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The Effect of Ethanol Concentration Variations on The Total Phenolic and Flavonoid Levels of *Bauhinia purpurea* L. Leaf Extract

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ABSTRACT

Bauhinia purpurea L. is an ornamental plant that is not widely known or explored by the public, despite its significant antioxidant properties. The constituents responsible for antioxidant activity are phenolic and flavonoid. Many factors affect the total flavonoid content, one of which is the concentration of the extraction solvent. This study aimed to evaluate the effect of 70% and 96% ethanol concentrations of *B. purpurea* L on the total phenolic and flavonoid levels. An experimental method was employed, using 70% and 96% ethanol. The extracts were subjected to organoleptic tests, phytochemical screening, Thin Layer Chromatography (TLC), and UV-Vis spectrophotometry. Total phenolic content was determined using gallic acid standards (mg GAE/g extract), while total flavonoid content was measured using quercetin standards (mg QE/g extract). The organoleptic test revealed that the 70% and 96% ethanol extracts of *B. purpurea* L had a blackish-green color, thick consistency, distinctive odor, and bland taste. Phytochemical screening indicated the presence of phenolics, flavonoids, and saponins. The total phenolic content for the 70% ethanol extract was 14.644±0.222 mg GAE/g, and the total flavonoid content was 25.519±0.921 mg QE/g. The total phenolic content for the 96% ethanol extract was 7.176±0.347 mg GAE/g, and the total flavonoid content was 11.208±0.412 mg QE/g. These results indicate a significant difference between 70% and 96% ethanol use in extracting total phenolic and flavonoid content from *B. purpurea* L, with the 70% ethanol extract showing higher levels of both compounds.

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1. Introduction

Skin aging is a gradual decline of skin function and regenerative capacity, influenced by intrinsic and extrinsic factors. Intrinsic factors include hormonal changes, cellular metabolism, and genetic predispositions, while extrinsic factors involve environmental exposures such as air pollution, infrared radiation, and ultraviolet radiation (Yusharyahya, 2021). The progression of skin aging can be mitigated or delayed by applying antioxidants. Plants contain secondary metabolites with antioxidant properties, notably phenolic and flavonoid compounds. One such plant is the butterfly tree leaf (*Bauhinia purpurea* L.), an ornamental species that remains underutilized despite its high antioxidant potential–and relatively unknown to the public. Research by Purwasari (2021) demonstrated that butterfly tree leaf extract exhibits an antioxidant activity of 23.601 µg/mL.

Additionally, Djuleng (2021) reported that the total phenolic and flavonoid content of a 70% ethanol extract of *B. purpurea* L. was 1.85% and 0.93%, respectively.

The secondary metabolites in butterfly tree leaves can be obtained through extraction processes, including maceration. The efficacy of the extraction process is influenced by several factors, including the concentration of the solvent used. Solvent concentration affects the polarity of the solvent, which in turn impacts the solubility of bioactive compounds such as phenolics and flavonoids (Suhendra et al., 2019). Studies indicate that varying ethanol concentrations yield different extraction efficiencies. For example, Aryantini (2021) found that a 96% ethanol extract of butterfly tree leaves yielded 8.228%, whereas Purwasari (2021) observed that a 70% ethanol extract yielded 22.427%. The higher yield obtained with 70% ethanol is attributed to its greater polarity, facilitated by a higher OH-group content compared to 96% ethanol (Pujiastuti & El'Zeba, 2021). Furthermore, Khairunnisa et al. (2022) reported that the total flavonoid content in *Centella asiatica* L. Urban extracted using 70% ethanol was higher than that extracted with 96% ethanol. However, no data are available regarding the total phenolic and flavonoid content of extract *B. purpurea* using variation concentration ethanol. The present study examines the impact of 70% and 96% ethanol concentrations on the total phenolic and flavonoid content of butterfly tree leaves (*B. purpurea* L.).

2. Methods

2.1 The Processing and Extraction of Butterfly Tree Leaves

Butterfly tree leaves (*B. purpurea L.*) was taken from Ngawu, Playen, Gunung Kidul, Yogyakarta (-7.9378510, 110.5445590), and the plant was verified by a botanist from the Plant Systematics Laboratory, Faculty of Biology, Gadjah Mada University, (number: 084/S.Tb/V/2022). The leaves were selected based on specific criteria: light green coloration, a position as the 2nd to 4th leaf from the top of the plant, and uniform size (approximately 5–7 cm). Wet sorting was conducted by washing the leaves under running water, followed by oven drying at a controlled temperature of 40–50°C until the leaves were completely dry. The dryness was confirmed by the leaves brittleness when crushed and a moisture content of less than 10%. The dried leaves were weighed, ground into powder using a grinder, and sieved through a 40-mesh sieve to ensure uniform particle size.

The butterfly tree leaf powder underwent maceration at a ratio of 1:10 (powder to solvent) for three days, stirring every six hours. The resulting filtrate was concentrated by evaporation at 40–50°C to produce a thick extract (Purwasari, 2021). The yield of the thick extract was subsequently calculated using the following formula:

$$\%\text{Yield} = \frac{\text{Weight of thick extract}}{\text{Simple Drug Weight}} \times 100\% \quad (1)$$

2.2 Organoleptic of Simple Drugs

Organoleptic testing was conducted using the five senses to assess the quality of the extract, evaluating attributes such as shape, taste, aroma, and color (Purwasari, 2021).

2.3 Phytochemical Screening

2.3.1 Alkaloid Test

A 10 mg sample of the 70% and 96% ethanol extract was dissolved in 2 mL of 70% and 96% ethanol, respectively. A few drops of 2 N HCl were added to this mixture. One mL of the mixture was then transferred into three separate test tubes, each receiving a different reagent: Wagner's reagent, Mayer's reagent, and Dragendorff's reagent. The presence of alkaloids in the extract is indicated by the formation of a yellow precipitate and a cloudy solution (Mayer), a brown precipitate (*Wagner*), and a brownish-orange precipitate (*Dragendorff*) (Harbone, 1997).

2.3.2 Phenolic Test

A 10 mg sample of the 70% and 96% ethanol extract was dissolved in 2 mL of 70% ethanol and 2 mL of 96% ethanol, respectively, and then heated. One mL of the mixture was taken and treated with three drops of 5% $FeCl_3$. The formation of a green or bluish-green color indicates the presence of phenolic compounds (Harbone, 1997).

2.3.3 Flavonoid Test

A 10 mg sample of the 70% and 96% ethanol extract was dissolved in 2 mL of 70% ethanol and 2 mL of 96% ethanol, respectively, and then heated. 1 mL of the mixture was taken, and a sufficient amount of magnesium powder was added, followed by three drops of concentrated HCl. The appearance of a yellow, red, or orange color indicates a positive result for the presence of flavonoids (Harbone, 1997).

2.3.4 Saponin Test

A 10 mg sample of the 70% and 96% ethanol extract was dissolved in 2 mL of 70% ethanol and 2 mL of 96% ethanol, respectively. To this mixture, 2 mL of hot water and three drops of 2 N HCl were added. The mixture was shaken for 10 seconds, and the formation of a stable foam of 1-10 cm in height for 10 minutes indicates a positive result (Harbone, 1997).

2.3.5 Thin Layer Chromatography (TLC) Test

The mobile phase used was a mixture of formic acid, acetone, and toluene (2:4:4. v/v/v), and the stationary phase was silica gel $60F_{254}$. Quercetin was used as the standard. A 10 mg sample of the ethanol extract was dissolved in 2 mL of methanol. The TLC plate (10 cm × 2 cm) was spotted with the extract and the quercetin standard (2 µL) using a capillary tube. The spots were examined under UV light at 254 nm and 365 nm. The TLC plate was then sprayed with 5% $AlCl_3$, and the spots were observed again. The Rf (Retention Factor) value was calculated using the formula: (Purwasari, 2021).

$$Rf = \frac{\text{analytical travel distance}}{\text{solvent travel distance}} \quad (2)$$

2.3.6 Determination of Total Phenolic Content

The determination of total phenolic content followed the procedure outlined by Chun et al., (2003).

2.3.7 Preparation of Gallic Acid Standard Solution

A standard gallic acid solution with a concentration of 1000 ppm was prepared. A series of concentrations, namely 100, 150, 200, 250, 300, 350, and 400 ppm, were then prepared by diluting the standard solution with sufficient methanol (p.a.) to a final volume of 5 mL.

2.3.8 Preparation of 7% Sodium Carbonate ($Na_2 CO_3$) Solution

Seven grams of $Na_2 CO_3$ were weighed and placed into a beaker. Sufficient warm distilled water is added to the beaker, and the mixture is sonicated using an ultrasound device to ensure thorough mixing. Distilled water is then added until the total volume of the solution reaches 100 mL.

2.3.9 Determination of Maximum Wavelength

A 0.1 mL aliquot of each concentration of gallic acid standard was taken and mixed with 0.1 mL of Folin-Ciocalteu reagent, 1 mL of 7% $Na_2 CO_3$ solution, and 5 mL of Water for Injection (WFI). The absorbance was then measured at 600 to 800 nm to determine the maximum wavelength.

2.3.10 Determination of Operating Time (OT)

A 0.1 mL aliquot of each concentration of gallic acid standard was taken and mixed with 0.1 mL of Folin-Ciocalteu reagent, 1 mL of 7% $Na_2 CO_3$ solution, and 5 mL of Water for Injection (WFI). The solution was then scanned at the maximum wavelength obtained for 2 hours, with readings taken at 1-minute intervals until a stable reaction was achieved.

2.3.11 Preparation of Standard Gallic Acid Calibration Curve

A total of 0.1 mL of Folin-Ciocalteu Reagent was mixed with 0.1 mL of the standard gallic acid solution at each concentration. After 4 minutes, 1 mL of 7% $Na_2 CO_3$ solution was added, followed by the addition of Water for Injection (WFI) to bring the total volume to 5 mL. The mixture was incubated for 2 hours at room temperature, and then the absorbance was read at the maximum wavelength. The procedure was repeated three times.

2.3.12 Determination of Total Phenolic Content (TPC)

A 70% ethanol extract with a concentration of 10.000 ppm and a 96% ethanol extract with a concentration of 15.000 ppm were prepared. A 0.1 mL aliquot of each extract was mixed with 0.1 mL of Folin-Ciocalteu reagent and shaken. After 4 minutes, 1 mL of 7% sodium carbonate solution was added, followed by Water for Injection (WFI) to bring the total volume to 5 mL. The mixture was allowed to stand at room temperature for the operating time (OT).

The absorbance was then measured at the maximum wavelength, and the procedure was repeated three times. The Total Phenolic Content (TPC) is expressed in milligrams of gallic acid equivalent (GAE) per 100 grams of sample. The phenolic content was determined using the following formula:

$$TPC = \frac{C.V.Fp}{g} \quad (3)$$

Where:
TPC = *Total Phenolic Content*
C = Concentration (x)
V = Extract volume (mL)
Fp = Dilution factors
g = Sample weight (g)

2.3.13 Determination of Total Flavonoid Content

The determination of flavonoid content followed the procedure outlined by Chang et al., (2002) as referenced in the research of Ahmad et al., (2014).

2.3.14 Preparation of the Quercetin Standard Stock Solution

A quercetin solution was prepared with a concentration of 1000 ppm. Subsequently, a series of solutions with concentrations of 40, 60, 80, 100, and 120 ppm were prepared. The absorbance of each solution was measured at the maximum wavelength.

2.3.15 Determination of the Maximum Wavelength

One mL of the quercetin stock solution was mixed with 8 mL of 5% CH_3COOH and 1 mL of 10% $AlCl_3$. The absorbance was then measured over a wavelength range of 350 to 450 nm.

2.3.16 Determination of Operating Time (OT)

One mL of 10% $AlCl_3$ and 8 mL of 5% CH_3COOH were added to 1 mL of the quercetin stock solution. The absorbance was scanned at the previously determined maximum wavelength for 1 hour, with readings taken at 1-minute intervals until a consistent reaction was observed.

2.3.17 Preparation of the Quercetin Standard Calibration Curve

One mL of each concentration of the quercetin stock solution was pipetted into separate containers. To each, 1 mL of 10% $AlCl_3$, 8 mL of 5% CH_3COOH, and 10 mL of methanol p.a. were added. The solutions were allowed to stand according to the operating time (OT) at room temperature. The absorbance was then measured at the maximum wavelength, and the procedure was repeated three times.

2.3.18 Determination of Total Flavonoid Content

A 70% ethanol extract was prepared with a concentration of 10.000 ppm, and a 96% ethanol extract was prepared with a concentration of 7.000 ppm. One mL of each extract was placed into separate measuring flasks, to which 8 mL of 5% CH_3COOH, 1 mL of 10% $AlCl_3$, and methanol p.a. were added to reach a final volume of 10 mL. The mixture was shaken until homogeneous and then incubated at room temperature. The absorbance was measured at the previously determined maximum wavelength, and the measurement was repeated three times. The flavonoid levels were calculated using the following formula:

$$TFC = \frac{C.V.Fp}{g} \quad (4)$$

Remarks:
TFC = Total Flavonoid content
C = Concentration (x)
V = Extract volume (mL)
Fp = Dilution factors
g = Sample weight (g)

2.4 Data Analysis

The data obtained were presented as total phenolic and flavonoid levels. These levels were tested for homogeneity and normality. Following this, the Independent T-test was performed for further analysis.

3. Results and Discussion

In this experiment, we prepared the extract of *B. purpurea* leaves using 70% and 96% ethanol. Based on Table 1, the yield of 70% ethanol is greater than 96% ethanol because 70% ethanol has a higher percentage of water than 96% ethanol. The hydroxyl group in the water is a very polar compound, so it is more effective in extracting all polar compounds, especially phenolic and flavonoid compounds (Wahyudi & Minarsih, 2023). The yield obtained has met the requirements of FHI because the extract obtained is not less than 10% (Kementrian Kesehatan RI, 2017).

Table 1. Results of ethanol extract yield of butterfly tre leaf

Sample		Raw Weight (g)	Yield (%)	Farmakope Herbal Indonesia, (2017)
70% Ethanol Extract	1st Maceration	22.016	22.016	>10%
	2nd Maceration	20.868	20.863	
	3rd Maceration	21.303	21.303	
96% Ethanol Extract	1st Maceration	11	11	
	2nd Maceration	12	12	
	3rd Maceration	16.512	16.5	

3.1 Phytochemical Screening

Phytochemical testing is an analytical method used to identify and detect the presence of specific chemical compounds in butterfly tree leaves. This method includes tests for alkaloids, phenolics, flavonoids, and saponins. The results of the phytochemical test indicate that butterfly tree leaves contain phenolic, flavonoid, and alkaloid compounds. The phytochemical test results for the 70% and 96% ethanol extracts of butterfly tree leaves are presented in Table 2.

Table 2. Phytochemical screening

Identification	Sample		Djuleng, (2021)
	70% Ethanol	96% Ethanol	
Alkaloid			
Mayer	-	-	-
Wagner	-	-	-
Dragendroff	-	-	-
Phenolic	+	+	+
Flavonoid	+	+	+
Saponin	+	+	+

Notes:
(+) positive: contains compound group
(-) negative: does not contain compound group

The test for alkaloid compounds showed negative results as no color change and no colored precipitate formed when Mayer, Wagner, and Dragendorff reagents were added. The addition of 2 N HCl in this test is intended to neutralize the basic properties of the alkaloids so that they can be extracted with acidic solvents. The absence of alkaloids in this study is probably due to the formation of a potassium alkaloid complex that does not reach the saturation limits and therefore does not form a precipitate. In addition, the low alkaloid content in the extract may result in the compound not being fully extracted during the extraction process (Minarno, 2015). The test for phenolic compounds was carried out by adding $FeCl_3$ solution, which aims to identify the presence of phenolic groups in the sample. Table 2 and the reaction shown in Figure 1 shows positive results with a blackish-green color, indicating that the sample contains phenolic compounds. This color change is because phenols can form complex compounds with $FeCl_3$ (Mukhriani *et al* ., 2019).

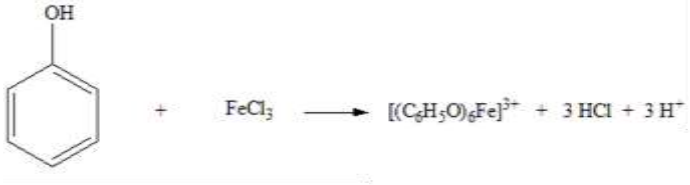

Figure 1. The reaction between phenol and $FeCl_3$ (Mukhriani et al ., 2019).

The flavonoid test unequivocally demonstrates the presence of an orange hue following reduction with concentrated hydrochloric acid and magnesium powder, which yields a complex compound and indicates the presence of positive flavonoid compounds belonging to the flavone, aurone, or chalcone group (Oktavia & Sutoyo, 2021).

The saponin test yielded positive results. The addition of 1% HCl resulted in the formation of a foam measuring 2 cm in height. These findings demonstrate that saponin compounds are readily soluble in water and produce foam when shaken. The observed foam is attributed to the interaction of saponin with air or agitation (Aryantini, 2021).

3.2 Thin Layer Chromatography

The Thin Layer Chromatography (TLC) test was used to identify flavonoid compounds in butterfly tree leaves. The mobile phase (eluent) consisted of a mixture of formic acid, acetone, and toluene in a 2:4:4 (v/v/v) ratio. Silica gel 60 F254 was used as the stationary phase. The separation of compounds was observed under UV light at two different wavelengths, 254 nm and 365 nm. The visible spots were then analyzed, and the RF values were calculated, as presented in Table 3.

Table 3. Rf value (*Retardation Factor*)

Quercetin	70% Ethanol Extract from *B. purpurea* L leaf	96% Ethanol Extract from *B. purpurea* L leaf	Purwasari, (2021)
0.812	0.825	0.837	0.875

The quercetin standard displayed a brownish-yellow color, the 70% ethanol extract exhibited a greenish-yellow color, and the 96% ethanol extract presented a faded greenish-yellow color. The appearance of greenish-yellow spots indicates the presence of flavonoid compounds (Figure 2).

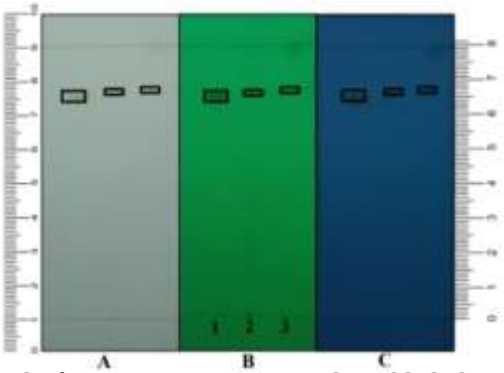

Figure 2. TLC of *B. purpurea* L. leaf extract. A. Detection with visible light; B. Detection with UV light 254 nm; C. Detection with UV light 365 nm. (1) Quercetin; (2) Ethanol extract 70%; (3) Ethanol extract 96%.

According to Ayu *et al*. (2019), a color change to greenish-yellow in spots observed after the thin-layer chromatography (TLC) separation process confirms the presence of flavonoids. TLC observations in this study confirmed that both the 70% and 96% ethanol extracts contained flavonoid compounds. Furthermore, as shown in Table 3. the Rf values of the samples and standards were nearly identical, suggesting that the flavonoid compounds in the extracts are comparable to the standard. A difference in *Rf* values is considered positive if≤0.05 and negative if>0.05 (Oktaviantari et al., 2019).

The determination of total phenolic content was conducted using the Folin-Ciocalteu method, while the total flavonoid content was measured using the calorimetric method with $AlCl_3$. The Folin-Ciocalteu method operates on the principle that phenolic compounds undergo oxidation when

reacted with the Folin-Ciocalteu reagent, resulting in a blue-colored solution. This blue color arises from the interaction between phenolic compounds and the reagent, which can be quantified using a UV-Vis spectrophotometer within the wavelength range of 600–800 nm. In this study, the maximum absorption wavelength of gallic acid, used as a standard phenolic compound, was determined to be 760 nm, based on its absorbance at a concentration of 300 ppm within this wavelength range.

The optimal reaction time for gallic acid was also determined by measuring its absorption every minute over two hours, resulting in an operating time of 1 hour and 44 minutes. Gallic acid was selected as the standard due to its high reactivity with the Folin-Ciocalteu reagent, which ensures reliable results–and stability as a natural phenolic compound. When gallic acid reacts with the Folin-Ciocalteu reagent, a yellow color initially appears, indicating the presence of phenolic compounds in the sample. The reaction is conducted under basic conditions to facilitate proton dissociation, which generates phenolate ions required for the oxidation process. A sodium carbonate solution ($Na_2\ CO_3$) was used to maintain these optimal basic conditions in the reaction mixture.

The determination of total flavonoid content was conducted using the colorimetric method, which involves measuring the color intensity of a solution with a UV-Vis spectrophotometer. This method requires using a complexing agent, such as aluminium chloride ($AlCl_3$), to enhance the absorbance value and stabilize the color of the flavonoid extract solution, thereby improving the accuracy and reliability of measurements. Flavonoid extract solutions typically range in color from light green to brown and exhibit an absorption peak within the wavelength range of 350–450 nm. Adding $AlCl_3$ increases the absorbance value and stabilizes the color for easier measurement (Pratiwi et al., 2022).

The colorimetric method relies on forming a complex between $AlCl_3$ and flavonoids. Specifically, $AlCl_3$ reacts with the keto group at the C-4 atom and the hydroxyl groups adjacent to the C-3 or C-5 atoms in the flavonoid structure, leading to a wavelength shift and a color change to a more intense yellow. The intensity of this colored complex is directly proportional to the flavonoid concentration in the sample; higher concentrations result in higher absorbance values (Masúd & Puspitasari, 2017).

In this study, the wavelength for gallic acid was determined using a solution with a concentration of 80 ppm, measured within the range of 350–450 nm. The maximum absorption wavelength of gallic acid was found to be 416 nm. The optimal reaction time for flavonoid analysis was established at 28 minutes, based on measurements taken at 1-minute intervals over 45 minutes. Standard curves were prepared to calculate the total phenolic and flavonoid content in extracts of butterfly tree leaves (*B. purpurea L.*) obtained with 70% and 96% ethanol. The standard curve equation for gallic acid was determined to be y=0.002x+0.0377. with a correlation coefficient (r) 0.9996. while for quercetin, the equation was y=0.0048x+0.0556. with a correlation coefficient of 0.9991.

The results of the total phenolic and flavonoid content analysis for the extracts are presented in Tables 4 and 5.

Table 4. Total phenolic content of butterfly tree leaf

Sample	Replication	Total Phenolic Content (mg GAE/g)	Average±SD (mg GAE/g)
70% Ethanol	1	14.413	14.644±0.222
	2	14.663	
	3	14.855	
96% Ethanol	1	7.287	7.176±0.347

Table 5. Total flavonoid content of *B. purpurea* L leaf

Sample	Replication	Total Flavonoid Content (mg GAE/g)	Average±SD (mg GAE/g)
70% Ethanol	1	25.024	25.519±0.921
	2	24.951	
	3	26.583	
96% Ethanol	1	10.746	11.208±0.412
	2	11.340	
	3	11.539	

The research findings indicate that the total phenolic and flavonoid content in the 70% ethanol extract of butterfly tree leaves (*B. purpurea L.*) is higher than that in the 96% ethanol extract. This suggests that 70% ethanol is more effective in extracting phenolic and flavonoid compounds from butterfly tree leaves. The higher polarity of 70% ethanol, due to its greater number of OH groups, enhances its ability to dissolve polar phenolic and flavonoid compounds more efficiently. Furthermore, the study revealed that the total flavonoid content in the extracts was higher than the total phenolic content.

This finding contrasts with the results of Djuleng (2021), who reported higher total phenolic content than flavonoid content. The observed discrepancy could be attributed to the inherent instability of gallic acid and variations in experimental conditions, such as operating time and wavelength settings, which may influence the extraction results. Supporting evidence from Krishnaveni (2014) demonstrated that flavonoid content exceeded phenolic content in butterfly tree leaves extracted with water, with total flavonoid content reported at 160.0±6.9 QE mg/g and total phenolic content at 126.66±6.11 GAE mg/g.

The findings underscore the significant potential of butterfly tree leaves, rich in phenolic and flavonoid compounds, as antioxidants. These compounds are known to confer various health benefits by mitigating oxidative damage. Statistical testing was conducted to determine the differences between each concentration of 70% ethanol and 96% ethanol. The results of this data analysis are presented in Table 6 and Table 7.

Table 6. Total phenolic content data analysis

Sample	Normality Test	Homogeneity Test	T-Test
70% Ethanol	0.463	0.728	0.000a
96% Ethanol	0.373		

Remarks: (a) Significant Gap

Table 7. Total flavonoid content data analysis

Sample	Normality Test	Homogeneity Test	T-Test
70% Ethanol	0.783	0.119	0.000a
96% Ethanol	0.924		

Remarks: (a) Significant Gap

The statistical analysis revealed that the data were normally distributed and homogeneous, as indicated by a p-value of<0.05. The results of the T-test showed a significant two-tailed value of <0.05 (0.000), demonstrating a statistically significant difference between the use of 70% and 96% ethanol in the extraction process to the total phenolic and flavonoid content of butterfly tree leaves (*B. purpurea L.*). These findings confirm that the concentration of ethanol used in the extraction process significantly affects the yield of these bioactive compounds, emphasizing the critical role of solvent selection in optimizing the extraction efficiency of phenolic and flavonoid compounds.

4. Conclusion

Variations in ethanol concentration (70% and 96%) significantly influence the total phenolic and flavonoid content in butterfly tree leaf (*B. purpurea* L.) extracts. The total phenolic content obtained with 70% ethanol was 14.644±0.222 mg GAE/g, while the total flavonoid content was 25.519±0.921 mg QE/g. In contrast, extraction with 96% ethanol yielded a total phenolic content of 7.176±0.347 mg GAE/g and a total flavonoid content of 11.208±0.412 mg QE/g. Further research is required to determine the phenolic and flavonoid content of butterfly leaves by varying conventional extraction methods (maceration) with modern ones such as Ultrasonic-assisted Extraction (UAE).

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REFERENCES

Ahmad, A. R., Sakinah, Wisdawati, & Asrifa, W. O. (2014). Study of antioxidant activity and determination of phenol and flavonoid content of pepino's leaf extract (*Solanum muricatum* Aiton). *International Journal of PharmTech Research*, 6(2), 600–606.

Aryantini, D. (2021). Aktivitas Antioksidan dan Kandungan Tanin Total Ekstrak Etanol Daun Kupu-Kupu Bauhinia purpurea L.). Diakses pada 10 Januari 2024 pukul 15.00 WIB. *Jurnal Farmagazine*, 8(1), 54. https://doi.org/10.47653/farm.v8i1.537.

Chang, C. C., Yang, M. H., Wen, H. M., & Chern, J. C. (2002). Estimation of total flavonoid content in propolis by two complementary colometric methods. *Journal of Food and Drug Analysis*, 10(3), 178–182. https://doi.org/10.38212/2224-6614.2748.

Chun, O. K., Kim, D. O., & Lee, C. Y. (2003). Superoxide Radical Scavenging Activity of the Major Polyphenols in Fresh Plums. *Journal of Agricultural and Food Chemistry*, 51(27), 8067–8072. https://doi.org/10.1021/jf034740d.

Djuleng, A. (2021). *Identifikasi Senyawa Total Fenolik Dan Total Flavonoid Ekstrak Larut Etanol Daun Kupu-Kupu (Bauhinia purpurea) Dengan Spektrofotometri UV-Vis.* Universitas Jenderal Achmad Yani Yogyakarta.

Harbone, J. B. (1997). *Metode Fitokimia: Penuntun Cara Modern Menganalisis Tumbuhan* (S. I. Penerjemah: Padmawinata K (ed.); 3rd ed.). Bandung: ITB.

Kementrian Kesehatan RI. (2017). *Farmakope Herbal Indonesia* (II). Kementrian Kesehatan RI.

Khairunnisa, S., Hakim, A. R., & Audina, M. (2022). Perbandingan Kadar Flavonoid Total Berdasarkan Perbedaan Konsentrasi Pelarut Etanol Dari Ekstrak Daun Pegagan (Centella asiatica [L] Urban). *Journal Pharmaceutical Care and Sciences*, 3(1), 121–131. https://doi.org/10.33859/jpcs.v3i1.236.

Krishnaveni, M. (2014). Antioxidant potential of bauhinia purpurea (L) leaf. *International Journal of Pharmacy and Pharmaceutical Sciences*, 6(7), 558–560.

Masúd, F., & Puspitasari, P. (2017). Studi Pendahuluan Ekstraksi Bertingkat Minyak Biji Mangga Arumanis (Mangifera Indica) Menggunalan Pelarut N-Heksan dan Etanol. *INTEK: Jurnal Penelitian*, 4(1), 42. https://doi.org/10.31963/intek.v4i1.92.

Minarno, B. E. (2015). Skrining Fitokimia dan Kandungan Total Flavanoid Pada Buah Carica pubescens Lenne & K. Koch Di Kawasan Bromo, Cangar, dan Dataran Tinggi Dieng. Diakses pada 7 Maret 2024 pukul 11.00 WIB. *El-Hayah*, 5(2), 73–82. https://doi.org/10.18860/elha.v5i2.3022.

Mukhriani, M., Rusdi, M., Arsul, M. I., Sugiarna, R., & Farhan, N. (2019). Kadar Fenolik dan Flavonoid Total Ekstrak Etanol Daun Anggur (Vitis vinifera L). Diakses pada 7 Maret 2024 pukul 11.00 WIB. *Ad-Dawaa' Journal of Pharmaceutical Sciences*, 2(2), 95–102. https://doi.org/10.24252/djps.v2i2.11503.

Oktavia, F. D., & Sutoyo, S. (2021). Skrining Fitokimia, Kandungan Flavonoid Total, Dan Aktivitas Antioksidan Ekstrak Etanol Tumbuhan Selaginella doederleinii. Diakses pada 15 maret 2024 pukul 15.30 WIB. *Jurnal Kimia Riset*, 6(2), 141. https://doi.org/10.20473/jkr.v6i2.30904.

Oktaviantari, D. E., Feladita, N., & Agustin, R. (2019). Identifikasi Hidrokuinon Dalam Sabun Pemutih Pembersihwajah Pada Tiga Klinik Kecantikan Di Bandar Lampung Dengan Metode Kromatografi Lapis Tipis dan Spektrofotometri UV-Vis. *Jurnal Analis Farmasi* , 4(2), 91–97.

Pratiwi, D. N., Utami, N., & Pratimasari, D. (2022). Karakterisasi dan Penetapan Kadar Flavonoid Total Ekstrak dan Fraksi Bunga Pepaya Jantan (Carica papaya L.) dengan Spektrofotometri UV-Vis. *Jurnal Ilmiah Farmasi*, 18(2), 219–233. https://doi.org/10.20885/jif.vol18.iss2.art20.

Pujiastuti, E., & El'Zeba, D. (2021). Perbandingan Kadar Flavonoid Total Ekstrak 70% Ethanol Dan 96% Kulit Buah Naga Merah *Hylocereus polyrhizus*) Dengan Spektrofotometri. *Cendekia Journal of Pharmacy*, 5(1), 28–43. https://doi.org/10.31596/cjp.v5i1.131.

Purwasari, F. (2021). *Uji Peredaman Radikal Bebas DPPH (2.2 diphenyl-1-pikrilhidrazil) Ekstrak Etanol Daun Kupu-kupu (Bauhinia purpurea L.)*. Universitas Jenderal Achmad Yani Yogyakarta.

Suhendra, C. P., Widarta, I. W. R., & Wiadnyani, A. A. I. S. (2019). Pengaruh Konsentrasi Etanol Terhadap Aktivitas Antioksidan Ekstrak Rimpang Ilalang ((Imperata cylindrica (L) Beauv.) pada Ekstraksi Menggunakan Gelombang Ultrasonik. *Jurnal Ilmu Dan Teknologi Pangan*, 8(1), 27–35.

Yusharyahya, S. N. (2021). Mekanisme Penuaan Kulit sebagai Dasar Pencegahan dan Pengobatan Kulit Menua. *EJournal Kedokteran Indonesia*, 9(2), 150. https://doi.org/10.23886/ejki.9.49.150.

Wahyudi, T. A., & Minarsih, T. (2023). Pengaruh Ekstraksi dan Konsentrasi Etanol terhadap Kadar Flavonoid Total dan Aktivitas Antioksidan Ekstrak Jahe Emprit (Zingiber officinale var. Amarum). Diakses pada 1 Juli 2024 pukul 14.00 WIB. *Indonesian Journal of Pharmacy and Natural Product*, 6(1), 30–38. https://doi.org/10.35473/ijpnp.v6i01.2208.