Butterfly Pea (Clitoria ternatea L.) Flower Water and Ethanol Extract: Phytochemical Screening, FTIR Analysis, and Antioxidant Activity Estimation using comparison of ABTS, DPPH, and FRAP Assays

by Perpustakaan IIK Bhakti Wiyata

Submission date: 31-Jul-2025 07:58AM (UTC+0700)
Submission ID: 2501130511
File name: nita_Antioksidan_Telang_Mei_2024_-_anggraini_dyah_Setiyarini.pdf (390.14K)
Word count: 8473
Character count: 42798

ISSN 0974-3618 (Print)
0974-360X (Online)
www.rjptonline.org

RESEARCH ARTICLE

Butterfly Pea (*Clitoria ternatea* L.) Flower Water and Ethanol Extract: Phytochemical Screening, FTIR Analysis, and Antioxidant Activity Estimation using comparison of ABTS, DPPH, and FRAP Assays

Rachma Nurhayati[1], Fenita Shoviantari[1]*, Tristiana Erawati Munandar[2], Mochammad Yuwono[2]

[1]Faculty of Pharmacy, Institut Ilmu Kesehatan Bhakti Wiyata, Jalan KH Wahid Hasyim 65 Kediri, East Java, Indonesia, 64117.

[2]Faculty of Pharmacy, Universitas Airlangga, Gedung Nanizar Zaman Joenoes Kampus C UNAIR Jalan Mulyorejo Surabaya, East Java, Indonesia, 60115.

*Corresponding Author E-mail: **fenita.shoviantari@iik.ac.id**

ABSTRACT:

Background: The community has empirically employed butterfly pea flower, one of the species, extensively as a traditional beverage with pharmacological benefits. The flavonoid content of these plants, particularly the anthocyanins, is assumed to be the source of the pharmacological effects. The content of the extract to be obtained will be impacted by the variation in solvent polarity. In this investigation, the butterfly pea flowers will be extracted utilizing the solvents of water and Ethanol. **Objective:** The goal of this study was to qualitatively assess the content of butterfly pea petal extract in water and ethanol solvents using phytochemical screening and FTIR analysis, followed by testing for antioxidant activity using three different methods, including ABTS, DPPH, and FRAP. **Method:** The extraction with water and ethanol solvents, phytochemical screening tests, antioxidant activity testing with DPPH assay, antioxidant capacity testing with ABTS, and quantitative FRAP assays utilizing UV-Vis spectrophotometry are all steps of this study. **Results:** When tested for antioxidant activity, ethanol extract produced IC_{50} of 113.31 0.142ppm and water extract produced IC_{50} of 86.67 0.485 ppm, placing water extract in the strong category and ethanol extract in the medium category. Using the FRAP assay, the antioxidant capacity of water extract and ethanol extract from butterfly pea flowers was tested, and the results were 52.8957 0.0881mgQE/g extract and 32.0051 0.0441mgQE/g extract, respectively. The antioxidant capacity test of the butterfly pea flower's ethanol and water extracts was performed using the ABTS assay, and the results were 71,497 3.29mgQE/g extract and 114,195 0.279mgQE/g extract, respectively. **Conclusion:** The water dan ethanol extract of butterfly pea flowers differs from one another in terms of antioxidant activity and phytochemical composition.

KEYWORDS: Anthocyanins, Flavonoid, Phytochemical, IC_{50}.

INTRODUCTION:

Along with the times, the pattern of human life has changed, including lifestyle changes to unhealthy and causing the body to be exposed to harmful substances that will cause disease and degenerative conditions. Excessive oxidation reactions in the human body mainly precede the disease. *Oxidation reactions* are defined as the reduction of electrons which can cause the formation of free radicals[1].

Free radicals are atoms or compounds that lose electron pairs. Free radicals are unstable, highly reactive, and consistently trying to find new electron pairs. Free radicals in average amounts in the body can be beneficial to health, one of which is as an anti-inflammatory and antibacterial. However, in excess amounts, free radicals in the body can result in oxidative stress. This situation can cause damage to the body both at the cellular and organ levels, supporting the acceleration of the ageing process and the emergence of various diseases in the body. Free radicals can oxidize protein-nucleic acids to initiate degenerative diseases and cell damage and damage body cells[2,3]. By preventing the start of oxidative chain reactions,

Received on 16.09.2023 Modified on 09.12.2023
Accepted on 06.02.2024
Research J. Pharm. and Tech 2024; 17(5):1973-1982.
DOI: 10.52711/0974-360X.2024.00313

antioxidant substances are known to protect the b against free radicals by preventing or decreasing the oxidation of biological compounds, including lipids, proteins, nuc acids, or other molecules. Based on this mechanism, antioxidants play an essential role in the body's absorption and neutralization of free radicals[4].

One plant that can act as anaturalanti oxidant is the butterfly peaflower (*Clitoria ternatea* L.), comes from the Fabaceae family.In several Asian countries, eating butterfly pea flowers (BPF) or drinking its infusion as herbal tea is believed to make the skin younger and prevent ageing[5]. The flowers of this tree have bright, dark blue, and white color. They are often used as natural dyes in local food and animal feed because they taste good, and its nutritional value. Parts of plants, such leaves, flowers, and roots, have various pharmacological activities such as analgesic, antipyretic, anti-inflammatory, antioxidant, antidiabetic, antimicrobial, anthelminthic, hepaprotective, and antiasthmatic[6,7].

The BPF is known traditionally as a medicine for the eyes and as a food coloring that gives it a blue color. The color produced by BPF indicates the presence of anthocyanins[8]. Anthocyanins in BPF are poly-alkylated anthocyanins or have more than two acyl groups with delphinidin as their aglycone. poly-alkylated anthocyanins have higher stability than anthocyanins that do not have acyl groups. Anthocyanins are a class of water-soluble pigments having a variety of useful applications. Anthocyanin is a member of the flavonoid component, which has the most potent antioxidant activity. Anthocyanins help fight free radicals because they can give hydrogen to radicals and stop their chain reactions[9,10].

The large number of activities of the BPF extract makes it interesting to study its ingredients. One of the most used techniques for explaining compounds and identifying chemical components is FTIR. The infrared spectrum may be measured quickly and accurately using FTIR, and it has been employed as a required method to identify medications in the pharmacopeia of several nations[11]. ABTS, DPPH, and FRAP are several methods to measure antioxidant activity[12,13]. This study examines several easurement methods, namely ABTS and FRAP, to determine the antioxidant capacity of samples equivalent to quercetin (QE) and the DPPH assay to determine each sample's antioxidant activity (IC_{50}). Antioxidant capacity and activity have almost the same meaning, which refers to the ability of a compound/samp o inhibit or ward off the activity of free radicals[14]. Based on the above background, this study will examine the content of phytochemicals, FTIR analysis and comparison of ABTS, DPPH, and FRAP testing to estimate the antioxidant activity of water and ethanol extracts of BPF.

MATERIALS AND METHODS:

Materials:

Clitoria ternatea were provided by Omah Sehat Mandiri Pangan Plantzone plantation in the Krakal, Klemunan, Wlingi District, Blitar, East Java, Indonesia, DPPH, ABTS (Sigma, US), ethanol 96% pa (Smart- Lab, Indonesia), quercetin (Sigma, US), $FeCl_3$(Smart-Lab, Indonesia), potassium acetate, potassium persulfate (Loba Chemie PVT. LTD, Mumbai), TPTZ(Sigma, US), water pro analytical (Onemed, Indonesia), distilled water (Bratachem, Indonesia), Wagner's, dragendorf's and Mayer's reagent (Asahimas Chemical, Banten), HCl, magnesium powder, H_2SO_4, NaCl and NaOH.

Extraction of Butterfly Pea Flower:

Water Extract of *Clitoria ternatea*L.(WEC):

After being thoroughly cleaned, fresh BPF are dried at 40°C for 12 hours. The flowers are then chopper-smoothed before being added to the distilled water. They are then filtered using doubled filter paper and placed in an ultrasonic for 15 minutes (stopping every 5 minutes to prevent overheating). The extract was compressed with a rotary evaporator and dried with a freeze-dryer.

Ethanol Extract of *Clitoria ternatea* L. (EEC)

After being thoroughly cleaned, fresh BPF are dried at 40°C for 12 hours. The flowers are then placed in 96% ethanol, blended with a chopper, ultrasonically processed for 15 min (stopping every 5 minutes to prevent overheating), and then filtered through double filter paper. The extract was compressed in a rotary evaporator.

Phytochemical screening of extract:

The water and ethanol extract of *Clitoria ternatea* L. blossom were subjected to different tests to identify the nature of bioactive chemical constituents present in e plant material. Using standard analysis methods[15–18], the crude extracts were screened qualitatively for the phytochemical constituents.

Test for Alkaloids:

40mg of extract was mixed with 1mL of 2N HCl, 9mL of water, and 3 parts were created after homogenization. When Part 1 was added to Mayer's reagent, a white or yellowish-white precipitate formed as a sign of success. Wagner's reagent was applied in Part 2, and the appearance of a brown precipitate was a good sign. Dragendorf reagent was added to part three, and the formation of a red-orange precipitate served as a positive outcome indicator.

Test for Flavonoids:
In boiling water, 40mg of the extract was dissolved, simmered for 5 minutes, and then filtered. The filtrate was diluted to a maximum of 5ml, followed by the addition of 0.05mg of magnesium powder, 1mL of concentrated HCl, and a vigorous shake. If the solution turns red, yellow, or orange, the results are positive.

TestforSaponins:
In boiling water, 40mg of the extract was dissolved, simmered for 5 minutes, and then filtered. The filtrate was diluted to a maximum of 5ml, followed by the addition of 1mL of concentrated HCl and 0.05mg of magnesium powder, which was then rapidly shaken. If the solution turns red, yellow, or orange, it has produced successful outcomes.

Test forTerpenoids:
The extract, 40mg, was dissolved in water. Three drops of concentrated HCl and one drop of concentrated H2SO4 were added to 2mL of the extract solution. The development of red or purple color is a sign of success.

Test for Tannins:
In a test tube, the extract was added, and then it was dissolved in hot water and agitated. 2Ml of 10% NaCl were added to the extract solution, then it was filtered. A few drops of $FeCl_3$ were added to the filtrate that resulted. A change in the solution's color to dark blue, yellowish-brown, or greenish-black indicates a successful outcome.

Test for Anthocyanins:
The sample is heated with 2M HCl for 2 minutes at 100°C in the first procedure, after which the sample's color is assessed. Anthocyanins are present when the sample's red color remains constant throughout time. The second method involves gradually adding the sample containing 2M NaOH. Anthocyanins are present when the red color gradually fades to blue-green and changes.

FT-IR Spectroscopic Analysis:
A Shimadzu Fourier transform infrared spectrometer (Japan) with a frequency range of 4000-500 cm-1 was used to record the FT-IR spectra. FT-IR spectroscopic measurement of the WEC and EEC of a sample was used to ascertain the characteristics of a molecule's vibrational modes.

Total Flavonoid Content (TFC):
Standard quercetin and aluminium chloride colorimetric techniques were modified by Putra et al.[19] to estimate the total flavonoid concentration.

a. Making a standard solution of quercetin.
100mL of ethanol was used to dissolve 10mg of standard quercetin every hour. A 5–60ppm standard series concentration was created. To make up to 10mL of distilled water, 2mL of each concentration was taken and mixed with 3mL of methanol pa, 0.2mL of 10% $AlCl_3$, and 0.2mL of 1M potassium acetate. After allowing the solution to stand at room temperature for thirty minutes, the absorbance was measured at the longest wavelength.

b. The sample's total flavonoid content
Each WEC and EEC were combined with 4mL of methanol per gramme, 0.2mL of 10% $AlCl_3$, 0.2mL of 1M potassium acetate, and 2mL of water to make a total of 10mL. The absorbance was then measured at the longest wavelength after the solution had been allowed to settle at room temperature for 30 minutes.

Total Anthocyanin Content (TAC):
a. Making a potassium chloride buffer is step one. 10.465g of potassium chloride was dissolved in distilled water and put into a 250mL volumetric tube. When the pH approaches 1, continue adding additional HCl solution.
b. Making Sodium Acetate Buffer. 8.2g of sodium acetate are dissolved in distilled water at pH 4.5 in a volumetric tube with a capacity of 250mL. When the pH hits 4.5, continue adding HCl solution.
c. Total amount of anthocyanins
The method of Lee[20] was modified to determine the total anthocyanin content. This technique makes use of the pH difference between a potassium chloride buffer (pH of 1) and a sodium acetate buffer (pH of 4.5). Using the same dilution factor (1 part for the test sample and 4 parts for the buffer), samples were diluted with both buffers prior to analysis. Both buffers were tested against a blank cell filled with distilled water after standing for 60 minutes at the longest wavelength and 700nm (for haze correction). The following equation was used to determine the total anthocyanin content:

$$\text{Total anthocyanin}\left(\frac{mg}{L}, CyE, ME\right) = \frac{A * MW * DF * 10^3}{\varepsilon * 1}$$

$A = (A520nm - A700nm)pH1.0 - (A520nm - A700nm)pH4.5$;
for malvidin-3-glucoside: MW= 493.43g/ mol; ε = 28000 molar extinction coefficients, in $L \cdot mol^{-1} \cdot cm^{-1}$
for cyanidin-3-glucoside : MW=449.2 g/mol;ε = 26900 molar extinction coefficients, in $L \cdot mol^{-1} \cdot cm^{-1}$;
DF =dilution factor established in D;
l = pathlength in cm;
and 10^3= factor forconversionfromg tomg.

Antioxidantactivity determinations of Butterflypea Flowerusing the FRAP assay:

The FRAP assay was conducted with the modifiedBenzie and Strain[21] technique. The stock solutions comprised of methanol, a solution of $FeCl_3.6H_2O$ w a concentration of 20mM, and a solution of (2, 4, 6-tripyridyl-s-zine) with a concentration of 10mM. To prepare the fresh working solution, a mixture was created by combining one milliliter of TPTZ solution, one milliliter of $FeCl_3.6H_2O$ solution, and ten milliliters of methanol at a ratio of 1:1:10. A volume of 100 of BPF extract in solution was subjected to heating at a temperature of 37°C for a duration of 30 minutes under conditions of darkness, subsequent to the reaction with 3mL of the FRAP solution and 1mL of water. Subsequently, the colored substance (ferrous tripyridyl triazine complex) was subjected to measurements at a wavelength of 593nm. The observed relationship between the concentration of quercetin and its corresponding response followed a linear trend within the concentration range of 5 to 120 parts per million (ppm). The outcomes are displayed in units of milligrams per gram of extract, denoted as quercetin uivalent (QE). If the observed FRAP value exceeded the linear range of the standard curve, more dilution was necessary.

Antioxidantactivity determinations of Butterfly pea Flowerusing the ABTSassay:

With a few adjustments, the Arnao et al[22] technique was used for the ABTS assay. The stock solutions contained 7mg of potassium persulfate solids and 36m of ABTS. Potassium persulfate and ABTS were each dissolved in 10.0mL of distilled water. After mixing the two stock solutions in equal parts, the working solution was created by adding ethanol until it reached a volume of 25mL for analysis. After that, give them 12hours at room temperature and no light to react. For each test, a brand-new $ABTS^{\circ+}$ solution was created. BPF racts in solution were given two hours in the dark to react with 850mL of the $ABTS^{\circ+}$solution. The absorbance at nm was then measured using a spectrophotometer. The standard curve was linear for quercetin concentrations between 20 and 600ppm. The result is displayed as quercetin equivalent (QE)/g for the extract. More dilution was necessary if the measured ABTS value was higher than the linear range of the standard curve.

Antioxidantactivity determinations of Butterfly pea Flower using the DPPHassay:

With a few adjustments, the Cahyaningsih et al.[23] technique was used for the DPPH assay. The stock solution contained 100mL of ethanol and 5mg of DPPH, and it was kept at -20°C until it was required. A working solution with an absorbance of 0.35764 units at 515nm is created by combining 2mL of the stock solution with 2 mL of the ethanol mixture using a spectrophotometer. The quercetin was left to get sensitized with 2mL of DPPH solution at a concentration of 8 to 40ppm for two hours in the dark. Each WEC and EEC (2mL) was left sensitive with 2mL of DPPH solution for 2 hours in the dark with a concentration of 40 to 150ppm; absorbance was obtained at 520nm with an absorbance of 0.74738. The standard curves for quercetin and the extract are linear. Results are presented as IC_{50} values.

RESULT:

Phytochemical Screening of BPF Extract:

Table 1: Phytochemical screening of butterfly pea flowers extract

S.No	Phytochemicals	Observation	
		Ethanol 96 % Extract	Water Extract
1	Alkaloid	-	-
2	Anthocyanin	-	+
3	Flavonoid	+	+
4	Saponin	+	+
5	Tannin	+	+
6	Terpenoid	-	+

Note:(-)Absent;(+) Present

Total Flavonoid Content (TFC)

The quercetin standard curve isobtained from aconcentrationseries of 5, 10, 20, 30, 40, 50 and 60ppm Measure data wave length o 432nm. The line arregressione quation obtained is y=0.0726x+0.0758 with a value of r = 0.9986. From the results of linear regression on the standard curve, quercetin solution was then usedto measure thetotal flavonoid contentin WEC and EEC. The test results found that the total flavonoids in WEC were 7,804±0.08mgQE/g extract, and in EEC, were 3,722±0.01mgQE/g extract. The amount of flavonoids in WEC is more significant when compared to EEC.

Total Anthocyanin Content (TAC):

The maximum wavelength measurement result of anthocyanins is at a wavelength of 543 nm. From these results, it can be determined the total anthocyanin concentration of WEC and EEC.

Table 2: Calculation of the total anthocyanincon centration of WEC and EEC

Sample	*Anthocyanin Monomer of Extract*	TAC *(mgME/gextract; mgCyE/gextract)*
WEC	*Malvidin*	4.159± 0.705
	Cyanidin	3.941± 0.670
EEC	*Malvidin*	- 3.618 ±0.081
	Cyanidin	- 3.428 ±0.077

Spectroscopic Analysis Using FT-IR

FT-IR is helpful to identify the characteristics of vibrational modes of a molecule in sample. Figure 1 displays the FT-IR spectra of WEC and EEC.

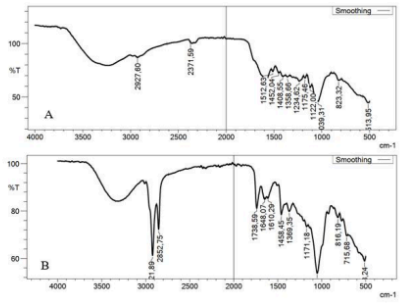

Fig. 1: FTIR spectra of (A) water extract (B) ethanol extract of *Clitoria ternatea* L

A broad band between 3000 and 3500 might suggest the presence of O-H. The two extracts have different absorptions in the range of 2800 – 2900. Two sharp peaks in the ethanol extract appeared in this range, indicating the presence of aliphatic C-H absorption, whereas in the aqueous extract, there were none. The wave number 1600 – 1650 in the ethanol extract indicates the presence of C=C (aromatic ring). Wave numbers 1000 – 1100, with sharp and strong peaks from both extracts, indicate the presence of C-O. The spectra of the EEC showed more specific absorption indicating the presence of flavonoids[24]. Whereas the WEC showed more specific absorption indicating the presence of anthocyanin content[25]

Antioxidant activity measured using FRAPassay:

At a wavelength of 593 nm, quercetin concentrations of 5, 10, 20, 40, 50, 60, 70, 80, 90, 100, 110, and 120ppm were measured and used to create standard curves. The derived linear regression equation has a value of r=0.9986 and is written as y = 0.0131x + 0.0614. The antioxidant content of the WEC and EEC is determined using the results of the linear regression equation derived from the quercetin solution standard curve.

Table 3: Calculation of the antioxidant capacity of WEC and EEC usingFRAPassay

Sample	Replication of extractsample	Absorbance	Concentrations (ppm)	Antioxidantactivity (mg QE/gextract)	Mean ± SD
WEC	1	0.753	52.7939	52.7939	52.8957± 0,0881
	2	0.755	52.9466	52.9466	
	3	0.755	52.9466	52.9466	
EEC	1	0.480	31.9542	31.9542	32.0051± 0.0441
	2	0.481	32.0305	32.0305	
	3	0.481	32.0305	32.0305	

Antioxidant activity measured using ABTSassay

Standard curves for quercetin were created using concentrations of 20, 60, 80, 120, and 180 ppm, and the linear regression equation y = -0.0043x + 1.7172 was created, with an r-value of 0.9964. The outcomes of the linear regression equation, which were derived from the quercetin solution standard curve, were used to determine the antioxidant levels in the WEC and EEC.

Table 4: Calculation of the antioxidant capacity of WEC and EEC using ABTS Assay

Sample	Replicationof extractsample	Absorbance	Concentrations (ppm)	Antioxidant activity (mgQE/g extract)	Mean± SD
WEC	1	1.458	60.279	72.335	71.497 ±3.29
	2	1.474	56.558	67.869	
	3	1.451	61.907	74.288	
EEC	1	1.309	94.930	113.916	114.195±0.279
	2	1.308	95.163	114.196	
	3	1.307	95.395	114.474	

Furthermore, to determine the antioxidant ability of WEC and EEC in inhibiting free radicals of ABTS°+, it is necessary to calculate the % inhibition of quercetin and BPFextract. Based on the curve of % inhibition of quercetin, the equation of the linear regression was obtained y = 0.2514x + 0.4389 with an r-value of 0.9964, where "x" is the sample concentration value and "y" is the % sample inhibition value. For the next, the % inhibition of the extract samples was calculated, and the average concentration of WEC was 59.58 ppm with a % inhibition value of 15.42%. At the same time, the EEC obtained concentration of 95.16 ppm with a % inhibition value of 24.36%.

Table 5: Calculation of % inhibition of quercetin and butterfly peaflower extract

Quercetin concentrations (ppm)	Quercetin absorbance	% Inhibition of quercetin	Average extract concentration (ppm)		% Inhibition of extract	
			Water	Ethanol	Water	Ethanol
20	1.608	6.77				
60	1.480	14.19				
80	1.364	20.91	59.58	95.16	15.42	24.36
120	1.221	29.21				
180	0.918	46.77				

Table 6: Calculation of the antioxidant activity of quercetin using DPPH assay

Concentrations (ppm)	Absorbance			%Inhibition			IC50			Mean ±SD
	I	II	III	I	II	III	I	II	III	
8	0.189	0.188	0.188	47.059	47.339	47.339				
10	0.187	0.187	0.187	47.619	47.619	47.619				22.056 ±0.196
20	0.179	0.178	0.180	49.860	50.140	49.580	22.08	22.24	21.85	
30	0.17	0.172	0.170	52.381	51.821	52.381				
40	0.169	0.169	0.168	52.661	52.661	52.941				
				Blank:0.357						

Table 7: Calculation of the antioxidant activity of WEC using DPPH assay

Concentrations (ppm)	Absorbance			%Inhibition			IC50			Mean ±SD
	I	II	III	I	II	III	I	II	III	
40	0.527	0.525	0.523	29.451	29.987	29.987	86.67	87.15	86.18	
50	0.469	0.470	0.467	37.216	37.082	37.483				86.67±0.485
60	0.43	0.431	0.43	42.436	42.303	42.436				
100	0.331	0.332	0.331	55.689	55.556	55.689				
150	0.208	0.208	0.208	72.155	72.155	72.155				
				Blank:0.747						

Table 8: Calculation of the antioxidan tactivity of EEC using DPPH assay

Concentrations (ppm)	Absorbance			%Inhibition			IC50			Mean±SD
	I	II	III	I	II	III	I	II	III	
40	0.586	0.586	0.587	21.553	21.553	21.419	113.15	113.41	113.38	113.31 ±0.142
50	0.558	0.557	0.559	25.301	25.435	25.167				
60	0.51	0.511	0.511	31.727	31.593	31.593				
100	0.421	0.422	0.423	43.641	43.507	43.373				
150	0.265	0.265	0.265	64.525	64.525	64.525				
					Blank:0.747					

Statistical Analysis:

Table 9: Statistical Analysis

	Normality Test *(Shapiro-Wilk)*	HomogenityTest	Parametric or Non-ParametricTest	Result
TFC	-	-	Mann-WhitneyUTest	Therearedifferences
TAC	√	√	Independentt-Test	Therearedifferences
ABTS	√	√	Independentt-Test	Therearedifferences
DPPH	√	√	Independentt-Test	Therearedifferences
FRAP	√	√	Mann-WhitneyUTest	Therearedifferences
Requirement	Sig. > 0.05	Sig. > 0.05	Sig. < 0.05	

Antioxidant activity measured using DPPH assay:

Quercetin standard curves were obtained from concentrations of 8,10, 20, 30, and 40 ppm, the equation of line. The resultant linear regression equation derived from the standard curve of the quercetin solution is utilized for the computation of antioxidant activity. (Table 6).

Water extract standard curves were obtained from concentrations of 40, 50, 60, 100, and 150ppm, the equation of line. The results of the linear regression equation obtained from the standard curve of the water extracts solution are for calculating the antioxidant performance. (Table 7).

Ethanol extracts standard curves were obtained from concentrations of 40, 50, 60, 100, and 150ppm, the equation of line. The findings of the linear regression equation derived from the water extract solution standard curve are used to determine the antioxidant activity. (Table 8).

DISCUSSION:

Total Flavonoid Content (TFC)

In order to determine the aggregate flavonoid concentrations in WEC and EEC, a calculation is required. The formation of a complex between aluminium chloride with keto groups and nearby hydroxy groups from the flavone and flavonol groups, which results in a shift in wavelength towards visible and is marked by a yellow colour in the solution, is the basis for determining flavonoid levels by the colorimetric method. The intensity of the yellow colour produced increases with concentration[26]. Because flavonoid molecules comprise a conjugated aromatic structure that exhibits high absorption bands in the ultraviolet and visible light spectrum regions, total flavonoid levels are measured using UV-Vis spectrophotometers[27]. The chemical used as a standard solution is quercetin, a flavonoid of the flavonol group with a ketone group at C-4 and a hydroxyl group at the neighboring C-3 or C-5 atom of flavones and flavonols.While the addition of $AlCl_3$ reagent aims to

form an acid-resistant complex between hydroxy groups and adjacent ketones and form an acid-resistant complex with ortho hydroxy groups on flavonoids so that AlCl3 reagents can detect flavone and flavonol groups in flavonoid compounds, the addition of potassium acetate aims to maintain wavelengths in the visible range. Additionally, methanol acts as a solution binder to read the absorption of flavonoid compounds in the visible, UV wavelength area[28–30]. To ensure the reaction is flawless and the colour intensity produced is maximised, incubate the sample for 1 hour prior to the measurement[31–33].

The maximum wavelength of quercetin was initially measured with a wavelength range of 400-450nm in order to ascertain the overall flavonoid concentration. Based on the highest absorption result, quercetin's maximum wavelength measurement result is 432nm. The absorption of typical quercetin solutions is measured using these wavelengths. In order to derive a linear regression equation with an r-value of 0.9986, the measurement results of quercetin standard absorption are plotted with levels. The equation is y = 0.0726x + 0.0758. The total flavonoid content in WEC and EEC can be compared using the quercetin calibration curve equation. The average total flavonoid concentration of WEC was higher than the average total flavonoid content of EEC, according to the study's findings. The Mann-Whitney U Test results on the total flavonoid levels of WEC and EEC revealed a significant value of p = 0.034 (0.05), indicating that there was a significant difference between the total flavonoid levels of the WEC and EEC.

Total Anthocyanin Content (TAC):

The pH differential spectrophotometry method, which calculates differences in visible light absorbance at different pHs, at pH 1.0 and pH 4.5, was used to determine the anthocyanin levels. The absorption correction factoris calculated using the absorbance data at the wavelength of 700nm. The extracted sample's maximum wavelength measurement result was at a wavelength of 543nm. According to Harborne[27], anthocyanin pigments have a maximum wavelength range of 515-545nm as evident by the appearance of red, sensitive red, purple, and blue. On each anthocyanin monomer, including malvidin and cyanidin, distilled water solvents produced the highest overall anthocyanin content. The average total anthocyanin content of ethanol solvent also turned negative at the same time. This suggests that ethanol solvents cannot be used to extract anthocyanin monomer compounds, such as malvidin-3-glucoside and cyanidin-3-glucoside[34]. This outcome is consistent with the findings of anthocyanin screening performed on an ethanol extract of BPF, which revealed low levels of anthocyanin chemicals. The compatibility between the polarity of the solvent and the substance being dissolved also affects the value of total anthocyanin levels, leading anthocyanins to dissolve easily and create high anthocyanin levels[35]. The Independent t-test results on the total amount of anthocyanin in WEC and EEC produced a significant value of p = 0.000 (0.05), indicating that there is a significant difference between the anthocyanin levels of the flowers' total water and ethanol extracts. Anthocyanin compounds are stable at acidic pH. Therefore, adding HCl is used as a solvent in making buffers. The function of adding HCl is to hydrolyze anthocyanins in the material, usually in the form of aglycone so that it can be measured properly. The more acidic state approaching pH one will cause more anthocyanin pigments to be in the form of flavilium cations or colored oxonium, which represent the number of anthocyanins, and absorbance measurements will show a greater amount of anthocyanins[34]. While at pH 4.5, this flavilium cation changes to be more stable half thick, and there is a decrease in color intensity until it is colorless so that the absorption that reads small or does not cause absorption so that the absorption is an absorption that represents the number of disturbing compounds.

Antioxidant activity measured usingFRAP ass

Test antioxidant activity with the FRAP method is based on the power of antioxidant compounds to reduce Fe3+-TPTZ ions to Fe2+-TPTZ form, which is characterized by intensive blue discoloration with an increase in absorbance value as the magnitude of antioxidant activity of the tested sample. TPTZ is a colorant, and Fe(III) is a free radical[36]. The addition of FeCl3 also aims to form a green-to-blue complex (berlin blue). In this study, the method used was a modification of the procedure reported by Benzie and Strain[21], where methanol was used as a TPTZ solvent.TPTZ compounds dissolve in methanol. According to Ou et al[37], antioxidant substances that may reduce Fe(III)TPTZ under thermodynamic reaction circumstances and have a reasonable reaction rate can be accurately measured using the FRAP test. As a result, the FRAP assay's incubation procedure is carried out at a temperature of 37°C. In addition, testing the antioxidant activity of the sample must be done in a dark place because antioxidants are very sensitive to changes in the surrounding environment, such as light exposure, so if exposed to light, these antioxidant compounds will be easily damaged. This can decrease antioxidant activity[38]

The FRAP assay is carried out semi-quantitatively and quantitatively. Semi-quantitatively, the FRAP test assay can be used as a rapid screening method to detect antioxidant redox reactions in samples by mixing drop-by-drop samples of water and ethanol extract into a

small part of the volume of the FRAP reagent. A solution that turns blue within a few minutes indicates that samples of WEC and EEC contain antioxidant activity. Quantitatively, testing the strength of antioxidant activity of WEC and EEC was carried out by FRAP assay using a UV-Vis spectrophotometer. The results of wavelength measurements are at a wavelength of 593nm. This result corresponds to the theoretical maximum wavelength of the Fe^{2+}-TPTZ complex is 593 nm, because the sample absorbance reading is best at that wavelength. Absorbance readings can also still be carried out in the wavelength range ranging from 560-620nm[14].

WEC has a higher antioxidant activity power than EEC. The amount of antioxidant activity in BPF can be caused by the presence of secondary metabolite compounds in the extract. This is also influenced by the selection of the solvent type used in the BPF sample extraction process. This type of solvent is based on the degree of polarity where the law of like dissolves like applies[27,39]. Flavonoid and anthocyanin compounds are polar, so they will dissolve in polar solvents like water, while ethanol solvents are semi-polar. Thus, water solvents can extract flavonoid and anthocyanin compounds better than ethanol solvents. The results of statistical tests on the antioxidant performance of WEC and EEC using the Mann-Whitney U Test showed a significant value of p = 0.043 (<0.05), which means there was a significant difference in the antioxidant activity of WEC and EEC tested by the FRAP assay.

Antioxidant activity measured using ABTS assay:

The capacity of a sample to reduce or neutralize free radical cations ($ABTS^{\bullet+}$) is used to measure the antioxidant activity of a sample using the ABTS (2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) test.[40]. In this method, electrons will be transferred from antioxidant compounds in the form of one or two electrons, which can reduce radical cations. In this case, radical oxidation occurs where the intensity of the $ABTS^{\bullet+}$ color will decrease from the original blue-green color. If the concentration becomes lighter or clearer, a decrease in the intensity of the color will cause a decrease in absorbance[41]. The decrease in $ABTS^{\bullet+}$absorbance will be the basis of reference in determining the antioxidant activity of a sample. Quercetin standard is used as a positive control because it has been shown to have strong antioxidant activity[42]. Based on the absorbance measurement of the quercetin standard solution that has been carried out, the regression equation results are y = -0.0043x + 1.7172 with a value of r = 0.9964. From the quercetin standard curve, a correlation value (r) is obtained, which is ≤ 1 that the linearity of the reference standard meets the standard for measuring the test substance. The linear regression equation obtained was then used to calculate the concentration of WEC and EEC.

Based on the data values, it can be seen that the antioxidant activity of the EEC is higher than the WEC. Differences can influence differences in concentration and antioxidant activity in extract samples in the number of compounds that act as antioxidants in each sample. The EEC is likely to contain compounds with antioxidant abilities in larger amounts than the WEC. These results were also supported by the calculation of the % inhibition of the BPF extract, which was equivalent to a quercetin standard, to determine the free radical inhibition ability of the extract samples. Percentage of inhibition (% inhibition) is one of the calculation methods to determine a sample's high or low antioxidant activity against free radical inhibition. The greater the percentage inhibition value, the higher the antioxidant activity of the samples tested[43]. Based on the data obtained, it is known that the average concentration of the WEC, which is 59.58ppm, has a % inhibition value of 15.42%. The EEC with an average concentration of 95.16 ppm has a free radical inhibition value or % inhibition of 24.36%. Secondary metabolites likely to provide antioxidant activity in the EEC are aglycone flavonoids such as isoflavones, flavanones and flavones which tend to be more soluble in semipolar solvents such as ethanol. Sequentially, these compounds have various antioxidant mechanisms, such as the mechanism of donating hydrogen ions, scavenging, and inhibition of oxidant enzymes[44–46]. In addition, there may also be condensed tannins in the EEC, where the tannins consist of a flavonoid polymer known as proanthocyanidin, one of whose pharmacological activities is antioxidant[47]

In the ABTS assay, the EEC has higher antioxidant activity. These results are inversely proportional to the FRAP and DPPH assays which show that the WEC has high antioxidant activity in inhibiting every radical. One of the causes of these differences is the reaction mechanism between each of the radicals and antioxidants analyzed. One of the weaknesses of the ABTS assay is that several types of antioxidants can provide different radical inhibition values, depending on the method of preparation of ABTS and the length of time the reaction takes place between the ABTS radicals and the sample. Several antioxidants can react quickly, moderately or slowly in reducing ABTS radicals[44,48]. Therefore, while calculating the antioxidant capacity, the reaction time needed for the ABTS test must be taken into account. Only the reaction's stoichiometry and the HAT or SET mechanism, which indicates which is dominant, are provided by the drop in absorbance[49]. However, this cannot predict and explain the mechanism and reaction rate between ABTS radicals and the antioxidants analyzed[14,50].

Antioxidant activity measured using DPPH assay:

A free radical employed in research on antioxidants is called DPPH. This approach has the benefits of being straightforward, quick, simple, accurate, dependable, and practical. The capacity of these compounds to contribute electrons to free radicals is related to their capacity to scavenge them; as a result, the DPPH colour will fade, turning from purple to yellowish. To determine this maximum wavelength, testing was done initially. The maximum wavelength attained falls within the 500–520 nmmaximum wavelength range utilized in the DPPH test[51]. Because, it can be affected by a number of variables, including temperature, humidity, sample reading time, and light, the maximum wavelength was determined twice in this study, namely during the antioxidant activity test on quercetin and the sample. The amount of measured light will grow as more light enters the cuvette. The sample's absorbance at 520nm and quercetin's maximum wavelength of 515nm, respectively, have been previously measured for the antioxidant activity test, and the test is lengthy enough to perform a recheck at the maximum wavelength.

According to the IC_{50} value parameter, where a value of less than 50ppm is considered in the extremely powerful antioxidant category, the results showed that the IC_{50} of replicating quercetin I, II, and III with an average of 22,056ppm. These findings suggest that quercetin is highly effective at scavenging free radicals. The average IC_{50} values for EEC from replications I, II, and III were 113.31ppm and 86.67ppm, respectively, indicating that these substances fall under the strong and moderate antioxidant categories, respectively. The findings of this study differ from those of Andriani and Murtisiwi's[36] study on the antioxidant activity test of BPF in 70% ethanol solvent, which used an extract from the BPF with an IC_{50} value of 41.36±1.191g/mL, including very potent antioxidants. This variation is likely the result of variations in the altitude at which they are grown, as well as variations in the concentration of ethanol used as a distiller. Each region will have a different phytochemical content of a plant's secondary metabolites, such as flavonoids, depending on environmental parameters such light, temperature, pH, height, and temperature. The secondary metabolites in the extract, including flavonoids, can activate the antioxidant activity in BPF. The study's findings demonstrated that the EEC lacked the antioxidant strength that the WEC did. Due to the fact that flavonoids are polar and will dissolve in polar solvents like water, whereas ethanol solvents are semi-polar, this is caused by the dissolving principle. So, the water solvent is more effective in extracting flavonoids than ethanol.

CONCLUSION:

Different solvents for the extraction process of butterfly pea flowers (*Clitora ternatea* L.) have different contents when analyzed using phytochemical screening or FTIR analysis. Anthocyanins and terpenoids could not be detected in the EEC.The FRAP and DPPH assays yielded the same results in this antioxidant activity test of WEC and EEC: the WEC had stronger antioxidant activity than the EEC. The ABTS assay, on the other hand, yielded different results, demonstrating that the EEC had higher antioxidant activity than the WECs. The variances could be the result of different antioxidant types that were present in the samples and had varied reactions to the radicals used. The mechanisms underpinning antioxidant tests differ for each approach. It becomes crucial to think about the best method for the samples.

CONFLICT OF INTEREST:

The reis no conflict of interest in this research.

ACKNOWLEDGMENTS:

This research was funded by the Directorate of Research, Technology and Community Services Ministry of Education, Culture, Research and Technology of Republic Indonesia in accordance with Contract Agreement Number 070/SP2H/PT/LL7/2022;412/R/PN/V/2022 Fiscal Year 2022

REFERENCES:

1. Zuo, Prather, Stetskiv, et al. Inflammaging and Oxidative Stress in Human Diseases: From Molecular Mechanisms to Novel Treatments. Int J Mol Sci. 2019;20(18):4472. doi:10.3390/ijms20184472
2. Phaniendra A, Jestadi DB, Periyasamy L. Free Radicals: Properties, Sources, Targets, and Their Implication in Various Diseases. Indian Journal of Clinical Biochemistry. 2015; 30(1): 11-26. doi:10.1007/s12291-014-0446-0
3. Ghanshyam B. Jadhav; Ravindranath B. Saudagar. Free radical Scavenging and Antioxidant Activity of Punica granatum Linn. Asian J Res Pharm Sci. 2014; 4(2).
4. Gaber NB, El-Dahy SI, Shalaby EA. Comparison of ABTS, DPPH, permanganate, and methylene blue assays for determining antioxidant potential of successive extracts from pomegranate and guava residues. Biomass Convers Biorefin. 2023; 13(5): 4011-4020. doi:10.1007/s13399-021-01386-0
5. Zakaria NNA, Okello EJ, Howes MJ, Birch-Machin MA, Bowman A. In vitro protective effects of an aqueous extract of Clitoria ternatea L. flower against hydrogen peroxide-induced cytotoxicity and UV-induced mtDNA damage in human keratinocytes. Phytotherapy Research. 2018; 32(6): 1064-1072. doi:10.1002/ptr.6045
6. Fatimah I, Hidayat H, Nugroho BH, Husein S. Ultrasound-assisted biosynthesis of silver and gold nanoparticles using Clitoria ternatea flower. S Afr J Chem Eng. 2020; 34: 97-106. doi:10.1016/j.sajce.2020.06.007
7. Drisya M. K; Sapna Shrikumar. GC- MS Analysis of Ethylacetate extract of leaves of Clitoria ternatea Linn. Asian Journal of Pharmaceutical Analysis. 2022; 12(1).
8. Muhammad Ezzudin R, Rabeta MS. A potential of telang tree (Clitoria ternatea) in human health. Food Res. 2018; 2(5): 415-420. doi:10.26656/fr.2017.2(5).073
9. Marpaung AM, Lee M, Kartawiria IS. The Development of Butterfly pea (Clitoria ternatea) Flower Powder Drink by Co-crystallization. Indonesian Food Science & Technology Journal. 2020; 3(2): 34-37. doi:10.22437/ifstj.v3i2.10185

10. Priyanga Rangasamy; Vadakkenchery Salimudheen Hansiya; Palanisamy Uma Maheswari; Thamburaj Suman; Natesan Geetha. Phytochemical Analysis and Evaluation of In vitro Antioxidant and Anti-urolithiatic Potential of various fractions of Clitoria ternatea L. Blue Flowered Leaves. Asian J Pharm Ana. 2019; 9(2): 67-76.
11. Liu H xia, Sun S qin, Lv G hua, Chan KKC. Study on Angelica and its different extracts by Fourier transform infrared spectroscopy and two-dimensional correlation IR spectroscopy. Spectrochim Acta A Mol Biomol Spectrosc. 2006; 64(2): 321-326. doi:10.1016/j.saa.2005.07.026
12. Thaipong K, Boonprakob U, Crosby K, Cisneros-Zevallos L, Hawkins Byrne D. Comparison of ABTS, DPPH, FRAP, and ORAC assays for estimating antioxidant activity from guava fruit extracts. Journal of Food Composition and Analysis. 2006; 19(6-7): 669-675. doi:10.1016/j.jfca.2006.01.003
13. Imene Guediri; Chérifa Boubekri; Ouanissa Smara; Touhami Lanez. Total phenolic contents and determination of Antioxidant activity by DPPH, FRAP, and cyclic voltammetry of the fruit of Solanum nigrum (black nightshade) growing in the south of Algeria. Asian J Research Chem. 2021;14(1).
14. Benzie IFF, Devaki M. The ferric reducing/antioxidant power (FRAP) assay for non•)enzymatic antioxidant capacity: concepts, procedures, limitations and applications. In: Apak R, Capanoglu E, Shahidi F, eds. Measurement of Antioxidant Activity and Capacity: Recent Trends and Applications. Wiley Published. 2018.
15. Abayomi Sofowora. Medicinal Plants and Traditional Medicinal in Africa. 2nd ed. Spectrum Books Ltd, 1993.
16. William Charles Evans. Pharmacognosy. 9th ed. Saunders Ltd.; 2009.
17. Ermi Abriyani; Lia Fikayuniar. Screening Phytochemical, Antioxidant Activity and Vitamin C Assay from Bungo perak-perak (Begonia versicolar Irmsch) leaves. Asian J Pharm Res . 2020;10(3).
18. Rangarajan Narasimhan; Sathiyamoorthy. M. Phytochemical Screening and Antioxidant Studies in the Pulp Extracts of Cucurbita maxima. Asian J Pharm Res. 2016; 6(1).
19. Tuan Putra TNM, Zainol MK, Mohdisa NS, Mohdmaidin N. Chemical characterization of ethanolic extract of butterfly pea flower (Clitoria ternatea). Food Res. 2021;5(4):127-134. doi:10.26656/fr.2017.5(4).744
20. Lee J, Durst RW, Wrolstad RE, et al. Determination of Total Monomeric Anthocyanin Pigment Content of Fruit Juices, Beverages, Natural Colorants, and Wines by the PH Differential Method: Collaborative Study.; 2001.
21. Benzie IFF, Strain JJ. The Ferric Reducing Ability of Plasma (FRAP) as a Measure of "Antioxidant Power": The FRAP Assay. Anal Biochem. 1996;239(1):70-76. doi:10.1006/abio.1996.0292
22. Arnao MB, Cano A, Acosta M. Analytical, Nutritional and Clinical Methods Section The hydrophilic and lipophilic contribution to total antioxidant activity. Food Chem. 2001;73:239-244.
23. Cahyaningsih E, Yuda PESK, Santoso P. Skrining Fitokimia dan Uji Aktivitas Antioksidan Ekstrak Etanol Bunga Telang (Clitoria ternatea L.) dengan Metode Spektrofotometri UV-VIS. Jurnal Ilmiah Medicamento. 2019;5(1). doi:10.36733/medicamento.v5i1.851
24. Widiyantoro A, Susanti R, Hadari Nawawi JH. Karakterisasi Senyawa Flavonoid Dari Fraksi Etil Asetat Bunga Nusa Indah (Mussaenda erythrophylla) Dan Aktivitas Sitotoksik Terhadap Sel Kanker Payudara T47D. 2017;6(2):83-88.
25. Hardeli, Zainul R, Isara LP. Preparation of Dye Sensitized Solar Cell (DSSC) using anthocyanin color dyes from jengkol shell (Pithecellobium lobatum Benth.) by the gallate acid copigmentation. In: Journal of Physics: Conference Series. Vol 1185. Institute of Physics Publishing; 2019. doi:10.1088/1742-6596/1185/1/012021
26. Pękal A, Pyrzynska K. Evaluation of Aluminium Complexation Reaction for Flavonoid Content Assay. Food Anal Methods. 2014;7(9):1776-1782. doi:10.1007/s12161-014-9814-x
27. Harborne JB, Sudiro I, Padmawinata K, Niksolihin S. Metode Fitokimia : Penuntun Cara Modern Menganalisis Tumbuhan. (Sofia Niksolihin, ed.). Penerbit ITB; 1996.
28. Chang CC, Yang MH, Wen HM, Chern JC. Estimation of total flavonoid content in propolis by two complementary colometric methods. J Food Drug Anal. 2020; 10(3). doi:10.38212/2224-6614.2748
29. Mursyidi A. Analisis Metabolit Sekunder. Universitas Gadjah Mada; 1990.
30. Muthusamy Senthil Kumar; Srinivasan Balachandran; Shibani Chaudhury. Influence of Incubation Temperatures on Total Phenolic, Flavonoids Content and Free Radical Scavenging Activity of Callus from Heliotropium indicum L. Asian J Pharm Res. 2012; 2(4).
31. Mahboubi M, Kazempour N, Boland Nazar AR. Total Phenolic, Total Flavonoids, Antioxidant and Antimicrobial Activities of Scrophularia Striata Boiss Extracts. Jundishapur Journal of Natural Pharmaceutical Product. 2013; 8(1).
32. Preeti Tiwari. Phenolics and Flavonoids and Antioxidant Potential of Balarishta Prepared by Traditional and Modern Methods. Asian J Pharm Ana. 2014; 4(1).
33. Preeti Tiwari; Rakesh K. Patel. Estimation of Total Phenolics and Flavonoids and Antioxidant Potential of Ashwagandharishta Prepared by Traditional and Modern Methods. Asian J Pharm Ana. 2013; 3(4): 147-152.
34. Anggraeni VJ, Ramdanawati L, Ayuantika W. Penetapan Kadar Antosianin Total Beras Merah (Oryza nivara). Jurnal Kartika Kimia. 2018; 1(1). doi:10.26874/jkk.v1i1.11
35. Handayani H, Sriherfyna FH, Yunianta. Antioxidant Extraction of Soursop Leaf with Ultrasonic Bath (Study of Material: Solvent Ratio and Extraction Time. Jurnal Pangan dan Agroindustri . 2016; 4(1): 262-272.
36. Boligon AA. Technical Evaluation of Antioxidant Activity. Med Chem (Los Angeles). 2014; 4(7). doi:10.4172/2161-0444.1000188
37. Ou B, Huang D, Hampsch-Woodill M, Flanagan JA, Deemer EK. Analysis of Antioxidant Activities of Common Vegetables Employing Oxygen Radical Absorbance Capacity (ORAC) and Ferric Reducing Antioxidant Power (FRAP) Assays: A Comparative Study. J Agric Food Chem. 2002; 50(11): 3122-3128. doi:10.1021/jf0116606
38. Gordon MH. Measuring antioxidant activity. In: Pokorny J, Yanishlieva N, Gordon M, eds. Antioxidant in Food: Practical Applications. Woodhead Publishing Ltd and CRC Press LLC; 2001.
39. Shriner RL, Hermann CKF, Morrill TC, Curtin DY, Fuson RC. The Systematic Identification of Organic Compounds, 8th Edition. 8th ed. Wiley Publisher; 2003.
40. Munteanu IG, Apetrei C. Analytical Methods Used in Determining Antioxidant Activity: A Review. Int J Mol Sci. 2021; 22(7): 3380. doi:10.3390/ijms22073380
41. Herlina Nasir N, Artikel I, Pusmarani J. Uji Aktivitas Antioksidan Ekstrak Metanolik Daging Buah Semangka (Citrullus lanatus (Thunb.) Matsum. & Nakai) dengan Metode ABTS dan FRAP. Jurnal Mandala Pharmacon Indonesia. 2021;7(2). doi:10.35311/jmpi
42. Cahyono B, Prihatini CS, Suzery M, Bima DN. Penentuan Aktivitas Antioksidan Senyawa Kuersetin dan Ekstrak Lengkuas Menggunakan HPLC dan UV-Vis. ALCHEMY. 2021; 8(2): 24-32. doi:10.18860/al.v8i2.10594
43. Atuani MT, Sudewi S, Wewengkang DS. ANALISIS FRAKSI Aktif Ekstrak Daun Gedi Hijau (Abelmoschus manihot L.) Dalam Menangkal Radikal bebas Dpph. Pharmacon. 2019; 8(1): 187. doi:10.35799/pha.8.2019.29253
44. Zheng L, Zhao M, Xiao C, Zhao Q, Su G. Practical problems when using ABTS assay to assess the radical-scavenging activity of peptides: Importance of controlling reaction pH and time. Food Chem. 2016; 192: 288-294. doi:10.1016/j.foodchem.2015.07.015
45. Bodet C, La VD, Epifano F, Grenier D. Naringenin has anti-inflammatory properties in macrophage and ex vivo human whole-blood models. J Periodontal Res. 2008; 43(4): 400-407. doi:10.1111/j.1600-0765.2007.01055.x
46. Kashyap P, Shikha D, Thakur M, Aneja A. Functionality of apigenin as a potent antioxidant with emphasis on bioavailability, metabolism, action mechanism and in vitro and in vivo studies: A review. J Food Biochem. 2022; 46(4). doi:10.1111/jfbc.13950
47. Atmira Sariwati. Bioassay Bioaktivitas Metabolisme Sekunder Tumbuhan. Insan Cendekia Mandiri.; 2021.
48. Tian X, Schaich KM. Effects of Molecular Structure on Kinetics and Dynamics of the Trolox Equivalent Antioxidant Capacity Assay with ABTS. J Agric Food Chem. 2013; 61(23): 5511-5519. doi:10.1021/jf4010725
49. Schaich KM, Tian X, Xie J. Hurdles and pitfalls in measuring antioxidant efficacy: A critical evaluation of ABTS, DPPH, and ORAC assays. J Funct Foods. 2015; 14: 111-125. doi:10.1016/j.jff.2015.01.043
50. Kavya Kulkarni; Govindaiah. Evaluation of Anti-oxidant properties of some medicinal plant products by ABTS Radical Scavenging Assay. Research Journal of Science and Technology. 2022; 14(4).
51. Molyneux P. The use of the stable free radical diphenylpicryl hydrazyl (DPPH) for estimating antioxidant activity. Songklanakarin J Sci Technol. 2004;26(2).

Butterfly Pea (Clitoria ternatea L.) Flower Water and Ethanol Extract: Phytochemical Screening, FTIR Analysis, and Antioxidant Activity Estimation using comparison of ABTS, DPPH, and FRAP Assays

ORIGINALITY REPORT

19%	17%	10%	%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	www.rjptonline.org Internet Source	7%
2	perpustakaan.poltekkes-malang.ac.id Internet Source	1%
3	storage.googleapis.com Internet Source	1%
4	pmc.ncbi.nlm.nih.gov Internet Source	<1%
5	www.tjnpr.org Internet Source	<1%
6	www.frontiersin.org Internet Source	<1%
7	jurnalfkip.unram.ac.id Internet Source	<1%
8	old.vniipp.ru Internet Source	<1%
9	aggie-horticulture.tamu.edu Internet Source	<1%
10	www.myfoodresearch.com Internet Source	<1%
11	www.contaipolytechnic.com Internet Source	<1%
12	www.scribd.com Internet Source	

		<1 %
13	ljkzedo.ba Internet Source	<1 %
14	journal.umpr.ac.id Internet Source	<1 %
15	www.pgia.pdn.ac.lk Internet Source	<1 %
16	orcid.org Internet Source	<1 %
17	A.J. Afolayan, F.O. Jimoh, M.O. Sofidiya, S. Koduru, F.B. Lewu. " Medicinal Potential of the Root of . ", Pharmaceutical Biology, 2008 Publication	<1 %
18	portal.arid.my Internet Source	<1 %
19	jurnal.fkip.untad.ac.id Internet Source	<1 %
20	Iris F.F. Benzie, Malegaddi Devaki. "The ferric reducing/antioxidant power (FRAP) assay for non-enzymatic antioxidant capacity: concepts, procedures, limitations and applications", Wiley, 2017 Publication	<1 %
21	Jérôme Govin, Cécile Caron, Emmanuelle Escoffier, Myriam Ferro et al. "Post-meiotic Shifts in HSPA2/HSP70.2 Chaperone Activity during Mouse Spermatogenesis", Journal of Biological Chemistry, 2006 Publication	<1 %
22	journal.walisongo.ac.id Internet Source	<1 %
23	uad.portalgaruda.org Internet Source	<1 %

24	www.cambridge.org Internet Source	<1%
25	Mahinda Abeywardena. "Polyphenol-enriched extract of oil palm fronds (Elaeis guineensis) promotes vascular relaxation via endothelium-dependent mechanisms", Asia Pacific Journal of Clinical Nutrition, 10/2002 Publication	<1%
26	Nurmahani MohdMaidin, Maria Jose Oruna-Concha, Paula Jauregi. "Surfactant TWEEN20 provides stabilisation effect on anthocyanins extracted from red grape pomace", Food Chemistry, 2019 Publication	<1%
27	Mortada El-Sayed, Maher El-Hashash, Heba Mohamed, Ezzat Abdel-Lateef. "Phytochemical Investigation and in vitro Antioxidant Activity of Different Leaf Extracts of Salix mucronata Thunb.", Journal of Applied Pharmaceutical Science, 2015 Publication	<1%
28	link.springer.com Internet Source	<1%
29	repository.ub.ac.id Internet Source	<1%
30	research.library.mun.ca Internet Source	<1%
31	www.pvj.com.pk Internet Source	<1%
32	Erkan, N.. "Antioxidant activities of rosemary (Rosmarinus Officinalis L.) extract, blackseed (Nigella sativa L.) essential oil, carnosic acid, rosmarinic acid and sesamol", Food Chemistry, 20080901 Publication	<1%

33	Hasyrul Hamzah, Sylvia Utami Tunjung Pratiwi, Asriullah Jabbar, Aldyba Syaqilla Hafifah et al. "Bioactivity Tracing of the Ethanol Extract of Bajakah Tampala (Spatholobus littoralis Hassk.) Typical Plant of Kalimantan Island as Antibiofilm of Staphylococcus aureus", Open Access Macedonian Journal of Medical Sciences, 2023 Publication	<1 %
34	Hrachya G. Hovhannisyan, Lusine Danielyan, Naira Chichoyan, Maria Pashayan et al. "Improvement of functional and sensory properties of fermented dairy drink Narine using raw apricot gum", Functional Foods in Health and Disease, 2024 Publication	<1 %
35	Mijat Božović, Dragana Petrović, Danka Caković, Milan Mladenović, Rino Ragno. "Phenolic Content and Antioxidant Potential of Wine Extracts", Natural Product Communications, 2025 Publication	<1 %
36	Nishigandha Sa, Khider Alkhayer, Anindita Behera. "Efficient removal of environmental pollutants by green synthesized metal nanoparticles of Clitoria ternatea", Heliyon, 2024 Publication	<1 %
37	Ousman Brahim Mahamat, Fadoua Asraoui, Ayoub Farihi, Mohamed Bouhrim et al. " Phytochemical analysis and antioxidant activity of A. Juss from the Republic of Chad: and studies ", Open Chemistry, 2025 Publication	<1 %
38	Zhao, H.. "Evaluation of antioxidant activities and total phenolic contents of typical malting	<1 %

barley varieties", Food Chemistry, 20080301
Publication

39	Zheng, Lin, Mouming Zhao, Chuqiao Xiao, Qiangzhong Zhao, and Guowan Su. "Practical problems when using ABTS assay to assess the radical-scavenging activity of peptides: Importance of controlling reaction pH and time", Food Chemistry, 2016. Publication	<1 %
40	interesjournals.org Internet Source	<1 %
41	jurnal.uii.ac.id Internet Source	<1 %
42	mail.qascf.com Internet Source	<1 %
43	repositorio.ucv.edu.pe Internet Source	<1 %
44	Akshani Anjula Wijesooriya, Srianthie A. Deraniyagala, Chamari M. Hettiarachchi. "Antioxidant, Anti-Inflammatory and Antibacterial Activities of the Seeds of A Sri Lankan Variety of Carica Papaya", Biomedical and Pharmacology Journal, 2019 Publication	<1 %
45	Antonio Cano, Marino B. Arnao. "ABTS/TEAC (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)/Trolox®-Equivalent Antioxidant Capacity) radical scavenging mixed-mode assay", Wiley, 2017 Publication	<1 %
46	Nisha, P.. "A comparative study on antioxidant activities of different varieties of Solanum melongena", Food and Chemical Toxicology, 200910 Publication	<1 %

47	Xinyue Cao, Li Li, Yiwen Liu, Xianjiang Zhou, Ran Qiu, Shihui Dai, Fengqing Wang, Xuyan Zong. "NIRS combined with chemometrics for non-destructive quality monitoring of components during soymilk boiling process", LWT, 2025 Publication	<1 %
48	ejournal.uin-malang.ac.id Internet Source	<1 %
49	jmp.ir Internet Source	<1 %
50	jrespharm.com Internet Source	<1 %
51	lib.uky.edu Internet Source	<1 %
52	mdpi-res.com Internet Source	<1 %
53	mediatum.ub.tum.de Internet Source	<1 %
54	psecommunity.org Internet Source	<1 %
55	thefutureoffoodjournal.com Internet Source	<1 %
56	www.agro.cmu.ac.th Internet Source	<1 %
57	www.mdpi.com Internet Source	<1 %
58	Ji-Yun Bae, Sung-Sil Hong, Mi-Ja Kim. "Antioxidant activity and anti-adipogenic effect of ethyl acetate fraction of cumin seeds on 3T3-L1 adipocytes differentiation", Food Science and Biotechnology, 2024 Publication	<1 %

59	Ni Ketut Esati, Elisabeth Oriana Jawa La, Ni Putu Sudiasih, Ni Nyoman Dina Saniasih. "Total Flavonoid Levels in n-hexane and Ethyl Acetate Fractions of Rosmarinus officinalis L. Leaves and Their Antibacterial and Antioxidant Activities", Borneo Journal of Pharmacy, 2024 Publication	<1%
60	Johnson, SS, FT Oyelola, T Ari, and H Juho. "*In vitro* inhibitory activities of the extract of *Hibiscus sabdariffa* L. (family malvaceae) on selected cytochrome p450 isoforms", African Journal of Traditional Complementary and Alternative Medicines, 2013. Publication	<1%

Exclude quotes	On	Exclude matches	Off
Exclude bibliography	On		