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Chemometric Analysis of Phytochemical Content and Antioxidant Activity of 70% Ethanol and Ethyl Acetate Extracts of Butterfly Pea Flowers

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Abstract: Antioxidants are essential for the body to inhibit or ward off free radicals. Naturally, the body can produce antioxidants, but they can also be obtained from external sources. Interestingly, antioxidants can also be found in plants, one of which is the butterfly pea flower (*Clitoria ternatea L.*). Compounds contained in butterfly pea flowers, such as flavonoids and phenolics, were macerated with 70% ethanol and ethyl acetate solvents. This study aims to analyze the phytochemical content (flavonoids and phenolics) and evaluate the antioxidant activity of butterfly pea flower extract and their relationship with the PCA (Principal Component Analysis) chemometric method. The total flavonoid content test was carried out with quercetin as a comparator, while the total phenolic content test was carried out with gallic acid as a comparator. Meanwhile, antioxidant activity was carried out using the DPPH method. In this study, the total flavonoid content in the 70% ethanol extract was 4.109 ± 0.027 mg QE/mL, while in the ethyl acetate extract it was 6.616 ± 0.22 mg QE/mL. The total phenolic content produced was 28.276 ± 1.36 mg GAE/mL for the 70% ethanol extract and 31.579 ± 1.59 mg GAE/mL for the ethyl acetate extract, respectively. Meanwhile, the IC_{50} value for antioxidant activity showed that the ethanol extract had a value of 54.23 ± 2.37 µg/mL, while the ethyl acetate extract had a value of 39.38 ± 3.14 µg/mL. The ethyl acetate extract had higher flavonoid, phenolic, and antioxidant activity than the 70% ethanol extract. Chemometric analysis showed a positive correlation between flavonoids and phenolics in the ethanol solvent. Chemometric analysis showed a relationship between flavonoid and phenolic content in both the ethanol and ethyl acetate solvents and the antioxidant activity of the DPPH method.

Keywords: Antioxidant activity; Clitoria ternatea L.; Chemometric analysis; Phytochemical Content; DPPH Test.

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1. Introduction

Antioxidants are the body's defense system against free radicals. An imbalance between the amount of antioxidants and the amount of free radicals in the body can lead to damage such as cancer, coronary heart disease, rheumatism, cataracts, and neurodegenerative diseases. Supplementation with antioxidants from external sources is also necessary to reduce the damaging capacity of free radicals. Antioxidants are produced naturally by the body, known as endogenous antioxidants, and those obtained from outside the body, known as exogenous antioxidants. Natural antioxidants include enzymes produced internally, while exogenous antioxidants can be found in vitamins and minerals obtained through food and supplements. Antioxidants can also be found in plants, one of which is the butterfly pea flower (Nurkhasanah et al., 2023).

The butterfly pea flower (*Clitoria ternatea L.*) has diverse potential, including as a raw material for herbal medicines, natural dyes, and health supplements. The butterfly pea flower is characterized by its distinctive purple petals, which are rich in anthocyanins. This flower is known to contain phytochemical compounds that have the potential as antibacterial agents (Besan et al., 2023), antioxidants, antidepressants, analgesics, antihistamines, antimicrobials, anticancer, antiparasitic, anticholesterol, and antidiabetic (Fadel et al., 2023). The chemical

compounds contained in butterfly pea flowers include carbohydrates, tannins, triterpenoids, saponins, flavonoids, phenols, proteins, cardiac glycosides, anthraquinones, alkaloids, flavonol glycosides, anthocyanins, stigmast-4ene-3,6-dione, steroids, and essential oils (Fadel et al., 2023). Compounds contained in butterfly pea flowers that have antioxidant properties include flavonoids and phenols. Several studies on total flavonoid and total phenolic levels have shown varying results. Rahayu et al., (2021) stated that the flavonoid levels obtained were 59.37 QE/g and 63.09 QE/g. According to research by Andriani & Murtisiwi (2018), the phenolic content of butterfly pea flowers is 19.43 GAE/g.

Antioxidant activity can be identified using several methods, one of which is the DPPH test. The DPPH method, also known as 11,1-diphenyl-β picrylhydrazyl, is used to assess the potential of antioxidants to neutralize free radicals (Mu'nisa, 2023). This study used the DPPH method because it is simple, fast, and easy to perform, can be used on small samples, is susceptible to low concentrations of samples, and is relatively stable compared to other methods. However, DPPH can only be dissolved in organic solvents such as methanol, ethanol, and ethyl acetate, making it somewhat difficult to analyze hydrophilic compounds (Apriani & Pratiwi, 2021).

Various studies have been conducted to evaluate the antioxidant activity of butterfly pea flower extract using the DPPH method. Several studies have examined the relationship between phytochemical content and antioxidant activity in plants in general (Setyowati et al., 2020), but research specifically examining the compound content and antioxidant activity of butterfly pea flower extract is still limited. Therefore, further analysis is needed to determine the relationship between the two, one of which is through chemometric methods that can provide more accurate and reliable analysis (Kucharska-ambroú & Karpinska, 2019). Chemometric analysis is a method of chemical data analysis that detects functional groups using infrared techniques processed using statistical and mathematical approaches (Kucharska-ambroú & Karpinska, 2019). In addition to being used to detect counterfeiting in various products, both in the food, beverage, and pharmaceutical sectors (Ary Prabowo et al., 2022; Kucharska-ambroú & Karpinska, 2019; Yulia et al., 2017), chemometrics also plays a crucial role in evaluating the relationship between phytochemical content and plant activity. Based on this background, researchers are interested in conducting further research on chemometric analysis of the phytochemical content and antioxidant activity of 70% ethanol and ethyl acetate extracts of butterfly pea flowers (*Clitoria ternatea L.*).

2. Research Methods

This research was conducted through an experimental process at the ITEKES Cendekia Utama Pharmacy Laboratory using quantitative methods. Samples in the form of 4 kg of fresh butterfly pea flowers (*Clitoria ternatea L.*) obtained from the Blora area, Central Java, were dried and ground into simplicia powder. The simplicia powder was extracted using two types of solvents, namely 70% ethanol solvent and ethyl acetate. Extraction was carried out using the maceration method for three days, with a material and solvent ratio of 1:10. Measurement of total flavonoid levels, total phenolics and antioxidant activity using the DPPH method was carried out using UV-Vis spectrophotometry. Total flavonoid levels were determined at a wavelength of 373 nm with quercetin as a standard reference, total phenolic levels were determined at a wavelength of 760 nm using gallic acid as a standard reference, while antioxidant activity tests were carried out using the DPPH method at a wavelength of 515 nm. The results obtained were analyzed statistically using Principal Component Analysis (PCA) in Minitab software.

3. Results and Discussion

Research Results

Plant Determination Results

The butterfly pea flower used in this study was identified in the Biology Laboratory of Ahmad Dahlan University under the registration number 104/Lab.Bio/B/II/2025. The identification results showed that the analyzed specimens had morphological characteristics consistent with Clitoria ternatea L., known as the butterfly pea flower according to the Flora reference (Steenis, 1958). The identification results are as follows::

1b – 2b – 3b – 4b – 6b – 7b – 9b – 10b – 11b – 12b – 13b – 14a – 15b – 197b – 208b – 219b – 220b – 224b – 225b – 227b – 229b – 230b – 234a Papilionaceae
1b – 5b – 16b – 19b – 20b – 21a Clitoria

Clitoria ternatea L.

Results of Determination of Water Content of Simplex Powder

Table 1. Water Content Results

Material	Powder Weight	Replication I	Replication II	Replication III	Average
Butterfly Pea Flower	5 gr	3,26%	3,26%	3,26%	3,26%
Water Content Requirement <10%					

Extract Results

Table 2. Butterfly Pea Flower Extract Results

Solvent	Powder Weight	Extract Weight	Yield	Characteristics		
				Shape	Color	Smell
Etanol 70% 2L	200 gr	71,1391	35,5695%	Thick	Blackish purple	Typical butterfly pea flowers
Etil Asetat 2L	200 gr	3,4984	1,7492%	Thick	Dark brown	Typical butterfly pea flowers

Ethanol Free Test Results

Table 3. Ethanol Free Test Results

Extract	Reagent	Result	Conclusion
Etanol 70% Butterfly Pea Flower	Extract + CH_3COOH + H_2SO_4	No ester odor is detected	Negative
According to the Indonesian Pharmacopoeia Edition VI (2020), the requirements for an ethanol-free test are that no esters are detected.			

Total Flavonoid Result

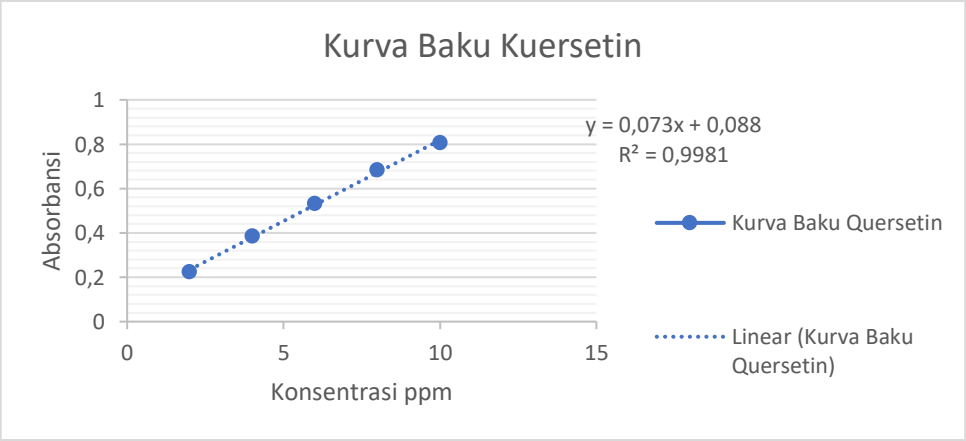

Figure 1. Calibration Curve of Total Flavonoids

Table 4. Total Flavonoid Result

Sample	Replication	Sample Absorbance	Total Flavonoid Content	Average total flavonoids ± SD
70% Ethanol Extract of Butterfly Pea Flowers	Rep 1	0,386	4,082	4,109 ± 0,027
	Rep 2	0,39	4,136	
	Rep 3	0,388	4,109	
Ethyl acetate extract of butterfly pea flower	Rep 1	0,589	6,863	6,616 ± 0,229
	Rep 2	0,556	6,410	
	Rep 3	0,568	6,575	

Total Phenolic Results

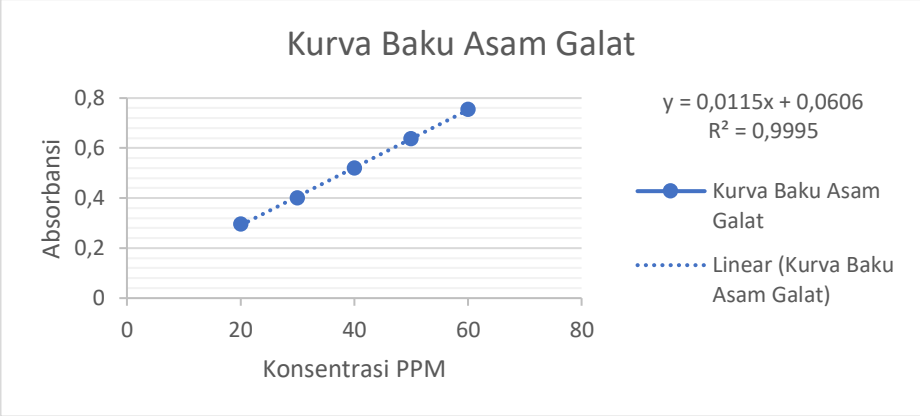

Figure 2. Total Phenolic Calibration Curve

Table 5. Total Phenolic Results

Sample	Reolication	Sample Absorbance	Total Phenolic Content	Average total phenolic ± SD
70% Ethanol Extract of Butterfly Pea Flowers	Rep 1	0,378	27,582	28,276 ± 1,36
	Rep 2	0,376	27,408	
	Rep 3	0,404	29,84	
Ethyl acetate extract of butterfly pea flower	Rep 1	0,412	30,53	31,579 ± 1,59
	Rep 2	0,445	33,408	
	Rep 3	0,415	30,8	

Antioxidant Results

Table 6. Antioxidant Results

Extract	IC50 value (µg/ml)			Average(µg/mL) ± SD	Antioxidant category
	Rep1	Rep 2	Rep 3		
Etanol 70% Bunga telang	51,92	54,09	56,67	54,23 ± 2,37	Strong
Ethyl acetate extract of butterfly pea flower	42,84	36,70	38,59	39,38 ± 3,14	Very strong
Kuersetin	3,68	3,67	-	3,677	Very strong

Chemometric Analysis Results

Loading plot

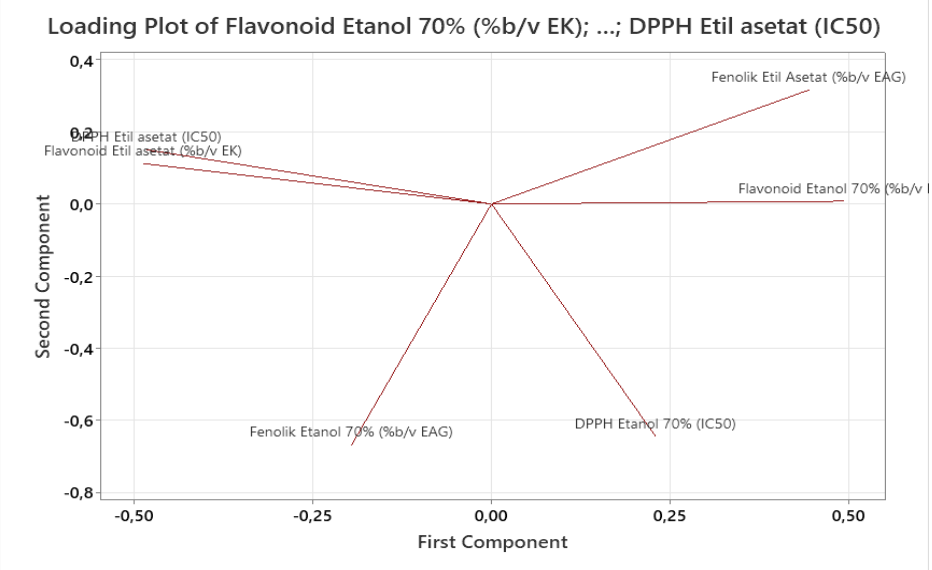

Figure 3. Loading Plot

Discussion

Plant Determination

Before being used in the research, butterfly pea plants were identified at the Biology Laboratory of Ahmad Dahlan University, Yogyakarta. This determination was carried out to ensure that the plants studied were indeed butterfly pea plants and to avoid errors in their use in the research. The results of the determination indicated that the plants used were indeed butterfly pea plants, possessing morphological characteristics consistent with the description of the species according to the Flora literature.

Sample Processing and Moisture Content Determination

Fresh purplish-blue butterfly pea flowers were sorted to remove any parts damaged by pests. They were then washed and dried to a moisture content below 10% to prevent microbial growth and improve the quality of the herbal preparations, ensuring they are less susceptible to spoilage and can be stored for a long time (Ministry of Health of the Republic of Indonesia, 1986). After drying, dry sorting was performed to remove any remaining impurities. The butterfly pea flowers were ground and sieved using a 40-mesh sieve to obtain a finer-grained powder for easier extraction.

Extract Preparation and Ethanol Beaps Test

The extraction process in this study used the maceration method, chosen because flavonoid and phenolic compounds are susceptible to structural damage at high temperatures. Therefore, the maceration method was used as a low-temperature extraction technique (cold extraction) to prevent damage to these compounds (Tara et al., 2021). The extraction was carried out using two types of solvents: 70% ethanol and ethyl acetate. 70% ethanol was chosen as the solvent because of its universal properties, which allow it to attract polar, semi-polar, and non-polar compounds, is effective in extracting various types of secondary metabolites, is resistant to microbial growth, and is affordable (Maryam et al., 2020). Meanwhile, ethyl acetate was used in the extraction because it is able to attract semi-polar compounds. Based on Table 2, 200 grams of powder was macerated using 70% ethanol to obtain 71.1391 grams of extract with a yield of 35.56%, while ethyl acetate produced 3.4984 grams of extract with a yield of 1.74%.

The ethanol-free test was conducted to determine whether the extract was solvent-free. Furthermore, the presence of ethanol indicates that the evaporation process was incomplete (Aulani, 2018). In Table 3, the results of the ethanol-free test for butterfly pea flowers showed a negative result, as evidenced by the absence of an ether odor after the addition of sulfuric acid and acetic acid. This indicates that the extract complies with the

requirements of the Indonesian Pharmacopoeia, Edition VI (2020), which requires no ester odor to be detected in the ethanol-free test, which is a negative indicator of ethanol.

Determination of Phytochemical Content

Total Flavonoids

After the extract was free of ethanol, the flavonoid and total phenolic content were determined using UV-Vis spectrophotometry with a colorimetric method. Flavonoid levels were determined using quercetin as a reference standard. Total flavonoid compounds were determined using the $AlCl_3$ reagent. $AlCl_3$ is used to identify the keto and hydroxy groups adjacent to the hydroxy-keto. The principle of this test involves aluminum chloride with a keto group at C-4 and a hydroxy group at C-4. In addition to $AlCl_3$, acetic acid is also used as a supporting reagent to aid color formation. Color intensity can then be detected after a specified incubation period (Nursamsiar et al., 2021).

The initial step is to determine the maximum wavelength and operating time. Determining the maximum wavelength aims to determine the wavelength at which absorption peaks, allowing measurements to be performed under optimal conditions. The first step is to find the maximum wavelength and the appropriate operating time to achieve maximum results. The wavelength was determined in the range of 350-450 nm, resulting in a result of 373 nm with an operating time of 17 minutes. The standard curve for quercetin was constructed using a series of concentrations of 2, 4, 6, 8, and 10 ppm, resulting in a regression of $y = 0.073x + 0.088$ with a correlation value of 0.998, as seen in Figure 1. The R2 value is close to 1, indicating that the absorbance follows a linear regression pattern that is directly proportional to the quercetin concentration.

Sample measurements were replicated three times with each solvent to ensure data accuracy. The results are shown in Table 4. Based on the results of this study, the total flavonoid content of butterfly pea flower extract using 70% ethanol was 4.109 ± 0.027 mg QE/mL and 6.616 ± 0.229 mg QE/mL using ethyl acetate. These results indicate that ethyl acetate is more effective in extracting flavonoids from butterfly pea flowers than 70% ethanol. This suggests that the type of solvent influences the amount of flavonoids in the extract.

Wayan et al. (2024) reported that the total flavonoid content of the 70% ethanol extract was 281.07 ± 0.74068 mg QE/100g. Meanwhile, according to research by Ngan (2025), the total flavonoid content of the ethyl acetate extract reached 15.39 ± 1.58 mg QE/g. These differences in flavonoid levels can be caused by several factors, including differences in extraction conditions, the form and condition of the crude drug, and the type of solvent used (Velisdeh et al., 2021). Furthermore, variations in plant origin, incubation time, and wavelength readings can also affect the results of total flavonoid measurements.

Total Phenolics

Following this, the total phenolic content is determined to determine the phenolic content of the extract. Total phenolic compounds are determined using the Folin-Ciocaleun method, where the Folin reagent reacts with the phenolic compounds, producing a color change from yellow to blue. The identity of the blue color is determined by the amount of phenolic compounds present in the sample; the greater the compound content, the more intense the blue color produced (Hudz et al., 2019). This method works based on the principle of the formation of blue compounds that can be measured at specific wavelengths, corresponding to the concentration of the compounds contained in the sample. This reagent undergoes hydrolysis of phenolates (alkali salts) or phenolic-hydroxy groups, reducing heteropoly acids (phosphomalibates), which are components of the Folin-Ciocalteu reagent, into a molybdenum-tungsten complex (Alfian & Susanti, 2015; Hudz et al., 2019).

The addition of sodium carbonate is necessary because phenolic compounds react with Folin-Ciocalteu in alkaline conditions. Total phenolic content was determined using gallic acid as a standard because this compound has hydroxyl groups and conjugated double bonds on each benzene ring, which effectively form complexes with the Folin-Ciocalteu reagent (Wijayanti, 2024). The first determination was carried out in the same manner as the total flavonoid test, namely by finding the maximum wavelength and appropriate operating time to ensure measurement under optimal conditions. The maximum wavelength was determined within the range of 400-800 nm, and the result was 760 nm, with an operating time of 25 minutes.

The wavelength and OT data were then used to determine the standard curve for quercetin and total phenolic content. The standard curve was created to calculate the phenolic content in the sample by determining a linear regression equation. The regression obtained was y = 0.0115x + 0.0608 with a correlation coefficient of 0.9995, as seen in Figure 2. Gallic acid solution was measured at concentrations of 20 ppm, 30 ppm, 40 ppm, 50 ppm, and 60 ppm, and the absorbance of the standard solution at each concentration was then obtained. A linear line was obtained, which was later used to determine the total phenolic content in butterfly pea flower extract (*Clitoria ternatea* L.).

The total phenolic content was measured in Gallic Acid Equivalent (GAE), which represents the number of milligrams of gallic acid in a sample. The determination of total phenolic content was replicated three times for each extract. The results are shown in Table 5. The 70% ethanol extract of butterfly pea flowers contained 28.276 ± 1.36 mg GAE/mL, and the ethyl acetate extract contained 31.579 ± 1.59 mg GAE/mL. Ethyl acetate was more effective in extracting phenolic compounds from butterfly pea flowers than 70% ethanol.

Previous research by Ngan (2025) reported that the total phenolic content of the ethyl acetate extract was 0.86 mg/g. Meanwhile, research by Andriani & Murtisiwi (2018) showed a much higher phenolic content, namely 19.43±1.621 mg/g. This difference in phenolic content can be caused by several factors, including differences in extraction conditions, the form and condition of the simplex, and the type of solvent used (Velisdeh et al., 2021). In addition, variations in plant origin, incubation time, and wavelength readings can also affect the results of total phenolic content measurements.

Antioxidant Activity Results Using the DPPH Method

The next test was the antioxidant activity test using the DPPH method. The DPPH method was used because it is simple, fast, easy to perform, can be used on small sample quantities, is susceptible to low concentrations of samples, and is relatively stable compared to other methods (Apriani & Pratiwi, 2021). The principle of DPPH is the neutralization of free radical molecules by antioxidant compounds, which is indicated by the fading of the DPPH solution due to a decrease in color intensity. The reduced color intensity indicates a reaction between the hydrogen atoms released by the sample and the DPPH radical molecules, resulting in the formation of 1,1-diphenyl-2-2-picrylhydrazine (Andriani & Murtisiwi, 2020). This antiradical test used quercetin as a positive control.

The DPPH method used antioxidant activity testing at a wavelength of 515 nm with an operating time of 37 minutes. The parameter used to assess antioxidant activity was the IC_{50}. The IC_{50} value is the extract concentration that provides 50% antiradical activity through a linear regression equation between the concentration and radical scavenging. The lower the IC_{50} value, the stronger the antioxidant activity. Based on the classification, antioxidant activity is divided into several categories: very strong (IC_{50} <50 μg/mL), strong (50-100 μg/mL), moderate (101-150 μg/mL), and weak (150-200 μg/mL) (Yumni et al., 2022). The results of the antioxidant activity test for the two extracts can be seen in Table 6.

The results of the antioxidant activity study showed that the IC_{50} value of the quercetin comparator was 3.677 μg/mL. This value is consistent with the literature stating that quercetin is a potent flavonoid with a polyphenolic structure that is effective in neutralizing free radicals through electron or hydrogen atom transfer (Carrillo-Martinez et al., 2024). Quercetin was chosen as a positive control for several reasons, including the fact that flavonoids are the dominant compound in butterfly pea flowers. Quercetin has a similar chemical structure to the antioxidant compounds in the extract, making it more relevant as a comparison. Quercetin is also more soluble in semi-polar solvents (Cunico et al., 2020).

Meanwhile, the IC_{50} values obtained from the 70% ethanol and ethyl acetate extracts were 54.23±2.37 μg/mL and 39.38±3.14 μg/mL, respectively. In this study, the ethyl acetate extract of butterfly pea flowers had a stronger IC_{50} value than the 70% ethanol extract. Although the standard deviation for ethyl acetate was slightly larger, this value was still within acceptable limits, resulting in relatively consistent results between replicates. This difference in antioxidant activity may be due to the different properties of the solvents used in the extraction process. Ethyl acetate is a semipolar solvent that is more selective in extracting phenolic and flavonoid compounds, which are bioactive compounds that play a crucial role in scavenging free radicals. Conversely, 70% ethanol, being polar, tends to extract a broader spectrum of compounds, including non-antiradical compounds.

Research by Riyanta et al. (2024) showed that the ethyl acetate fraction has antioxidant activity with an IC_{50} value of 29.51 μM. Other studies using ethyl acetate as a solvent also showed variations in IC_{50} values, namely 107.42±0.02 μg/mL in Rajamanickam et al. (2015) and 474.76 μg/mL in Ngan (2025). Meanwhile, Wulandari et al. (2023) stated that 70% ethanol extract had an IC_{50} value of 58.39±0.959 ppm. And in a study by Andriani & Murtisiwi (2020), the IC_{50} result was 41.36±1.191. This is due to differences in post-harvest conditions, storage, and extraction, such as drying and evaporation, which affect phenolic and flavonoid compounds.

Chemometric Analysis

The obtained data on total flavonoid content, total phenolic content, and antioxidant activity (IC_{50}) were then analyzed using the PCA (Principal Component Analysis) chemometric method with the aid of Minitab software (Windows). PCA is used to reduce correlated variables to linearly uncorrelated variables. This approach aims to retain as much information (variance) in the data as possible by using the minimum number of components (Arina & Shiyan, 2022).

The relationship between total flavonoids, total phenolics, and antioxidants can be analyzed using a loading plot. In this loading plot, each variable is depicted as a vector that indicates its contribution to the principal component. Two vectors forming an angle less than 90° indicate a positive correlation. Conversely, an angle of 90° indicates no significant correlation between the two variables. An angle greater than 90° or approaching 180° indicates a negative correlation (Widyastuti et al., 2021).

The chemometric analysis shows the PCA loading plot, which illustrates the relationship between variables based on their direction and contribution to the principal components. The DPPH vectors for ethyl acetate (IC_{50}) and flavonoid ethyl acetate form a narrow angle (less than 90°), indicating a very strong positive correlation between the two. This indicates that the higher the flavonoid ethyl acetate content, the higher the antioxidant activity, indicating a decrease in the IC_{50} value. Conversely, the phenolic ethyl acetate did not correlate with the antioxidant activity of the ethyl acetate solvent, as the vectors formed showed an angle greater than 90°, indicating no correlation.

The flavonoid and phenolic levels of the ethanol extract of butterfly pea flowers correlated with the antioxidant activity tested using the DPPH method with ethanol as the solvent. This is indicated by the position of the vectors forming an angle less than 90° to the DPPH vector of ethanol. In addition, flavonoids and phenolic ethyl acetate are also related to the antioxidant activity of the DPPH method with ethanol solvent, as well as phenolic ethanol is related to DPPH ethyl acetate.

4. Conclusions and Suggestions

This study showed that the highest total flavonoid content was obtained from the butterfly pea flower extract with ethyl acetate solvent of 6.616 ± 0.229 mg QE/mL, while the highest total phenolic content was also from the butterfly pea flower extract with ethyl acetate solvent of 31.579 ± 1.59 mg GAE/mL. Antioxidant activity based on the DPPH test showed the highest IC_{50} value in the butterfly pea flower extract with ethyl acetate solvent of 39.38 ± 3.14 μg/mL, which is classified as very strong. Chemometric analysis showed a positive correlation between flavonoids and phenolics in ethanol solvents and ethyl acetate solvents with antioxidant activity (IC_{50}) using ethanol solvents. Based on these findings, it is recommended that further research be conducted on the formulation of butterfly pea flower extract preparations in liquid and solid forms. In addition, further in vivo tests on animals and clinical trials on humans are needed to evaluate its effectiveness.

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