Potential of Flavonoid Content from *Clitoria ternatea* Flowers Extract as Natural Antioxidant Candidate and Its Correlation

Rissa Laila Vifta[1], Komang Sintya Trinadi[2], Suratno Suratno[3]
[1,2]Pharmacy Study Program, Faculty of Health, Universitas Ngudi Waluyo,
[3]Department of of Medical Laboratory Technology, Faculty of Health Science, Universitas Muhammadiyah Palangkaraya
[3]Department of Pharmacognosy, Semmelweis University

Article

Article History
Submitted: 2022-02-01
Accepted: 2022-03-23
Published:2022-03-24

Keywords: Antioxidants, Flavonoids, DPPH, Clitoria Ternatea

Abstract

Free radicals can cause various diseases such as retinal damage, cardiovascular, stroke, cancer, asthma and premature aging. One of the natural products with antioxidant secondary metabolite compounds is the Clitoria ternatea, known as Telang flowers.. Clitoria ternatea contains secondary metabolites such as phenols, flavonoids, anthocyanins, and glycoside flavonols as antioxidants. This research aimed to determine the effect of extraction methods on total flavonoid content and evaluate the antioxidant activity of Clitoria ternatea from Tabanan. The determination of total flavonoid content was done using $AlCl_3$ colorimetric method, and the antioxidant activity was carried out using DPPH (2,2-diphenyl-1-picrylhydrazyl). The results of total flavonoid content from highest to lowest were maceration 53,127 mg QE/g, reflux 24,527 mg QE/g, and soxhlet 21,060 mg QE/g). The IC50 results of Clitoria ternatea were 250,850 ppm from maceration, 289,612 ppm from reflux, and the weakest one was 336,75 ppm from soxhlet. Total flavonoid and antioxidant activity determination results have no significant difference between the three extraction methods. However, both of flavonoid and antioxidant activities have in-line correlation.

Abstract

Radikal bebas dapat memicu berbagai penyakit seperti kerusakan retina, kardiovaskular, stroke, kanker, asma hingga penuaan dini. Radikal bebas dapat diredam oleh adanya antioksidan. Salah satu bahan alam yang mengandung senyawa metabolit sekunder yang diduga berperan sebagai antioksidan adalah bunga telang (*Clitoria ternatea*). Bunga telang mengandung senyawa metabolit sekunder seperti fenol, flavonoid, antosianin, glikosida flavonol yang berfungsi sebagai antioksidan. Penelitian ini betujuan untuk mengetahui pengaruh metode ekstraksi terhadap kadar flavonoid total dan aktivitas antioksidan ekstrak bunga Telang (*Clitoria ternatea*) asal Tabanan. Pengujian Flavonoid total menggunakan metode kolometri $AlCl_3$ dan aktivitas antioksidan menggunakan DPPH (*2,2-Difenil-1-pikrilhidrazil*). Hasil pengujian flavonoid total dari

tertinggi hingga terendah adalah maserasi sebesar 53,127 mg QE/g), reflux sebesar 24,527 mg QE/g, dan soxhlet sebesar 21,060 mg QE/g. Hasil uji antioksidan bunga telang ekstrak maserasi memiliki nilai IC_{50} sebesar 250,850 ppm, reflux sebesar 289,612 ppm, dan paling lemah sokhlet sebesar 336,75 ppm. Hasil pengujian flavonoid total dan aktifitas antioksidan ekstrak bunga telang tidak berbeda signifikan antara ketiga metode ekstraksi, tetapi antara flavonoid total dan aktifitas antioksidan memiliki korelasi linier.

Introduction

The danger of free radicals is a severe problem in this modern era. Free radicals are harmful compounds to the body when the number of free radicals exceeds the body's capacity to neutralize them. Free radicals can bind to body cells and cause cell damage by oxidizing nucleic acids, proteins, lipids which will initiate cell degradation. Free radicals bind to normal cell electrons causing changes in DNA structure resulting in the formation of mutant cells (Kurniasih, 2019). Free radicals can trigger oxidative stress that causes various diseases such as cardiovascular, retinal damage, cataracts, hepatitis, arthritis, rheumatoid, stroke, asthma, diabetes mellitus, immunodepression, cancer, hyperoxia, dermatitis, premature (Andriani & Murtisiwi, 2020).

Antioxidants are compounds that can reduce free radicals by donating electrons so that free radicals become more stable (Parwata, 2016). The function of antioxidants in the body is to protect the body from the adverse effects of free radicals. Antioxidants consist of two types, namely natural and synthetic (vitamins A, E and C) (Erlidawati & Safrida, 2018). One source of natural antioxidants is the pea flower. According to (Al-Snafi, 2016), the secondary metabolites of the *Clitoria ternatea* flower are tannins, carbohydrates, saponins, triterpenoids, phenols, flavonoids, flavonol glycosides, proteins, alkaloids, anthraquinones, anthocyanins, cardiac glycosides, stigmast-4-ene-3,6- dione, essential oils, and steroids. These secondary metabolites have various therapeutic effects, one of which is an antioxidant. Research conducted by (Andriani & Murtisiwi, 2020) states that the *Clitoria ternatea* flower is a source of very potent antioxidants that can be developed as a source of natural antioxidants.

According to (Maesaroh et al., 2018), testing of antioxidant activity can be done by various methods. Each method has different advantages and disadvantages, and the resulting test results vary. The difference in test results is caused by differences in antioxidant structure, free radical sources, and physicochemical properties. The selection of the proper method and in accordance with the type of sample being tested will provide optimum test results. The selection of the DPPH method used in this study was based on research conducted by (Maesaroh et al., 2018) by comparing three antioxidant activity test methods DPPH (2,2-diphenyl-1-picrylhydrazyl), FRAF (Ferric Reducing Antioxidant Power) and FIC (Ferrous Ion Chelating) stating that the DPPH method is the most effective and efficient method among the three methods. Based on this background, researchers will conduct this research using a different extraction method. Extraction methods used in this study include maceration, reflux, and soxhletation methods.

Method

This research is an experimental laboratory study using the $AlCl_3$ colorimetric method to determine total flavonoids and the DPPH method (2,2-Diphenyl-1-picrylhydrazil) to measure antioxidant activity using UV-Vis spectrophotometry on 96% ethanol extract of Clitoria ternatea flower. The sample in this study was the *Clitoria ternatea* flower originating from Tabanan Bali with the criteria of perfect bloom, looking fresh and bluish-purple. The TLC identification of flavonoid was performed by preparing silica gel $G_{60}F_{254}$ stationary phase (TLC plate) with a length of 14 cm and a width of 5 cm. Next, the extract was weighed 10 mg dissolved in 1 mL of ethanol and spotted on the TLC plate. Preparation of mobile phase BAA (butanol: acetic acid: water) with a ratio (6:2:2) which has been modified from research conducted by (Vifta et al., 2019). The determination of total flavonoid content was determined by using $AlCl_3$ colorimetric method. The antioxidant activity of *Clitoria ternatea* flower extract was carried out using DPPH with vitamin C/ascorbic acid as a standard.

Results and Discussion

Extraction of Clitoria ternatea flower

The total weight of the Clitoria ternatea flowers simplicia was 800 g after being blended into a powder to obtain 643 g. A total of 600 g was extracted by three methods, namely maceration, reflux, and soxhlet, using 96% ethanol as solvent. Ethanol 96% can filter polar, semipolar, and nonpolar compounds to produce an optimal amount of extract. Furthermore, the filtrate is obtained, concentrated into a thick extract. The weight of maceration extract (66.41 g) with extract yield of 33.20%, reflux (55.76 g) with extract yield of 27.88%, and soxhlet (64.62 g) with extract yield of 32.31%.

Identification of Thin layer chromatography

Identification of flavonoids by TLC using the mobile phase of BAA (butanol: acetic acid: water) with a ratio of (6:2:2) shows that the first spot as quercetin and the other were samples of maceration, reflux, and soxhlet extract. These four spots have parallel propagation distances, as shown in Figure 1. The calculation result of the Rf value obtained for quercetine as a comparison (1^{st} spot) is 0.87. The calculation of the Rf value of these samples spots starts from the second, third, fourth, and fifth spots are 0.73, 0.65, 0.62, and 0.56, respectively. Based on these results in Figure 1, the Clitoria ternatea flower is suspected of containing flavonoid compounds, which is in line with research conducted by Al-Snafi (2016).

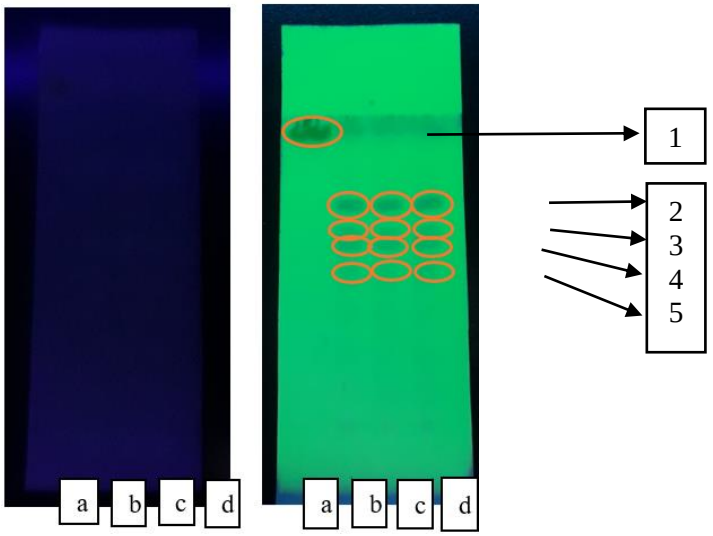

Figure 1. TLC scanner of flavonoid under UV-light (a) Quercetin, (b) maceration extract, (c) reflux extract, (d) soxhlet extract

Total flavonoid content of Clitoria ternatea flower

Quercetin was used as the standard for identifying flavonoids with a concentration of 20, 40, 60, 80, and 100 ppm given in **Table 1**. The choice of quercetin as a positive control solution was because quercetin is a flavonoid of the flavonol group (Aminah et al., 2017). Measurement of the maximum wavelength of quercetin was carried out at a wavelength of 350-450 nm. Measurement of the maximum wavelength of quercetin was carried out at a 350-450 nm wavelength, and the test results obtained that quercetin has a wavelength of 413.50 nm. Next, the operating time is carried to minimize the occurrence of measurement errors by knowing the optimum time required for the compound being tested to achieve reaction stability (Suharyanto & Prima, 2020). The results of the quercetin operating time obtained a stable absorbance value starting from the fourth minute.

After measuring the maximum wavelength and operating time, the next step is to determine the standard curve. The result showed that the higher the sample concentration, the higher the absorbance. Based on the absorbance and concentration data, it can be seen that there is a linear regression of quercetin with a value of y = 0.005x + 0.1027, R2 value = 0.9997, then r value = 0.9998. The linear regression equation was used to determine the total flavonoid content in the *Clitoria ternatea* flower sample.

The average levels of total flavonoids from the *Clitoria ternatea* flower extract given in **Table 1** were sequentially from the largest to the smallest are maceration extract with an average level of 53.127 mg QE/g, reflux extract with an average level of 24.527 mg QE/g, and soxhlet extract with mean level 21.060 mg QE/g. The maceration method has the highest flavonoid content because maceration is an extraction method without heating, while the reflux and Soxhlet methods use heating. Heating in the extraction process can reduce flavonoid levels by 15-78%. Increasing temperature can affect the phenolic content in the sample. When heated, phenol levels can increase up to a specific temperature but decrease with higher temperatures (Sa'adah et al., 2017). Furthermore, data processing of total flavonoid levels was carried out using IBM SPPS Statistics 23, namely the one-way ANOVA test. The results

showed significant differences in the test samples, then continued with post hoc tests.

Table 1. Total flavonoid content of *Clitoria ternatea* flower

	Replication	TFC (mg QE/g)	Average of TFC (mg QE/g)
Maceration	1	53,260	53,127
	2	50,260	
	3	55,860	
Reflux	1	24,060	24,527
	2	27,460	
	3	22,060	
Soxhlet	1	20,460	21,060
	2	21,260	
	3	21,460	

Based on the post hoc tests of total flavonoid content, there is a significant difference between the maceration method and the reflux and soxhlet methods, but the reflux method against the Soxhlet method has no significant difference; this is explained by the sig value obtained. The maceration method with reflux has a sig value. 0.001, and the maceration method with soxhlet has a sig value. 0.006 were both 0.05, which means there is a significant difference between the macerated extract with reflux and soxhlet. Meanwhile, reflux and soxhlet extracts have sig value. 0.396 (≥0.05) means no significant difference between extracts using reflux and Soxhlet methods.

Antioxidant activity of Clitoria ternatea extract

The antioxidant activity of *Clitoria ternatea* extract was carried out using the DPPH method and positive control, namely vitamin C. Determination of the maximum wavelength of the DPPH solution with a spectrophotometer at a wavelength of 400-800 nm obtained a DPPH wavelength of 517 nm. The antioxidant activity of vitamin C expressed by IC_{50} is 5.905 ppm, which means that vitamin C has very strong antioxidant levels, as shown in **Table 2**. Ascorbic acid as an antioxidant is essensial and has been widely used to keep the body healthy, fit, and protected from various diseases. Like antioxidants in general, vitamin C also donates electrons and prevents the formation of chain reactions from free radicals, so that vitamin C can reduce free radicals.

Table 2. Antioxidant activity of ascorbic acid as standard

Concentration (ppm)	Average of absorbance ±SD	Average of inhibition percentage	IC_{50} (ppm)
3	0,808±0,002	28,020	**5,905 ppm (very strong category)**
4	0,728±0,002	35,144	
5	0,641±0,002	42,891	
6	0,569±0,002	49,332	
7	0,475±0,005	57,703	
8	0,390±0,005	65,272	
9	0,260±0,008	76,877	

Based on the antioxidant activity test results of the Clitoria ternatea flower extract using the maceration, reflux, and soxhlet methods given in Table 3, the results were respectively showed that the IC_{50} values were 250.850, 289.612, and 336.575 ppm, which are classified as weak antioxidants. Compared with the results of previous studies conducted by Winarti (2020) and Rahayu et al., (2020) where the antioxidant activity of the *Clitoria ternatea* flower with the

FRAP method is a very strong antioxidant. The difference in the antioxidant activity results may be due to the different methods used.

Tabel 3. Antioxidant activity of *Clitoria ternatea* flower

Extraction method	Concentration (ppm)	Average of absorbance ±SD	Average of inhibition percentage	IC_{50} (ppm)
Maceration	100	0,695±0,003	36,093	
	200	0,594±0,004	45,324	
	300	0,494±0,005	54,554	**250,850 (weak category)**
	400	0,389±0,001	64,213	
	500	0,299±0,007	72,493	
	600	0,213±0,004	80,405	
Reflux	100	0,703±0,003	33,175	
	200	0,614±0,005	41,667	
	300	0,519±0,006	50,697	**289,612 (weak category)**
	400	0,422±0,002	59,854	
	500	0,324±0,001	69,202	
	600	0,231±0,002	78,074	
Soxhlet	100	0,774±0,003	26,426	
	200	0,648±0,002	38,403	
	300	0,561±0,003	46,705	**336,575 (weak category)**
	400	0,464±0,012	55,894	
	500	0,365±0,005	65,272	
	600	0,264±0,006	74,873	

Each extraction method has its own advantages and disadvantages that affect the antioxidant activity obtained. **Table 3** shows that the extract with the highest antioxidant content was maceration, followed by reflux and then soxhlet. Maceration has the highest antioxidant content, possibly because maceration is an extraction method without heating. The antioxidant compounds contained in the *Clitoria ternatea* flower are thought to have been damaged when extracted by reflux and soxhlet methods using heating.

Heating at high temperatures can damage antioxidant compounds; the higher the temperature used, the lower the antioxidant levels (Kusuma et al., 2019). The weak category of antioxidants in the sample can be caused by several factors, namely the extraction process is less than optimum, the extract drying process with a water bath is not optimal, the simplicia manufacturing process is not precise, and the antioxidant analysis method used is less precise.

Based on the one-way ANOVA test results, it is known that each method, namely maceration, reflux, and soxhlet, has a sig value. 0.749 (≥0.05) means that there is no significant difference. This result shows no significant difference (no difference in antioxidant activity) between each sample and shows no need for further post hoc tests.

The other result of the test, there was an in-line correlation between total flavonoid content and antioxidant activity from Clitoria ternatea flower extract. It can be seen by looking at the correlation value of total flavonoid levels and antioxidant activity, which shows a correlation value of 0.668 with a strong correlation category. A strong relationship between total flavonoid levels and antioxidant activity can be seen from the high correlation value, such as research conducted by Chayati and Miladiyah (2012), with a correlation value of 0.922.

Conclusion and Suggestions

The results of total flavonoid content from highest to lowest were maceration 53,127 mg QE/g, reflux 24,527 mg QE/g, and soxhlet 21,060 mg QE/g). The IC_{50} results of Clitoria ternatea were 250,850 ppm from maceration, 289,612 ppm from reflux, and the weakest one was 336.75 ppm from soxhlet. Total flavonoid and antioxidant results have no significant difference between the three extraction methods. However, both flavonoid and antioxidant activities have an in-line correlation.

Acknowledgements
This Work is totally funded by Ngudi Waluyo University and we are very thankful for providing Internal funding facilities in 2021 SK No. 002/KTR-PENELTIAN/LPPM/UNW/VI/2021 to achieve this research.

References

Al-Snafi, A.E., 2016. Pharmacological importance of *Clitoria ternate*a–A review. I*OSR Journal of Pharmacy*, 6(3), pp.68-83.

Aminah, A., Tomayahu, N. and Abidin, Z., 2017. Penetapan kadar flavonoid total ekstrak etanol kulit buah alpukat (*Persea americana* Mill.) dengan metode spektrofotometri UV-Vis. *Jurnal Fitofarmaka Indonesia*, 4(2), pp.226-230.

Andriani, D. and Murtisiwi, L., 2020. Uji aktivitas antioksidan ekstrak etanol 70% bunga telang (*Clitoria ternatea* L) dari daerah sleman dengan metode DPPH. *Pharmacon: Jurnal Farmasi Indonesia*, 17(1), pp.70-76.

Chayati, I., & Miladiyah, I. 2012. Kajian kadar flavonoid, aktivitas antioksidan, dan kapasitas antioksidan madu monoflora. *Jurnal Ilmiah Universitas Negeri Yogyakarta*, *1*(1), 17–18.

Erlidawati, & Safrida. 2018. *Potensi Antioksidan Sebagai Antidiabetes : Buku untuk mahasiswa*. Syiah Kuala University Press.

Kurniasih, E., 2019. Sosialisasi Bahaya Radikal Bebas Dan Fungsi Antioksidan Alami Bagi Kesehatan. *Jurnal Vokasi*, 3(1), pp.1-7.

Kusuma, I.G.N.S., Putra, I.N.K. and Darmayanti, L.P.T., 2019. Pengaruh suhu pengeringan terhadap aktivitas antioksidan teh herbal kulit kakao (*Theobroma cacao* L.). *Jurnal Ilmu Dan Teknologi Pangan (ITEPA)*, 8(1), p.85.

Maesaroh, K., Kurnia, D. and Al Anshori, J., 2018. Perbandingan metode uji aktivitas antioksidan DPPH, FRAP dan FIC terhadap asam askorbat, asam galat dan kuersetin. *Chimica et natura acta*, 6(2), pp.93-100.

Parwata, M.O.A., 2016. *Bahan Ajar Antioksidan. Kimia Terapan Program Pascasarjana* Universitas Udayana.

Rahayu, S., Vifta, R., and Susilo, J. 2021. Uji Aktivitas Antioksidan Ekstrak Etanol Bunga Telang (*Clitoria ternatea* L.) dari Kabupaten Lombok Utara dan Wonosobo Menggunakan Metode FRAP, *Generics: Journal of Research in Pharmacy*, vol. 1, no. 2, pp. 1 - 9, Oct. 2021.

Saadah, H., Nurhasnawati, H. and Permatasari, V., 2017. Pengaruh metode ekstraksi terhadap kadar flavonoid ekstrak etanol umbi bawang dayak (*Eleutherine palmifolia* (L.) Merr) dengan metode spektrofotometri. *Borneo Journal of Pharmascientech*, 1(1).

Suharyanto, S. and Prima, D.A.N., 2020. Penetapan Kadar Flavonoid Total pada Juice Daun Ubi Jalar Ungu (*Ipomoea batatas* L.) yang Berpotensi Sebagai Hepatoprotektor dengan Metode Spektrofotometri UV-Vis. *Cendekia Journal of Pharmacy*, 4(2), pp.110-119.

Vifta, R.L., Sunnah, I., Chanifah, N. and Advistasari, Y.D., 2019. Purifikasi Buah Parijoto (*Medinilla speciosa* Blume) dan Uji Bioaktivitasnya Sebagai Alternatif Pengobatan Diabetes Mellitus. *Media Informasi Penelitian Kabupaten Semarang*, 2(2), pp.185-199.

Winarti, N. (2020). Uji Aktivitas antioksidan Ekstrak Bunga Telang (*Clitoria ternatea* L.) Dengan Pelarut Etanol Dan Etil Asetat Menggunakan Metode FRAP (Ferric Reducing Antioxidant Power), *Skripsi*, Universitas Ngudi Waluyo.