract, which leads to an increase in anthocyanin concentration. The rehydrated extract, after dilution, returned to a state comparable to the fresh extract. To preserve the anthocyanin content in the concentrated extract, it

was stored at refrigerator temperature and covered with aluminum foil to protect it from light. Light exposure can accelerate the degradation of anthocyanins and influences both anthocyanin content and its antioxidant activity (Huyskens-Keil et al., 2020).

Antioxidants activity

As shown in Table 3, the antioxidant activity of the extracts varied significantly depending on the combination of extraction temperature and duration. Significant differences in antioxidant activity were observed in the fresh, concentrated, and rehydrated extracts for treatments K1T2, K1T3, K1T4, K3T3, and K3T4, as indicated by the use of distinct capital letters. In contrast, the remaining treatments did not exhibit statistically significant differences.

The highest antioxidant activity in the fresh extract was recorded at the lowest extraction temperature (25°C). However, extending the extraction duration at this temperature did not result in a significant enhancement in antioxidant activity (Table 3). At higher extraction temperatures, a notable decline in antioxidant activity was observed. This trend is consistent with prior findings, such as those reported by Suzery et al. (2020), who demonstrated that elevated extraction temperatures can lead to degradation of antioxidant compounds, as similarly noted in roselle (*Hibiscus sabdariffa* L.).

In the concentrated extract, antioxidant activity was not significantly different from that of the fresh extract, despite the concentration process involving evaporation at 70°C using a water bath. Although this process led to an increase in anthocyanin content, it concurrently caused a reduction in antioxidant activity. As previously discussed, exposure to high temperatures during processing can degrade heat-sensitive antioxidant compounds. In this context, not only the temperature but also the duration of concentration (70°C for 10h) likely contributed to the decline in antioxidant activity. Supporting this observation, Sunarya et al. (2024) reported that heating above 50°C for more than 30 minutes significantly reduced the antioxidant activity of anthocyanins extracted from black soybean seed coats.

Notably, the antioxidant activity observed in the fresh extract of this study (70.11 ± 13.42) was substantially higher than that reported by Havananda & Luengwilai (2019), whose values ranged from 26.7 ± 11 to 52.1 ± 20 mg TEAC g⁻¹ FW under similar ambient temperature extraction conditions (25°C). This suggests that differences in plant variety, extraction protocol, or sample morphology—such as the use of double-petaled blue *C. ternatea* in the present study—may contribute to enhanced retention of antioxidant compounds during extraction.

Colorimetric color test

The Lightness (L) value is used to measure the brightness of a color, ranging from 0 (black) to 100 (white). The fluctuating results in L value in the butterfly pea flower extracts can be attributed to both the high extraction temperatures and extended extraction periods. Additionally, the concentration process increases the dissolved anthocyanin pigments, which impact the color.

The higher the amounts of extracted pigments, the lower the L value, meaning the extract appears darker (Manasika & Widjanarko, 2014). This is because pigments absorb more visible light, which reduces the light reflected, hence decreasing the brightness.

A high L value often indicates that the pigment was not fully extracted, leading to a lighter color. The color stability can be influenced by factors like light, oxygen, temperature, pH, and more (Estiasih, 2016). This can explain why the brightness values (L*) of extracts vary based on extraction conditions, with higher temperatures or longer extraction times affecting the L value due to changes in pigment extraction and stability.

The a* value measures redness (ranging from -100 for green to +100 for red). The fresh extract in all tests showed a shift towards green, while the concentrated extract turned reddish, and rehydrated extracts returned to a greenish hue. This color shift could be attributed to changes in pH during the concentration process, which causes anthocyanins to form a reddish color. After rehydration, the color reverted to green, likely due to pH adjustments. Anthocyanins from purple cabbage turn bluish-green in neutral to alkaline pH and high pH leads to the instability of anthocyanins, causing them to degrade into colorless chalcones (Moulana et al., 2012; Susanti et al., 2019).

The b value indicates yellowness (ranging from -100 for blue to +100 for yellow). Positive values signify a yellowish color, while negative values indicate blue. Table 6 demonstrates that the interaction of temperature and extraction time significantly affected the b value in both fresh, concentrated, and rehydrated extracts. Specifically, the concentrated extract showed increased color intensity, with the blue color becoming more pronounced at higher temperatures. However, after rehydration, the color did not retain the same intensity as the fresh extract. Prolonged storage or higher temperatures can decrease color intensity by damaging the pigment structure and destabilizing the color components, which explains the observed changes in the b value (Winarti, 2008; Azzahra et al., 2013). In summary, the extraction process, temperature, and time all influence the color attributes of butterfly pea flower extracts, with higher temperatures tending to deepen the color intensity, while rehydration causes a loss of color vibrancy compared to the fresh extract.

CONCLUSION

The analysis showed that the extract with K2T4 treatment was the best for this study, as seen from the yield value in fresh and high-concentrated extracts. In addition, the rehydrated extract from the K2T4 treatment maintained the anthocyanin content, antioxidant activity, and color test results (brightness and color values a and b) compared to the fresh extract. The yield value of fresh extract was 89%, and concentrated extract was $25 \pm 0.02\%$. The anthocyanin content of fresh extract, concentrated extract, and rehydrated extract was 80.54 ± 6.78 ppm, 132.70 ± 9.76 ppm, 72.80 ± 13.18 ppm.

The antioxidant activities of fresh, concentrated, and rehydrated extracts were $45.84 \pm 23.71\%$, $28.21 \pm 11.88\%$, and $23.57 \pm 12.75\%$. In the color test, K2T4 treatment showed brightness values (L) in fresh extract, concentrated extract, and rehydrated extract of 30.15 ± 2.33 ; 25.18 ± 0.94 ; 35.54 ± 0.65 d B. Color value a of fresh extract, concentrated extract and rehydrated extract: -0.59 ± 0.79 ; -0.18 ± 0.23 ; -1.76 ± 0.50 . While the color value b of fresh extract, concentrated extract, and rehydrated extract: -2.28 ± 1.47 ; -5.32 ± 0.27 ; -3.30 ± 2.48 . Thus, the concentrated anthocyanin extract from butterfly pea flowers can be used as a ready-to-use natural blue dye for application in food and beverage products.

Further research is needed to test the color stability of anthocyanin extract during storage for a certain period. In addition, further research is needed on antioxidant content testing by adding avonoid and phenol tests. It is necessary to research the application of blue anthocyanin dye from butterfly pea flowers in food products with various concentrations of concentrated extract.

Acknowledgements: The author would like to thank dean of Agrotechnology Study Program, Faculty of Agriculture and Fisheries, Muhammadiyah University of Purwokerto and Research Center for Biosystematic and Evolution, BRIN.

Conflict of interest: The authors have declared that no conflicts of interest exist.

Authors' contributions: All authors contributed equally to both the research and the writing of the manuscript.

Ethical approval: Not applicable.

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