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The Effect of Variation in Solvents towards Total Flavonoid Content and Total Phenolic Content of Butterfly Pea (*Clitoria ternatea*) Flower Extract

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ABSTRACT

Butterfly pea flower (*Clitoria ternatea*) has many benefits including antioxidant. The antioxidant activity was contributed by flavonoid and phenolic components. The choice of solvent is important to obtain the metabolite components that contribute to antioxidant activity. Therefore this study was conducted to find the ability of different solvent to obtain total flavonoid content (TFC) and total phenolic content (TPC) in *C. ternatea*. The fine powder of dried flower was extracted by different solvents (ethanol 70%, ethanol 96%, and methanol). Quercetin was used as standard in determination of TFC and gallic acid was used as standard in determination of TPC. The TFC value of ethanol 70%, ethanol 96% and methanol extract were 46.23±0.39; 28.19±0.03; and 16.87±0.11 mg QE/g extract respectively. The TPC value of ethanol 70%, ethanol 96% and methanol extract were 93.91±0.16; 78.63±0.16; 84.46±0.16 mg GAE/g extract respectively. Ethanol 70% extract had the highest yield value and the highest amount of TPC and TFC compare to other solvents. Thus, the amount of flavonoid and phenolic in ethanol 70% extract showed the promising potential benefit of *C. ternatea* as antioxidant.

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1. Introduction

Since ancient times, a lot of medicinal plants have been used either as therapeutic, cosmetic or nutritional purposes. *Clitoria ternatea* plants or known as telang in Indonesia is widely distributed in Southeast Asia. *C. ternatea* or butterfly pea flower has beneficial potency for human health such as antidiabetic, antioxidants, anti-inflammatory and antimicrobial. The abundance amount of anthocyanins marked by blue color as part of flavonoid class in *C. ternatea* flower makes the plant beneficial as antioxidant and as a natural dyes (Jeyaraj et al., 2021; Oguis et al., 2019; Vidana Gamage et al., 2021). Previous research stated that *C. ternatea* flower in brewed beverage contained phenolic and flavonoid (Waruwu et al., 2023). Meanwhile butterfly pea flower extract contained phenolic total at 19.43±1.621 mg GAE/g sample (Andriani & Murtisiwi, 2018). Phenolic components and flavonoids are part of secondary metabolites widely found in plants that possess ability as antioxidant. The hydroxyl groups in phenolic and flavonoid compounds act as good electron donors that contribute to antioxidant activity. The ability of phenolic and flavonoid components are getting a great interest as it has versatile benefits not only as antioxidant but also

for anticancer, antibacteria, cardioprotective agents, and anti-inflammation (Bendary et al., 2013; Fattahi et al., 2014; Tungmunnithum et al., 2018).

Extraction studies is very important step to identify the variables that influence the extraction of phytochemical in plants. Conventional extraction has been the most common method and shown to be efficient. However nonconventional method also have been explored to obtain more time efficient. All difference extraction methods are used to customize different purposes such as increasing the yield value of extract. Besides extraction method, solvent used in extraction may contribute to antioxidant activity. Different polarity of solvents is used to observe the efficiency of the extraction process (Nabih et al., 2023). Previous research that have been conducted showed that different solvents might influence the amount of flavonoid and phenolic compounds extracted thus affect antioxidant activity (Do et al., 2014; Mehmood et al., 2022). Those metabolite components have different chemical compositions, characteristic, and polarities that will interact differently with each solvent (Jaafar et al., 2020). Therefore, the objective of this research was to determine the amounts of total flavonoid and total phenolic of *C. ternatea* extracts using three different solvents (ethanol 70%, ethanol 96% and methanol).

2. Methods

2.1. Extraction of butterfly pea flowers

Flowers were collected from farmers in Bambanglipuro District, Bantul Regency, Yogyakarta. Fresh blue petals were separated from sepal (the green part). The petals were dry cleaned from dirt or leaves part. The flowers were dried using oven around 40-50^{o}C for 2 days until it could be crushed. The dried flowers were grounded and sieved using 60 mesh sieve to obtain fine powder. A total of 300 gram powder were macerated with 3 L ethanol 70% for 2 days and remacerated with 1.5 L ethanol 70% for 1 day. Stirring was done every 6 hours. The filtrate was filtered and the solvent was evaporated at 40-50°C to gain crude extract. Extraction stage were repeated with different solvents (ethanol 96% and methanol). The yield of extract was calculated as follows.

$$\text{percentage yield (\%)} = \frac{\text{weight of extract}}{\text{weight of dried flower}} \text{ x } 100\% \qquad (1)$$

2.2. Characterization of extracts

a) Moisture content
   Around 1 gram of extract was placed on sample pan of moisture analyzer at 105°C. The percentage of moisture content was measured to determine the water content in crude extract.
b) Phytochemical screening
   1) Flavonoid
      0.5 gram extract was added with 5 drops of HCl and 0.1 gram of magnesium powder. Positive flavonoid was indicated with color change into yellow or red solution.
   2) Tannin
      0.5 gram extract was diluted with 5 mL water and boiled in test tube. After being cooled, 3 mL $FeCl_3$ solution (1%) was added. Positive tannin was indicated by color change into blue-black or dark green solution.
   3) Phenolic
      0.5 gram extract was mixed with 5 drops of ethanol 70% and 3 drops of $FeCl_3$ solution (5%) in test tube. Positive phenolic was indicated with color change into green, yellow, orange or red solution.
   4) Saponin
      0.5 gram of extract was diluted with 9 mL of aquadest in the test tube and boiled for 3-5 minutes. The test tube was shaked and added with 2 drops of HCl 2 N until foam was formed around 1-10 cm. Positive saponin was indicated with foam did not disappear after 7 minutes
   5) Alkaloid
      Around 0.5 gram extract was diluted with 10 mL of ethanol 70% and added with drops of HCl 1%. The mixtures are divided into 3 test tube. Each tube were added with different reagent (Mayer, Wagner and Dragendorff). Positive alkaloid was indicated with formation of deposits after addition of reagent. Addition of Mayer shows the formation brownish-yellow deposits. Addition of Wagner changed the solution into brownish-red deposits. Addition of Dragendorff shows the formation of brownish-red deposits.

2.3. Determination of total phenolic content

a) Preparation of standard calibration curve
Gallic acid was used as standard. A standard solution of gallic acid was prepared at concentration of 50, 100, 150, 200, and 250 µg/mL.

b) Sample preparation
Extract solutions of each solvent were prepared at 1000 µg/mL concentration. Concentration 1000 µg/mL was prepared by dissolving 0.01 gram extract into into 10 mL solvent.

c) Total phenolic content (TPC)
Around 0.2 mL sample (gallic acid or extract) was mixed with 4 mL aquadest and 0.2 mL Folin – Ciocalteu reagent. After 5 minutes incubation, 2 mL Na_2CO_3 7% was added to the mixture. The solution was incubated for 95 minutes and measured at 765 nm wavelength. the phenolic content was calculated from equation of calibration curve (y=0.0036x – 0.0224) and expressed as mg gallic acid equivalent per gram extract (mg GAE/g). The determination was analyzed in triplicate. After the absorbance was processed into the equation of calibration, the gallic acid value in µg/mL unit was converted into mg/mL and was able to be used in the equation 2.

$$\text{Total phenolic content (TPC)} = \frac{\text{Gallic acid equivalent } \left(\frac{\text{mg}}{\text{mL}}\right) \text{ x total volume of sample (mL)}}{\text{sample weight (g)}} \qquad (2)$$

2.4. Determination of total flavonoid content

a) Preparation of standard calibration curve
A stock solution of quercetin (1000 µg/mL) was prepared using ethanol as solvent. A standard quercetin solution was prepared by dilution at concentration of 50, 75, 100, 125 and 150 µg/mL.

b) Sample preparation
Extract solutions of each solvent were prepared at concentration 2000 µg/mL (ethanol 70% solvent) and 4000 µg/mL (ethanol 96% and methanol solvent). Concentration 2000 µg/mL was prepared by dissolving 0.02 gram extract with solvent into 10 mL measuring flask. Concentration 4000 µg/mL was also prepared by dissolving 0.04 gram extract in 10 mL measuring flask with solvent.

c) Total flavonoid content (TFC)
1 mL of sample (quercetin or extract solution) was mixed with 1 mL of 10% $AlCl_3$ and 8 mL of 5% acetic acid (Puspa Yani et al., 2023). The mixture solution was incubated for 25 minutes as operating time at room temperature. The absorbance of mixture was measured at 413 nm wavelength. The total flavonoid content was calculated from equation of calibration curve (y=0.0051x+0.1025) and expressed as mg quercetin equivalent per gram extract (mg QE/g). The determination was carried out in triplicate. After the absorbance was processed into the equation of calibration, the quercetin value in µg/mL unit is converted into mg/mL and was able to be used in the equation 3.

$$\text{Total flavonoid content (TFC)} = \frac{\text{Quercetin equivalent } \left(\frac{\text{mg}}{\text{mL}}\right) \text{ x total volume of sample (mL)}}{\text{sample weight (g)}} \qquad (3)$$

3. Results and Discussion

Yield value of extracts were able to be seen at Table 1. Each solvents showed different ability to extract secondary metabolite based on their polarity aspect. Plants components can be polar or non polar in nature. The presence of hydroxyl group makes phenolic components are more soluble in polar organic solvents (Aryal et al., 2019; Sultana et al., 2009). Therefore ethanol 70%, ethanol 96% and methanol are selected as solvents for extraction. The higher yield value, the more composition of metabolite contained. The ethanol 70% extract showed the highest yield value, followed by methanol and ethanol 96%. Higher yield value means ethanol 70% has best penetration across plasma membranes to facilitate the liberation of cellular contents into the solvents. The higher amount of water in ethanol 70% may contribute to extract a wide range of secondary metabolites with different polarity compare to other solvents (Hikmawanti et al., 2021). Phytochemical screening in Table 2 showed that all three extract contained flavonoid, tannin, phenolic, saponin and alkaloid at different intensity levels.

This different intensity is connected to the different ability of three solvents at extracting the metabolites based on the similar polarity. Meanwhile the correlation of yield value and flavonoid and phenolic content in extract is analyzed further. The moisture content of all extracts were below 10%. This is beneficial as less moisture content helps delaying the growth of microbial in extract.

Table 1. Yield value and characteristic of butterfly pea flower extract with variation of solvents

Solvent	Extract weight (g)	Yield (%)	Moisture content (%)
Ethanol 70%	129.8	43.27	2.10
Ethanol 96%	87.6	29.20	0.86
Methanol	95.8	31.93	6.00

Table 2. Comparison of phytochemical content in butterfly pea extracts with variation of solvents

Compound		Extract			Observation
		Ethanol 70%	Ethanol 96%	Methanol	
Flavonoid		+	+	+	Red solution
Tannin		+	+	+	Dark green solution
Phenolic		+	+	+	Green solution
Saponin		+	+	+	Foam forming
Alkaloid	Mayer	+	+	+	Brown deposit
	Wagner	+	+	+	Brownish red deposit
	Dragendorf	+	+	+	Brownish red deposit

Notes: + indicated the presence of secondary metabolites

Phenolic and flavonoid content of three extracts were shown at Table 3. All solvents are able to obtain TFC and TPC from *C. Ternatea* flower extract. Through maceration the total phenolic obtained are in the range of 84.46 – 93.91 mg GAE/g extract and the total flavonoid are in the range of 16.87 – 46.23 mg QE/g extract. Meanwhile previous research found the total flavonoid in butterfly pea flower extract with maceration method is around 53.127 mg QE/g sample and phenolic around 19.43 mg GAE/g sample (Andriani & Murtisiwi, 2018; Vifta et al., 2022).

Table 3. Total phenolic content and total flavonoid content of butterfly pea flower extract with variation of solvent

Solvent	Phenolic total (mg GAE/g ekstrak)	Flavonoid total (mg QE/g extract)
Ethanol 70%	93.91 ± 0.16^{a}	46.23 ± 0.39^{a}
Ethanol 96%	78.63 ± 0.16^{b}	28.19 ± 0.03^{b}
Methanol	84.46 ± 0.16^{c}	16.87 ± 0.11^{c}

Each value is represented as mean ± SD (n = 3). Means followed by a different letter in the same column are significantly different ($p < 0.05$)

Difference amount of TPC and TFC in this work compare to previous work may also indirectly be influenced by different plant location, harvest time and environmental factors such as climate and soil physicochemical properties (Chen et al., 2023; Mehrabani et al., 2023). The result of TFC in macerated extract is in the order of ethanol 70% > ethanol 96% > methanol. Meanwhile the TPC value is in the order of ethanol 70% > methanol > ethanol 96%. Polyphenols are classified into different classes include phenolic acids, flavonoids, lignans, tannin and stilbenes. The classification is based on the number of phenol rings that they contain and the structural elements that bind these ring (Pandey & Rizvi, 2009; Zhang et al., 2022). Theoritically, as flavonoid is class of phenolic, the amount of TPC in extract is always higher than TFC. The results is similar with the theory. The TFC measured in butterfly pea extracts is lower than TPC for all solvents.

As flavonoid is only a part among phenolic class, the lower amount flavonoid in extracts but higher amount of phenolic acid, lignans, tannin or stilbenes is possible. This indicates that higher phenolic is not always followed by higher flavonoid. In this research, ethanol 70% extract showed direct correlation, as higher TPC is contributed by higher TFC. But it didin't happen in TPC and TFC of ethanol 96% and methanol extract. Methanol extract has higher TPC than ethanol 70% extract, but methanol didn't have higher TFC than ethanol 70%. It might happen because methanol were better at withdraw other phenolic class than flavonoid. Different polarity among phenolic class toward each solvents may contribute to this result as these three solvents have different ability to withdraw phenolic.

(Poly) phenols interacted with Folin Ciolcalteau under basic condition (sodium carbonate solution) to create blue complexes (Pérez et al., 2023). The higher amount of phenol results stronger intensity of blue color in solution. Gallic acid as part of phenolic acid is used as standard. The TPC value measured by different method might give different output (Jaafar et al., 2020). Hence, different procedures in Folin Ciocalteau method might cause different TPC value. Meanwhile quantification of flavonoid is widely performed using aluminium chloride colorimetric assay based on the formation of yellow colored of Al(III) – flavonoid complexes at 1:1 ratio (Shraim et al., 2021). This complexes is performed in the presence of acetate acid (Abeysinghe et al., 2021). The stronger intensity of yellow color showed the higher amount of flavonoid in solution. Flavonoids are divided into some groups that consist of flavones, flavonols, flavanones, isoflavones, anthocyanins, flavanols or catechins and chalcones (Chen et al., 2023; Panche et al., 2016). Quercetin as part of flavonols class is used as standard at measuring TFC in extracts. The amount of flavonoid and phenolic of butterfly pea extracts were obtained by inserting the absorbance of extract into the standard curve equation (Figure 1).

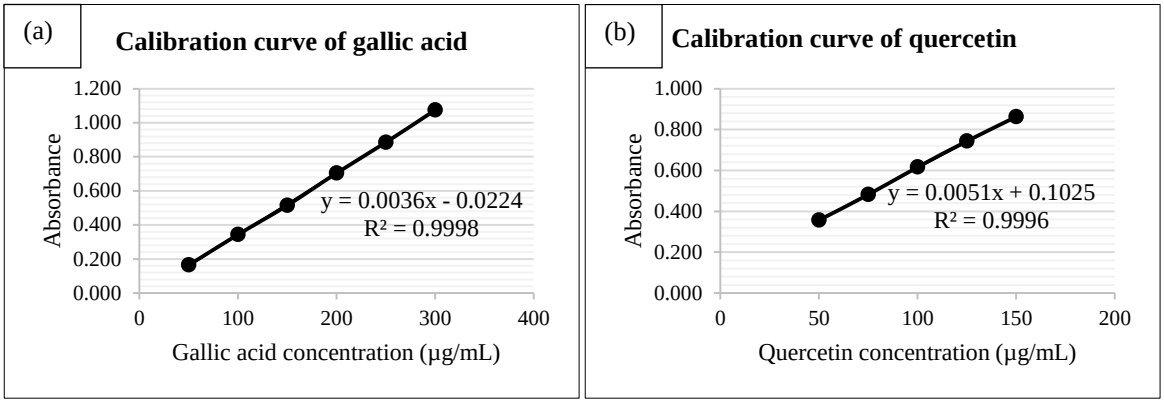

Figure 1. (a) Calibration curve of gallic acid for total phenolic content; (b) Calibration curve of quercetin for total flavonoid content.

The statistical analysis of TPC and TFC in Table 3 was performed using SPSS software. According to Kruskal Wallis result, it showed that the TFC and TPC value of three solvents differ significantly. The flavonoid and phenolic compounds interact differently with each solvent because of the difference interaction of functional group in extract metabolite compunds with solvent itself (Jaafar et al., 2020). Based on Table 1 and Table 3, as ethanol 70% has the highest yield value, it correlate directly with its highest value of TPC and TPC. Extraction using ethanol 70% is proved to draw flavonoid and phenolic best among other solvents. The hydroxyl group of phenolic compounds include flavonoid in butterfly pea extracts have the ability to scavenge free radical thus has potential to be explored as antioxidants

4. Conclusion

This study reports that all different solvents (ethanol 70%, ethanol 96% and methanol) are able to obtain flavonoid and phenolic in butterfly pea flower extracts. Ethanol 70% extract with the highest yield value showed the highest content of flavonoid and phenolic among other solvents.

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