Journal of TROPICAL BIODIVERSITY E-ISSN : 2774-4116

Analysis of Total Phenols, Total Flavonoids and Anthocyanin Levels in Blue Pea Flowers (*Clitoria ternatea L*)

Fira Kuswandari[1], Ernawati Sinaga[2,3], Nurbaiti[4], Ali Husni[5]

[1]*Department of Biology Program, Graduate School, Universitas Nasional, Jakarta, Indonesia*
[2]*Faculty of Biology, Universitas Nasional, Jakarta, Indonesia,*
[3]*Center for Medicinal Plant Research Universitas Nasional, Jakarta, Indonesia,*
[4]*Poltekkes Kemenkes Jakarta II Jakarta*
[5]*National Research and Innovation Agency, Jakarta, Indonesia*

Corresponding author: firakuswandari@gmail.com

Abstract

This study aims to determine the content of total phenol, total flavonoid, and anthocyanin levels in flower telang flower (*Clitoria ternatea L*). Observations were made using different methods and solvents. Telang flower (*Clitoria teratea L*) extracted by maceration using ethanol solvent and dry extraction using water solvent freezedry method showed different content of total phenol, total flavonoid and anthocyanin content. The extract obtained was analyzed for its content using a UV-Vis spectrophotometer. The results showed that the average total phenolic content of flower telang flower was 2459.94 mgGAE/100g, total flavonoids 1171.10 mg/100g and anthocyanin content 122.79mg/100g. Meanwhile, the freeze-dry extract of the flower of telang flower showed an average total phenol content of 1924.96 mgGAE/100g, a total flavonoid content of 763.88 mg/100g and anthocyanin content of 890.49 mg/100g. The content of total phenol, total flavonoid and anthocyanin from ethanol extract was higher than that from water solvent. The ethanol extract and the water extract of the flower of telang flower have antioxidant potential in terms of the parameters of the total phenol content

Keywords: *blue pea flowers, levels of anthocyanins, total phenols, total flavonoids*

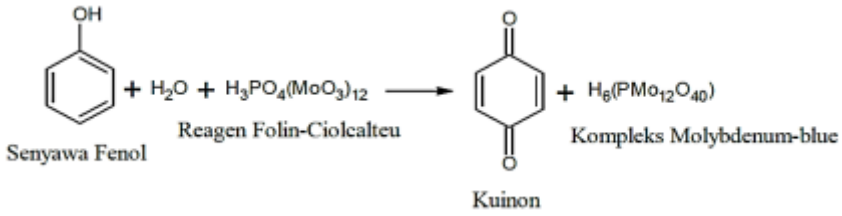
Submission	:	March, 09th 2022
Revision	:	May 17th 2022
Publication	:	August 30th 2022

INTRODUCTION

Blue pea (*Clitoria ternatea L*) is a member of the Fabaceae family which has cyclotides (protein components). From several studies that have been carried out previously, the blue pea contains flabatannin compounds, carbohydrates, saponins, triterpenoids, phenols, flavonoids, flavonol glycosides, proteins, alkaloids, anthraquinones, cardiac glycosides, stigmast-4-en-3,6-dione, essential oils and steroids which are compounds that have medicinal potential (Mukhreeje, 2007; Jiji *et al*, 2020). Polyphenols are one of the phytochemical components contained in blue pea which have several pharmacological activities. Phenolic compounds in natural ingredients are easily found in all plants, leaves, flowers and fruits. The chemical structures detected included flavonoids, simple monocyclic phenols, phenyl propanoids, polyphenols (lignin, melanin, tannins), and phenolic quinones (Fauziah, 2008). Two groups of compounds that are considered to make a major contribution to the antioxidant power and medicinal effects of natural materials are phenolic

compounds and flavonoids. This is because there is a linear relationship between total phenolic levels and antioxidant activity (Aryal, *et al*., 2019). In addition, research conducted by Johari and Khong (2019) stated that the higher the total phenolic content, the higher the antioxidant activity. Blue pea crown contains flavonoids, anthocyanins, flavanol glycosides, kaempferol glycosides, quercetin glycosides and myrisetin glycosides (Anthika *et al*., 2015). Research proves that the flower of blue pea contains phenolic acid which is an antioxidant compound that has the function of eliminating free radicals in the body (Wulandari, 2014).

Total phenol and flavonoid tests were carried out to determine the content of secondary metabolites contained in the ethanol and *freezedry* extracts. The principle of the reaction for determining phenol levels using the Folin Ciocalteu method is that phosphomolybdic acid phosphotungstate will be reduced by phenolic ions (Folin Ciocalteu reagent) under alkaline conditions to form a molybdenumtungsten complex compound which produces a blue color. Under basic conditions, phenolic ions will be formed through the dissociation of protons from an alkaline compound, namely sodium carbonate. Phenolic compounds are reacted in an alkaline environment so that proton dissociation occurs into phenolic ions. The reaction of phenol compounds with Folin-Ciocalteu can be seen in Figure 1.



Sumber : Mukhriani.,*et.al* 2019

Figure.1 Reaction of phenol compounds with folin ciocalteu reagent

Testing the total flavonoid content in the ethanol extract and *freeze-dried* extract of blue pea using the reaction principle, namely the formation of a stable complex between $AlCl_3$ and C-4 ketone groups, as well as with C-3 or C-5 hydroxyl groups of flavones and flavonols. Determination of total flavonoid levels using a comparison compound, namely quercetin. The method (Chang and When, 2002) used aluminum chloride and sodium acetate as reagents. Aluminum chloride can form more stable complexes with flavones and flavonols (Mabry *et al*., 1970). The percentage of total flavonoids obtained can state the percentage of flavones and flavonol groups contained in the ethanol extract and the freezedry dried kerng extract of the flower of the blue pea. The extract drying process can cause a reduction in compound components such as the amount of phenolic components that are reduced in the dried extract compared to the amount in the fresh plant. To prevent damage to bioactive compounds, especially phenolic compounds, a drying method that does not use high heat energy is needed because phenolic compounds are sensitive to heat treatment (Masqudi *et al*., 2014). Based on research by Réblová (2012), the higher the heating temperature, the higher the decrease in antioxidant activity of the two phenolic acids due to damage.

Flavonoids are natural compounds that have the potential as antioxidants that can counteract free radicals that play a role in the emergence of degenerative diseases through the mechanism of destroying the body's immune system, lipid and protein oxidation (Rais, 2015). In the development of this flavonoid health field, scientific research from 2011 has found more than 9000 flavonoids and have been used for health supplements (Wang *et al*., 2018). Anthocyanins are secondary metabolites that are soluble in water, have many benefits and can be found in various types of plants. Anthocyanins are a subclass of water-soluble flavonoids that are responsible for the red, purple and

blue colors of fruits, vegetables, cereals and flowers. Anthocyanins in the flower of the telang produce a blue/purple pigment. *Freeze drying* or lyophilization method or also known as *freezedrying* is a process in which frozen water is removed from the sample, initially this process begins with a sublimation process (primary drying) and then continues with a desorption process (secondary drying). The *freezedry* method is carried out by reducing simplicia temperature so that most of the simplicia vapor is deposited in all parts of the plant as a solid phase (Babu *et al*., 2014). This method has advantages in maintaining the quality of the product, both in terms of sensory characteristics, nutritional value, physical and chemical compared to drying. normally use thermal. This method is still rarely used because it has high costs for investment and operation. (Habibi, 2019).
Research on the analysis of total phenol levels, total flavonoids and anthocyanin levels, from aqueous extracts using the freeze-dry method of flower telang (*Clitoria ternatea L*) needs to be carried out as a basis for assessing the potential for their use in dry extracts.

METHOD

This research was carried out at the Research and Natural Materials Laboratory of the National University of Jakarta and Balai Besar Pasca Panen Bogor.

Test Material

Blue pea flower (*Clitoria ternatea L*) obtained from the Cibodas Farm Purwakarta community plantation.
The equipment used are analytical balance, blender, UV-Vis spectrophotometer PG Instruments Ltd, flask, water bath, and glassware which is common in the laboratory.

Chemical material

Toluene P, distilled water, filter paper, quercetin, methanol pro-analytical, ethanol pro-analytical (Brand), aluminum chloride (AlCl3), potassium acetate (CH3COOK), potassium hydroxide (KOH), hydrochloric acid (HCl), spirtus, chloral hydrate, reagent Dragendorf, Mayer's reagent, aqueous ammonium hydroxide (NH4OH), chloroform, Magnesium powder, amyl alcohol, iron (III) chloride reagent, sodium sulfate (Na2SO4), 1% gelatin solution, ether, 10% vanillin-sulfuric acid reagent, Lieberman's reagent -Bouchard, DPPH, Vitamin C, filter paper, ash-free filter paper and aluminum foil.

Simplisia making

1 kg of fresh blue pea flower is cleaned (no need to wash because it will cause the flower color to fade) and then dried in a food dehydrator at 50^0C for 24 hours. After that it was powdered and sieved through a 60 mesh sieve.

Blue pea extract

Blue pea flower powder as much as 100 g was macerated in 500 ml of 96% ethanol for 24 hours. After that, it was filtered using filter paper, and the filtrate was stored in an Erlenmeyer flask in a refrigerator at 2-8^{o}C. The residue was macerated twice using the same volume of 96% ethanol and in the same time. The resulting filtrate was collected and then concentrated using a vacuum rotary evaporator. Blue pea extract was stored in a closed glass container and stored at 2-8^{o}C until used.

Freezedrying of blue pea

Blue pea flower powder as much as 100 grams is brewed with 1000 mL of warm drinking water at 90°C then allowed to stand for 5 minutes. After that, it was filtered using filter paper. The filtrate was frozen in a freezer at -60°C, then *freeze-drying* was carried out to obtain a dry powder. Freeze-dried powder is stored in a closed glass container and stored in a refrigerator at 2-8°C until used.

Figure 2. The process of freeze-drying the flower of Blue pea *Quantitative analyze*

Fenol total

Measurements of *freeze-dry* total phenol levels and ethanol extract were carried out as the previous researchers (Abozed.*et al*, 2014, Wirasti, 2019) with slight modifications. A total of 50 mg of the sample was dissolved with distilled water to 5 mL to obtain a concentration of 10 mg/ml then diluted to obtain a concentration of 1 mg/ml. 0.2 ml of the solution was pipetted, then 15.8 ml of distilled water and 1 ml of Folin-Ciocalteu reagent were added and allowed to stand for 8 minutes. After that, 3 mL of 10% Na2CO3 was added to the solution and the solution was allowed to stand for 2 hours at room temperature. Then the absorption was measured by UV-Vis spectrophotometer at 765 nm. After measuring the absorbance of the sample, a standard curve was made by dissolving gallic acid in 85% methanol with various concentrations of 0 - 100 mg/L. Total phenol content was calculated using the linear regression equation of gallic acid, y = ax+b.

Flavonoid total

Freezedry total flavonoid levels and ethanol extract of blue pea flower were carried out using the $AlCl_3$ colorimetric method as carried out by Dyah *et al*. (2014) with the comparison compound quercetin.

1) Creation of a Quersetin Standard Curve

Quercetin was weighed as much as 25 mg was put into a 25 ml volumetric flask, then 80% ethanol was added to a volume of 25 ml (main solution 1000 g/ml). Then a series of standard solutions of 20 g/ml, 40 g/ml, 60 g/ml, 80 g/ml and 100 g/ml were made. Each 0.5 ml of the standard solution was pipetted with 1.5 ml of 95% ethanol, 0.1 ml of 10% aluminum chloride (AlCl3), 0.1 ml of 1 M potassium acetate and 2.8 ml of distilled water was added. After that, it was incubated for 30 minutes at 25oC. The absorption was measured at 434.2 nm using a UV-Vis spectrophotometer. Then a calibration curve is made by connecting the absorption value as coordinates (Y) and the concentration of the standard solution as abscissa (X)

2) Preparation of Test Solution

Freezedry and ethanol extract of the flower of telang were taken as much as 5.0 g and then added with 25 ml of ethanol. Then stirred for 24 hours using a stirrer at a speed of 200 rpm, after that it was filtered and the filtrate obtained was added to 25 ml of ethanol.

3) Determination of Sample Flavonoid Level

A total of 0.5 ml of the test solution was added with 1.5 ml of 95% ethanol, 0.1 ml of 10% aluminum chloride (AlCl3), 0.1 ml of 1 M potassium acetate and 2.8 ml of distilled water were added. After that, it was incubated for 30 minutes at 25oC. The absorption was measured at 434.2 nm using a UV-Vis spectrophotometer. Each absorption measurement was compared to the blank, which was 95% ethanol.

Anthocyanins level

Determination of anthocyanin levels was carried out using the differential pH method as done by (Giusti and Wrolstad, 2000). Each freezedried brew/ethanol extract of the flower of blue pea was weighed as much as 25 mg, diluted with 5.0 mL of ethanol which had been acidified to a pH of 1.0.

1 mL of the extract solution was put into 2 vials, each of which was added 5 mL of KCl buffer pH 1.0 and 5 mL buffer Na-acetate pH 4.5. Then shaken until dissolved for 30 minutes-1 hour. After that, the absorbance value was calculated at a maximum wavelength of 700 nm (Sompong et al., 2011).

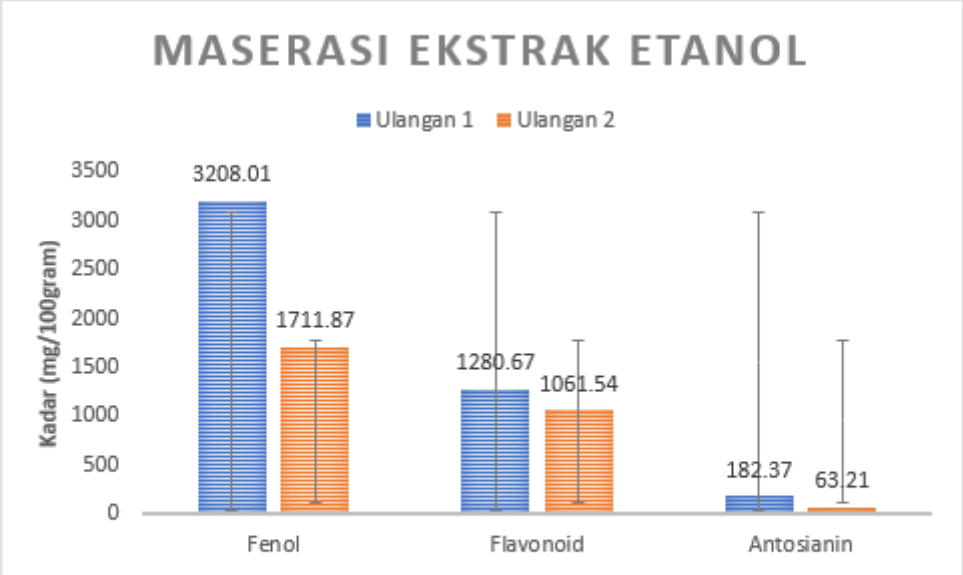
RESULT

Maceration extraction method

Total phenol content and total flavonoid and anthocyanin content of ethanol extract of flower blue pea are presented in Table-1.

Table – 1. Total Phenol, Total Flavonoid and Anthosine levels in the ethanol extract of Blue pea flower

Jenis sampel	Kadar Fenol Total (mgGAE/100g)	Kadar Flavonoid Total (mg/100g)	Kadar Antosianin (mg /100g)
Ulangan 1	3208,01	1280,67	182,37
Ulangan 2	1711,87	1061,54	63,21



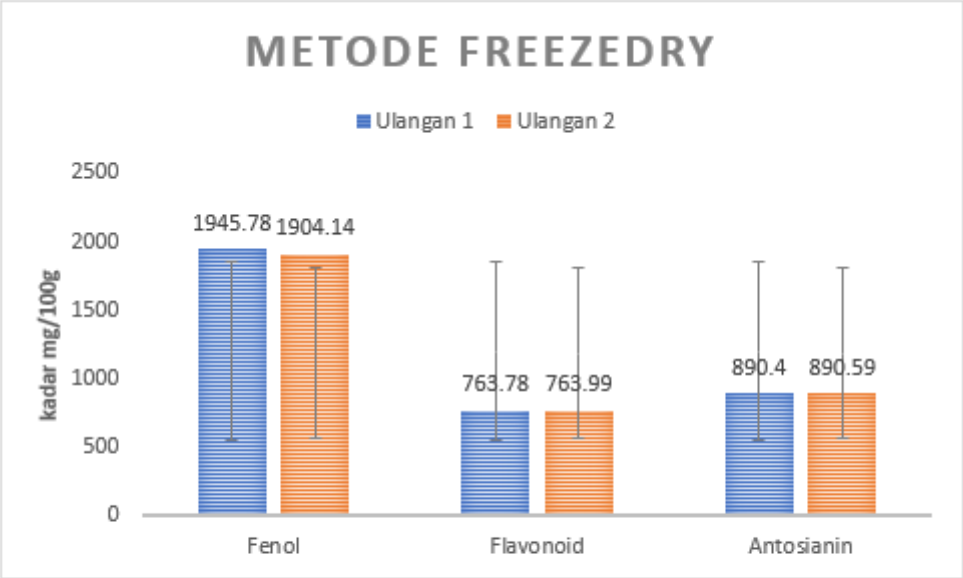
	Fenol	Flavonoid	Antosianin
Rerata	2459.94	1171.105	122.79
SD (±)	1057.931	154.9483	84.25884

Freezedry method

The total phenol and total flavonoid content of the freeze-dried extract of the blue pea flower are presented in Table-2.

Table – 2. Total Phenol, Total Flavonoid and Anthosine levels in the freeezedry extract of Blue pea flower

Jenis sampel	Kadar Fenol Total (mgGAE/100g)	Kadar Flavonoid Total (mg/100g)	Kadar Antosianin (mg /100g)
Ulangan 1	1945,78	763,78	890,40
Ulangan 2	1904,14	763,99	890,59



	Fenol	Flavonoid	Antosianin
Rerata	1924.96	763.885	890.495
SD (±)	29.44393	0.148492	0.13435

DISCUSSION

The content of total phenol, total flavonoid and anthocyanin from ethanol extract was higher than that from water solvent.. However, statistically, the results of the *Mann Whitney* test showed that there was no significant difference between the ethanol extract and the *freeze-dried* extract in all parameters ($p>0.05$). The results showed that ethanol solvent was effectively used to extract blue pea so as to produce the highest total phenol content. Ethanol solvent functions as a substance that makes compounds such as phenolic in plants extracted, because polar compounds will dissolve compounds that are also polar (Najoan.,J.J.J.R.R,Max, and S.W.Defney, 2016). 96% ethanol solvent has the ability to extract the compounds needed to test the activity of blue pea such as phenolics, flavonoids, terpenoids and steroids. The choice of ethanol as a solvent is because it is cheap, universal and food grade and has the ability to penetrate hydrophilic and lipophilic, which is able to penetrate cell membranes and then enter cells and interact with metabolites contained in cells. The extraction process with this maceration method produced ethanol and pulp filtrate. Ethanol extract can identify more metabolites than aqueous extract. The maceration method with 96% ethanol solvent followed by evaporation resulted in the total phenol content of the ethanol extract of 3208.01mg GAE/100g. This result is different from the research conducted by Disa *et.al* (2018) which tested the total phenol

content using the 70% ethanol extract method of blue pea which had a concentration of 19.43±1.621 GAE mg/g sample. These differences may occur due to the preparation treatment The selection of solvent concentrations and varieties used are different so that the content of the bioactive compounds produced is different.

Whereas in *freezedry* dry extract, the use of water as a solvent is a polar compound compared to other solvents, so that the polar components are also extracted and cause the total phenol per sample weight to be low (Septian and Asnani, 2012). In the *freeze-drying* results, the flower blue pea produced anthocyanin levels of 0.89 mg GAE/100g slightly different from the research conducted by Rianita and Joan (2022) that the total anthocyanin content was 0.71±0.02 mg/g in powders made using extracts with water solvent. These differences can occur due to the preparation treatment, the choice of solvent concentration and the variety used so that the content of the bioactive compounds produced is different. *Freezedry* method in producing dry extract has the best physicochemical characteristics. *Freezedry* dry extract has the potential to be developed in product quality that meets the physical quality requirements of powdered drinks so that the resulting *freezedry* form has the potential to be developed, one of which is as a beverage ingredient. However, the weakness of the *freeze-drying* method is that it produces a very hygroscopic powder so that after contact with outside air it becomes a thick extract.

CONCLUSION

Based on the results of research that has been carried out on the analysis of the extract of blue pea (*Clitoria ternatea L*) with ethanol and water solvents using the *freezedry* method, it can be concluded as follows:

1. Ethanol extract of flower blue pea (*Clitoria ternatea L*.) has a total phenol content of 2459.94 mgGAE/100g, total flavonoid content of 1171.10 mg/100g and anthocyanin content of 122.79 mg/100g
2. *Freezedry* extract of flower blue pea (*Clitoria ternatea L*.) has a total phenol content of 1945.78 mgGAE/100g, total flavonoid content of 763.885mg/100g and anthocyanin content of 890.495 mg/100g
3. There was no significant difference in mean between ethanol extract and *freeze-dry* extract on all parameters (P>0.05)

The *freeze-drying* method of flower blue pea has a good prospect to be applied appropriately and according to the characteristics of the dried material**.**

ACKNOWLEDGMENT

The authors are thankful to Universitas Nasional and Blue pea Research Team for their support to this research.

REFERENCES

Alnanda, R., D. Ulima, N. Merry, dan S. Purbaningsih (2017). Studi awal pemanfaatan kuntum *Clitoria ternatea L*. (kembang telang) sebagai pewarna alami makanan. Departemen Biologi, FMIPA UI, Kampus UI Depok, Jawa Barat

Dontha.S, (2016). A Review On Antioxidant Methods. Asian J Pharm Clin Res, Vol 9, Suppl. 2, 2016, 14-32

Habibi.N, Sarah.F, Citra.T, (2019). Perubahan Karakteristik Bahan Pangan pada Keripik Buah dengan Metode Freeze Drying (Review). JURNAL SAINS TERAPAN VOL. 5 NO. 2 2019

Najoan, J. J., J. R. R. Max, dan S. W. Defny. (2016). Uji fitokimia dan aktivitas antioksidan ekstrak etanol daun tiga (Allophylus cobbe L.). Pharmacon Jurnal Ilmiah Farmasi-Unsrat. 5(1) : 266 – 274

Septian, A.T., dan A. Asnani. (2012). Kajian sifat fisikokimia ekstrak rumput laut coklat Sargassum duplicatum menggunakan berbagai pelarut dan metode ekstraksi. Agrointek. 6(1) : 22 – 28

Rais, I. R. (2015). Isolasi dan penentuan kadar flavonoid ekstrak etanolik herba sambiloto (andrographis paniculata (burm. F.) Ness). Jurnal Fakultas Farmasi Universitas Ahmad Dahlan, 5(1), 101–106

Rianita, P dan Joan.P,. (2022). Karakterisasi Sifat Fisikokimia Ekstrak dan Bubuk Hasil Pengeringan Beku Antosianin Mahkota Bunga Telang (Clitoria ternatea L.). Agro Bali : Agricultural Journal. Vol. 5 No. 2: 304-312, July 2022

Réblová Z. (2012): Effect of temperature on the antioxidant activity of phenolic acids. Czech J. Food Sci., 30: 171–177

Siti Azima A M, Noriham A and Manshoor N., (2014). Anthocyanin Content In Relation to The Antioxidant Activity and Colour Properties of Garcinia Mangostana Peel, Syzigium Cumini and Clitoria Ternatea Extracts, International Food Research Journal, 21(6): 2369-2375

Wang, T., Li, Q., Bi, K., 2018. Bioactive flavonoids in medicinal plants: Structure, activity and biological fate. Asian J. Pharm. Sci. 13, 12–23