Indonesian Journal of Chemical Science and Technology (IJCST)

State University of Medan, https://jurnal.unimed.ac.id/2012/index.php/aromatika

IJCST-UNIMED 2026, Vol. 09, No. 2, Page; 387 – 400

Received : Jun 20th, 2026 Accepted : Jul 23rd, 2026 Web Published : Jul 31st, 2026

Phytochemical Screening and Antioxidant Activity of Ethanolic Extracts of Young and Mature Butterfly Pea (*Clitoria ternatea*) Leaves Using the DPPH Method

Rudi Munzirwan Siregar, Amelia Dwi Putri*, Tita Juwitaningsih, Murniaty Simorangkir, Ahmad Nasir Pulungan

Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Negeri Medan, Medan 20221, Indonesia

**Corresponding author : ameliadwiputri752@gmail.com*

ABSTRACT

Butterfly pea leaves (Clitoria ternatea) are one part of the plant that contains various secondary metabolites and have the potential to serve as a natural antioxidant source. This study aimed to determine the phytochemical content and compare the antioxidant activity of ethanolic extracts of young and mature butterfly pea leaves using the DPPH (2,2-diphenyl-1-picrylhydrazyl) method. The samples were extracted by maceration using 96% ethanol, followed by qualitative phytochemical screening and antioxidant activity testing at concentrations of 20, 40, 60, and 80 µg/mL. The results showed that both extracts were positive for phenolics, flavonoids, tannins, alkaloids, saponins, and steroids, but negative for triterpenoids. The IC_{50} values of young leaf extract, mature leaf extract, and vitamin C were 80.408, 58.023, and 5.655 µg/mL, respectively. Based on the IC_{50} values, both extracts were classified as strong antioxidants, while vitamin C was classified as a very strong antioxidant. The mature butterfly pea leaf extract showed higher antioxidant activity than the young leaf extract, indicating that leaf maturity affects the antioxidant potential of butterfly pea leaves.

Keywords: antioxidant activity, butterfly pea leaves, *Clitoria ternatea*, DPPH, phytochemical screening

1. INTRODUCTION

Free radicals are reactive molecules or atoms that contain one or more unpaired electrons and can react easily with biological components in the body. Excessive free radical formation may cause oxidative stress, a condition that occurs when the production of free radicals exceeds the capacity of the antioxidant defense system. Oxidative stress can damage cellular components such as lipids, proteins, and DNA, and is associated with the development of various degenerative diseases.[1,2] Antioxidants play an important role in preventing oxidative damage by donating electrons or hydrogen atoms to stabilize reactive molecules.[3] Therefore, antioxidant compounds are needed to maintain redox balance and protect the body from cellular damage caused by free radicals.

e-ISSN : 2622-4968, p-ISSN : 2622-1349

The human body naturally produces endogenous antioxidants, such as glutathione, superoxide dismutase, and catalase. However, these antioxidant defense systems are often insufficient under conditions of physiological stress, environmental exposure, and increased radical production.[3] For this reason, exogenous antioxidants from food or medicinal plants are needed to support the body's antioxidant defense. Synthetic antioxidants, such as butylated hydroxytoluene and butylated hydroxyanisole, are commonly used to inhibit oxidation, but their long-term use has raised safety concerns.[4] This has encouraged the exploration of natural antioxidants from plants because they are considered safer, more accessible, and environmentally friendly.

Butterfly pea (*Clitoria ternatea*) is a plant from the Fabaceae family that grows widely in tropical regions and has been used in agriculture, traditional medicine, and food applications.[5] This plant is commonly known for its flowers, which contain anthocyanin pigments and are widely used as natural colorants in food and beverages.[6] However, not only the flowers but also the leaves of *C. ternatea* contain various bioactive compounds. Previous phytochemical studies reported that butterfly pea leaves contain secondary metabolites such as phenolics, flavonoids, tannins, alkaloids, saponins, and steroids.[7] These compounds are known to contribute to antioxidant activity, especially phenolic and flavonoid compounds, which can scavenge free radicals through electron or hydrogen donation mechanisms. In addition, *C. ternatea* extract has been reported to show antioxidant potential, supporting its possible development as a natural antioxidant source.[8]

The content of secondary metabolites in plants can be influenced by genetic factors, environmental conditions, and physiological changes in plant organs. Leaves are metabolically active organs, and their maturity level may affect the accumulation of bioactive compounds. Young and mature leaves may have different phytochemical compositions because they undergo different physiological and metabolic processes during plant growth.[9] Several studies have shown that leaf age or maturity can influence phenolic content and antioxidant activity in plant leaves. However, information regarding the comparison of antioxidant activity between young and mature *C. ternatea* leaves is still limited. Therefore, it is important to conduct phytochemical screening and antioxidant activity testing on butterfly pea leaves at different maturity levels.

The DPPH (2,2-diphenyl-1-picrylhydrazyl) method is one of the most commonly used methods to evaluate antioxidant activity in plant extracts because it is simple, rapid, and effective for measuring free radical scavenging ability.[10],[11] In this method, antioxidant activity is indicated by the reduction of DPPH radicals, which causes a decrease in absorbance and a color change from purple to yellow. Based on this background, this study aimed to identify the phytochemical compounds and compare the antioxidant activity of ethanolic extracts of young and mature butterfly pea leaves using the DPPH method. It was expected that young and mature leaves would show different antioxidant activities due to differences in secondary metabolite accumulation. The results of this study are expected to provide scientific information on the antioxidant potential of *C. ternatea* leaves based on leaf maturity level.

2. EXPERIMENTAL

2.1. Chemicals, Equipment and Instrumentation

This research was conducted at the Research Laboratory, Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Negeri Medan, from February to June 2026. The samples used in this study were young and mature butterfly pea (*Clitoria ternatea*) leaves obtained from Titi Kuning, Medan Johor District, Medan City, North Sumatra. Young leaves were collected from the shoot area or leaves that were light green and had a softer texture, while mature leaves were collected from the middle to lower parts of the plant, fully developed, darker green in color, and firmer in texture. The chemicals used included 96% ethanol (C_2H_5OH), methanol p.a. (CH_3OH), DPPH powder (2,2-diphenyl-1-picrylhydrazyl) Merck, vitamin C ($C_6H_8O_6$), distilled water (H_2O), $FeCl_3$ solution 10 mg/mL, magnesium powder (Mg), concentrated hydrochloric acid (HCl), 1 N hydrochloric acid, NaCl solution 20 mg/mL, gelatin solution 10 mg/mL, chloroform, ammonia, concentrated sulfuric acid (H_2SO_4), glacial acetic acid (CH_3COOH), Mayer reagent, and Dragendorff reagent.

The equipment used in this study included an analytical balance (Fujitsu FSR-A320), covered glass jars, beakers, measuring cylinders, stirring rods, glass funnels, Whatman No. 1 filter paper, volumetric flasks, blender, measuring pipettes, glass bottles, micropipettes, dropper pipettes, vortex, vials, 40-mesh sieve, stopwatch, rotary evaporator, and UV-Vis spectrophotometer. The UV-Vis spectrophotometer was used to measure the absorbance of DPPH solution after reaction with the sample solution and reference solution.

e-ISSN : 2622-4968, p-ISSN : 2622-1349

2.2. Research Procedure

2.2.1. Preparation of Young and Mature Butterfly Pea (Clitoria ternatea) Leaf Samples

Young and mature butterfly pea leaves, each weighing 2 kg, were collected and subjected to wet sorting to remove damaged, wilted, or unsuitable leaves. The sorted samples were washed under running water to remove dirt, dust, and soil residues attached to the leaf surface. The leaves were then drained until no water remained on the surface.

The cleaned young and mature butterfly pea leaves were cut into small pieces to allow a more uniform drying process. Drying was carried out in a shaded place with good air circulation and without direct sunlight until the leaves became dry and easily crushed. The dried samples were ground using a blender to obtain powder, then sieved using a 40-mesh sieve to obtain uniform particle size. The simplicia powder obtained was stored in a tightly closed container and placed in a cool area protected from light before being used for the extraction process.[12]

2.2.2. Extraction of Young and Mature Butterfly Pea (Clitoria ternatea) Leaves

The extraction of young and mature butterfly pea leaves was carried out using the maceration method with 96% ethanol as the solvent. A total of 300 g of young butterfly pea leaf simplicia powder and 300 g of mature butterfly pea leaf simplicia powder were each placed into tightly closed glass containers. Then, 96% ethanol was added with a simplicia-to-solvent ratio of 1:3. The soaking process was carried out for 3 × 24 hours at room temperature with occasional stirring to optimize the diffusion of active compounds into the solvent.

After maceration was completed, the mixture was filtered using Whatman No. 1 filter paper to separate the filtrate and residue. The residue was then remacerated using fresh solvent with the same volume to extract the remaining active compounds. The filtrates from maceration and remaceration were combined and concentrated using a rotary evaporator at 40°C and 60 rpm until a thick extract was obtained. The ethanolic extracts of young and mature butterfly pea leaves were stored in tightly closed amber bottles at low temperature before being used for phytochemical screening and antioxidant activity testing.[13]

2.2.3. Phytochemical Screening of Ethanolic Butterfly Pea Leaf Extracts

Phytochemical screening was carried out qualitatively to identify the presence of secondary metabolites in the ethanolic extracts of young and mature butterfly pea leaves, including phenolics, flavonoids, tannins, alkaloids, saponins, steroids, and triterpenoids.[14]

a). Phenolic Test

A total of 40 mg of ethanolic butterfly pea leaf extract was added with 10 drops of $FeCl_3$ solution 10 mg/mL. The sample was considered positive for phenolic compounds if the color changed to green, blue, purple, red, or dark black.

b). Flavonoid Test

A total of 40 mg of ethanolic butterfly pea leaf extract was added with 100 mL of hot water, heated for 5 minutes, and filtered. Then, 5 mL of the filtrate was taken and added with 50 mg of magnesium powder and 1 mL of concentrated HCl, followed by vigorous shaking. The sample was considered positive for flavonoids if a yellow, orange, or red color was formed.

c). Tannin Test

A total of 40 mg of ethanolic butterfly pea leaf extract was added with 1 mL of NaCl solution 20 mg/mL and 5 mL of gelatin solution 10 mg/mL. The sample was considered positive for tannins if a precipitate was formed.

d). Alkaloid Test

A total of 40 mg of ethanolic butterfly pea leaf extract was added with 2 mL of chloroform and several drops of ammonia, then filtered. The filtrate was added with 3–5 drops of concentrated H_2SO_4 and shaken until two layers were formed. The acid layer was collected and separately added with 4–5 drops of Mayer and Dragendorff reagents. The sample was considered positive for alkaloids if a white precipitate was formed with Mayer reagent and a reddish-yellow precipitate was formed with Dragendorff reagent.

e). Saponin Test

e-ISSN : 2622-4968, p-ISSN : 2622-1349

A total of 40 mg of ethanolic butterfly pea leaf extract was added with 10 mL of water and shaken for 1 minute. Then, 2 drops of 1 N HCl were added. The sample was considered positive for saponins if stable foam was formed and persisted for approximately 7 minutes.

f). Steroid and Triterpenoid Test

A total of 40 mg of ethanolic butterfly pea leaf extract was added with 10 drops of glacial acetic acid and 2 drops of concentrated H_2SO_4. The sample was considered positive for steroids if the color changed to green or blue, while triterpenoids were indicated by the formation of a red or purple color.

2.2.4. Preparation of DPPH Solution

The DPPH solution was prepared by weighing 15.77 mg of DPPH powder, then dissolving it in methanol p.a. in a 100 mL volumetric flask until homogeneous. The resulting solution had a concentration of 0.4 mM. The DPPH solution was stored at low temperature and protected from light to prevent degradation before being used in antioxidant activity testing.[15]

2.2.5. Preparation of Sample Solutions and Vitamin C Reference Solution

The sample solutions were prepared from ethanolic extracts of young and mature butterfly pea leaves. Each extract was weighed as much as 5 mg, dissolved in methanol p.a., and placed into a 50 mL volumetric flask. Methanol p.a. was then added up to the mark and the solution was homogenized. The stock solution obtained was further diluted into several concentration variations, namely 20, 40, 60, and 80 µg/mL. All sample solutions were stored under light-protected conditions until used in antioxidant activity testing.[16]

The reference solution was prepared using vitamin C. A total of 50 mg of vitamin C was dissolved in 50 mL of distilled water until completely dissolved to obtain a stock solution with a concentration of 1000 ppm. From this stock solution, 10, 20, 30, and 40 µL were respectively pipetted into 5 mL volumetric flasks and diluted with distilled water up to the mark. The vitamin C standard solutions obtained had concentrations of 2, 4, 6, and 8 ppm. The vitamin C solutions were stored under light-protected conditions before being used as the reference in antioxidant activity testing.[16]

2.2.6. Determination of Maximum Wavelength and Operating Time of DPPH

The maximum wavelength of DPPH was determined by mixing 4 mL of 0.4 mM DPPH solution with 2 mL of methanol p.a. The solution was homogenized and incubated for 30 minutes at room temperature under light-protected conditions. After incubation, the absorbance of the solution was measured using a UV-Vis spectrophotometer in the wavelength range of 400–800 nm. The wavelength showing the highest absorbance peak was determined as the maximum wavelength and used as the working wavelength in antioxidant activity testing.[17]

The operating time was determined to identify the optimum reaction time between DPPH solution and antioxidant compounds. A total of 4 mL of 0.4 mM DPPH solution was mixed with 50 µL of vitamin C standard solution and homogenized using a vortex for 1 minute. The mixture was allowed to react at room temperature under light-protected conditions. Absorbance was measured every 5 minutes for 60 minutes at the maximum wavelength until a stable absorbance value was obtained.[16]

2.2.7. Antioxidant Activity Test Using the DPPH Method

Blank absorbance measurement was carried out by pipetting 1 mL of 0.4 mM DPPH solution, then adding ethanol p.a. up to a final volume of 5 mL in a volumetric flask. The mixture was homogenized and allowed to stand for 30 minutes under light-protected conditions. The blank absorbance was then measured at the maximum wavelength of DPPH.

The antioxidant activity test of vitamin C was carried out by mixing 4 mL of vitamin C solution at concentrations of 2, 4, 6, and 8 ppm with 1 mL of 0.4 mM DPPH solution. The mixture was homogenized and incubated for 30 minutes in the dark. After incubation, the absorbance of the solution was measured using a UV-Vis spectrophotometer at the maximum wavelength.

e-ISSN : 2622-4968, p-ISSN : 2622-1349

The antioxidant activity test of ethanolic extracts of young and mature butterfly pea leaves was carried out by taking 4 mL of sample solution from each test concentration, namely 20, 40, 60, and 80 µg/mL. Each sample solution was mixed with 1 mL of 0.4 mM DPPH solution and homogenized thoroughly. The mixture was incubated for 30 minutes under light-protected conditions to allow the reaction between antioxidant compounds in the extract and DPPH radicals. After incubation, the absorbance of the solution was measured using a UV-Vis spectrophotometer at the maximum wavelength.[16]

2.2.8. Data Analysis

Antioxidant activity was determined based on the percentage inhibition of DPPH radicals at each concentration of sample solution and reference solution. The percentage inhibition was calculated using the following formula:

$$\%Inhibition = \frac{\text{absorbance blank} - \text{absorbance sample}}{\text{absorbance blank}} x\ 100\ \%$$

where blank absorbance is the absorbance of DPPH solution without sample, while sample absorbance is the absorbance of DPPH solution after the addition of sample solution or reference solution.

The IC_{50} value was determined using a linear regression equation between the logarithm of sample concentration and percentage inhibition with the equation y = bx + a. The value of y = 50% was substituted into the regression equation to obtain the sample concentration required to inhibit 50% of DPPH free radicals. A lower IC_{50} value indicates stronger antioxidant activity. The IC_{50} values of ethanolic extracts of young and mature butterfly pea leaves were then compared with vitamin C as the reference standard.[18]

3. RESULTS AND DISCUSSION

3.1. Preparation and Extraction of Young and Mature Butterfly Pea Leaves

Sample preparation was carried out to obtain dried simplicia powder of young and mature butterfly pea (*Clitoria ternatea*) leaves for the extraction process. The young and mature butterfly pea leaves were collected from Titi Kuning, Medan Johor District, Medan City, North Sumatra. Young leaves were collected from the shoot area and were characterized by a light green color and softer texture, whereas mature leaves were collected from the middle to lower parts of the plant and were characterized by a darker green color, fully developed leaf structure, and firmer texture. Differences in leaf maturity may influence the content of secondary metabolites because young and mature leaves are at different physiological stages.[19]

The collected young and mature leaves were sorted, washed, cut into small pieces, dried, ground, and sieved using a 40-mesh sieve. Drying was conducted to reduce the moisture content of the samples, prevent microbial growth, and maintain the quality of the simplicia.[20] Grinding and sieving were performed to obtain a more uniform particle size. Smaller particle size can increase the contact surface area between the simplicia and the solvent, thereby improving the extraction of active compounds.[21] From 2 kg of young butterfly pea leaves, 317 g of simplicia powder was obtained, while 338 g of simplicia powder was obtained from 2 kg of mature butterfly pea leaves.

Extraction was carried out using the maceration method with 96% ethanol as the solvent. Maceration was selected because it is a simple extraction method and does not involve high heating, thereby minimizing the degradation of thermolabile secondary metabolites. Ethanol 96% was used because it can dissolve various secondary metabolites, including polar and semi-polar compounds, and has good penetration ability into plant cell walls.[22] A total of 300 g of young leaf simplicia powder and 300 g of mature leaf simplicia powder were each macerated using 96% ethanol with a simplicia-to-solvent ratio of 1:3. The filtrates obtained from maceration and remaceration were combined and concentrated using a rotary evaporator at 40°C and 60 rpm until thick extracts were obtained.[13]

The extraction results showed that the young butterfly pea leaf extract produced 19.8 g of thick extract with a yield of 6.6%, while the mature butterfly pea leaf extract produced 17.7 g of thick extract with a yield of 5.9%. The extraction yields of young and mature butterfly pea leaves are presented in Table 1.

e-ISSN : 2622-4968, p-ISSN : 2622-1349

Table 1. Yield of ethanolic extracts of young and mature butterfly pea leaves

No	Sample	Simplicia weight	Thick extract weight	Yield
1.	Young butterfly pea leaves	300 g	19.8 g	6.6%
2.	Mature butterfly pea leaves	300 g	17.7 g	5.9%

Based on Table 1, the yield of young butterfly pea leaf extract was higher than that of mature butterfly pea leaf extract. This difference may be caused by the softer tissue structure of young leaves, which allows the solvent to penetrate the cell matrix more easily and extract the compounds contained inside the cells. In contrast, mature leaves have thicker and firmer tissue structures, which may limit solvent penetration into the cells.[23] However, extraction yield is not always directly proportional to antioxidant activity, because yield only indicates the total amount of extract obtained, whereas antioxidant activity is more strongly influenced by the type and concentration of active compounds present in the extract.

3.2. Phytochemical Screening of Ethanolic Extracts of Butterfly Pea Leaves

Phytochemical screening was conducted to identify the secondary metabolites present in the ethanolic extracts of young and mature butterfly pea leaves. The screening included tests for phenolics, flavonoids, tannins, alkaloids, saponins, steroids, and triterpenoids. The presence of secondary metabolites was indicated by color changes, precipitate formation, or foam formation after the addition of specific reagents.[14] The phytochemical screening results of the ethanolic extracts of young and mature butterfly pea leaves are presented in Table 2.

Table 2. Phytochemical screening results of ethanolic extracts of young and mature butterfly pea leaves

No.	Test	Reagent	Young leaves	Mature leaves	Observation
1.	Phenolics	$FeCl_3$	+	+	Green solution
2.	Flavonoids	Mg + concentrated HCl	+	+	Orange solution
3.	Tannins	NaCl + gelatin	+	+	Blackish-green color
4.	Alkaloids	Mayer and Dragendorff	+	+	White and reddish-brown precipitates
5.	Saponins	Water + HCl 1 N	+	+	Stable foam for ±7 minutes
6.	Steroids	Glacial CH_3COOH + concentrated H_2SO_4	+	+	Green solution
7.	Triterpenoids	Glacial CH_3COOH + concentrated H_2SO_4	-	-	No red or purple color formed

Note: (+) = detected; (−) = not detected.

Based on Table 2, both young and mature butterfly pea leaf extracts were positive for phenolics, flavonoids, tannins, alkaloids, saponins, and steroids, but negative for triterpenoids. These results indicate that both maturity levels contain relatively similar groups of secondary metabolites. Differences in leaf maturity did not eliminate certain metabolite groups, but may influence the amount or level of active compounds accumulated in the extract.

The positive result for phenolics was indicated by the formation of a green color after the addition of $FeCl_3$ reagent. This color change occurs because Fe^{3+} ions react with hydroxyl groups in phenolic compounds, forming colored complexes. Phenolic compounds play an important role as antioxidants because they can donate electrons or hydrogen atoms to stabilize free radicals.[3]

The flavonoid test showed positive results in both extracts, indicated by the formation of an orange color after the addition of magnesium powder and concentrated HCl. The orange color indicates the reduction reaction of flavonoid compounds by magnesium under acidic conditions. Flavonoids are a group of phenolic compounds that contribute to antioxidant activity through electron donation and hydrogen atom donation mechanisms.[35]

Tannins were also detected in both young and mature leaf extracts. Tannins are polyphenolic compounds that contain several hydroxyl groups in their chemical structures, allowing them to contribute to free radical scavenging activity. In addition to phenolics, flavonoids, and tannins, both extracts also showed positive results for alkaloids, saponins, and steroids. Alkaloids are nitrogen-containing secondary metabolites with various biological activities. Saponins were indicated by stable foam formation due to their surface-active properties, while steroids were indicated by the formation of a green color after the addition of Liebermann–Burchard reagent.[14]

The negative result for triterpenoids indicates that triterpenoid compounds were not detected in the ethanolic extracts of either young or mature butterfly pea leaves using the qualitative method applied in this study. The absence of a positive reaction may be caused by the absence of triterpenoid compounds or by their concentration being too low to

e-ISSN : 2622-4968, p-ISSN : 2622-1349

produce a clear color change. Overall, the phytochemical screening results show that the ethanolic extracts of young and mature butterfly pea leaves contain secondary metabolites that may support antioxidant activity, particularly phenolics, flavonoids, and tannins.

3.3. Determination of Antioxidant Activity Using the DPPH Method

3.3.1. Maximum Wavelength of DPPH

The determination of the maximum wavelength was carried out to obtain the working wavelength that provides optimum absorbance for the DPPH solution. Measurement at the maximum wavelength is important because absorbance changes can be observed more clearly and the results become more accurate.[24] In addition, the use of the maximum wavelength increases analytical sensitivity because the solution gives the highest absorbance at that wavelength.[25]

Based on the spectral scanning of 0.4 mM DPPH solution in the wavelength range of 400–600 nm, the maximum wavelength was found at 517 nm with an absorbance value of 0.368. This result is consistent with the characteristics of DPPH, which generally shows optimum absorbance in the range of 515–520 nm when dissolved in organic solvents such as methanol or ethanol.[26]

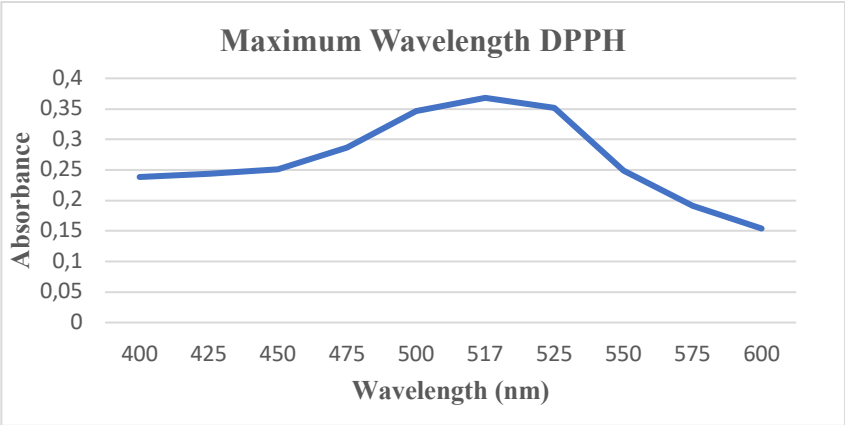

Figure 1. Maximum wavelength graph of DPPH

The maximum wavelength of 517 nm was then used as the working wavelength in the antioxidant activity assay. At this wavelength, the decrease in absorbance caused by the reaction between DPPH and antioxidant compounds can be measured more sensitively. A decrease in absorbance indicates that DPPH radicals are reduced into a more stable form after accepting hydrogen atoms or electrons from antioxidant compounds.

3.3.2. Operating Time of DPPH

The operating time was determined to identify the optimum reaction time required for DPPH radicals and antioxidant compounds to reach a stable reaction before absorbance measurement. Operating time indicates the period at which the reaction reaches a relatively stable condition, resulting in more consistent absorbance values.[27] This determination was based on absorbance changes at specific time intervals until relatively constant values were obtained.[28]

In this study, the operating time was determined using DPPH solution and vitamin C as a positive control at the maximum wavelength of 517 nm. Absorbance was observed every 5 minutes for 60 minutes. The results showed that the absorbance began to stabilize after 30 minutes of incubation. Therefore, an incubation time of 30 minutes was used in the antioxidant activity assay of young butterfly pea leaf extract, mature butterfly pea leaf extract, and vitamin C. The use of a 30-minute incubation time is also consistent with DPPH assays, which are commonly performed under light-protected conditions to ensure an optimum reaction.[29]

e-ISSN : 2622-4968, p-ISSN : 2622-1349

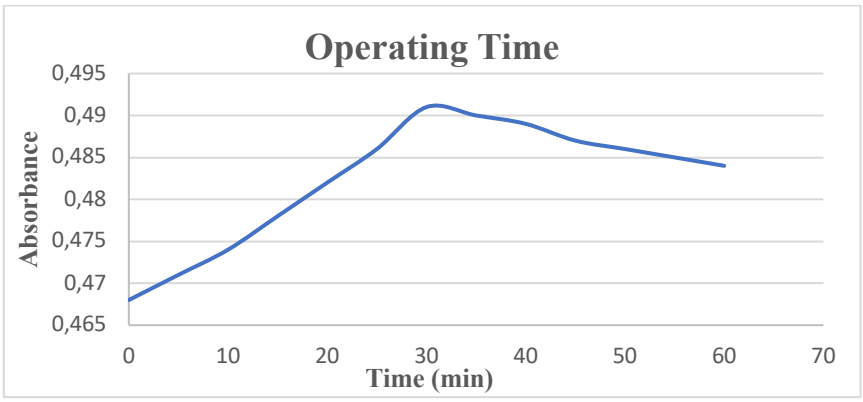

Figure 2. Operating time graph of DPPH

The stable absorbance value indicates that the reaction between DPPH radicals and antioxidant compounds had proceeded optimally. If measurement is carried out before the reaction stabilizes, the absorbance value may not accurately represent the maximum antioxidant capacity of the sample. Therefore, determining the operating time is an important step in antioxidant activity testing using the DPPH method.

3.3.3. Color Change of DPPH Solution after Sample Addition

The DPPH method was selected because it is simple, relatively rapid, requires only a small amount of sample, and can indicate antioxidant activity through color change and absorbance reduction.[30] DPPH is a stable free radical with a purple color due to the presence of an unpaired electron. After reacting with antioxidant compounds, DPPH accepts a hydrogen atom or electron and is reduced to DPPH-H, which has a paler color.[32,33]

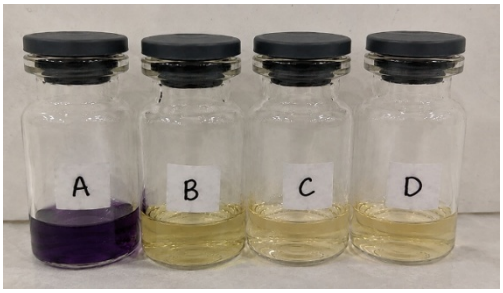

Figure 3. Color change of solutions: A = DPPH solution, B = vitamin C + DPPH solution, C = young butterfly pea leaf extract + DPPH solution, D = mature butterfly pea leaf extract + DPPH solution

Based on visual observation, the purple DPPH solution faded after the addition of vitamin C, young butterfly pea leaf extract, and mature butterfly pea leaf extract. This color change indicates a reaction between antioxidant compounds in the samples and DPPH radicals. The greater the fading of the solution color, the higher the ability of the sample to scavenge DPPH free radicals.[34]

The color change in the vitamin C solution was more apparent because vitamin C is a reference compound with very strong antioxidant activity. In the butterfly pea leaf extracts, the color did not completely turn pale yellow because the extracts still contained natural pigments. Nevertheless, the decrease in purple color intensity still indicates antioxidant activity in both young and mature butterfly pea leaf extracts.

3.3.4. Relationship between Concentration and Absorbance

The antioxidant activity assay was performed on young and mature butterfly pea leaf extracts at concentrations of 20, 40, 60, and 80 µg/mL. Vitamin C was used as a reference standard at concentrations of 2, 4, 6, and 8 µg/mL. The results showed that increasing sample concentration caused a decrease in absorbance values. The relationship between

e-ISSN : 2622-4968, p-ISSN : 2622-1349

concentration and absorbance of young and mature butterfly pea leaf extracts is shown in Figure 4, while the relationship between concentration and absorbance of vitamin C is shown in Figure 5.

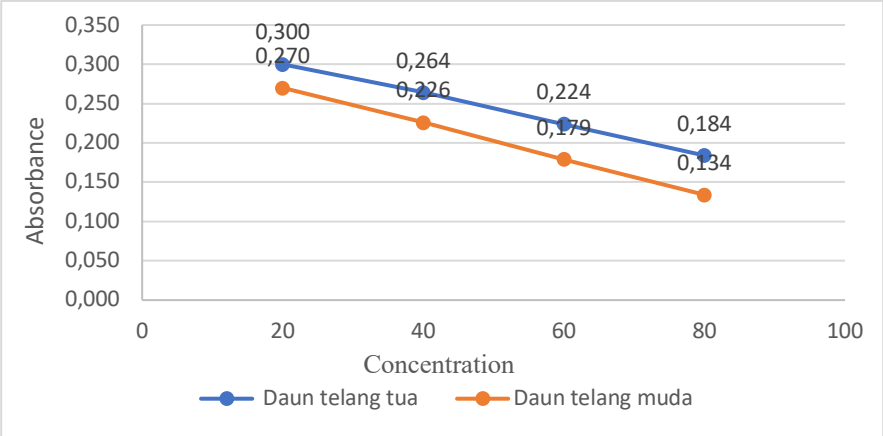

Figure 4. Graph of the relationship between concentration and absorbance of young and mature butterfly pea leaf extracts

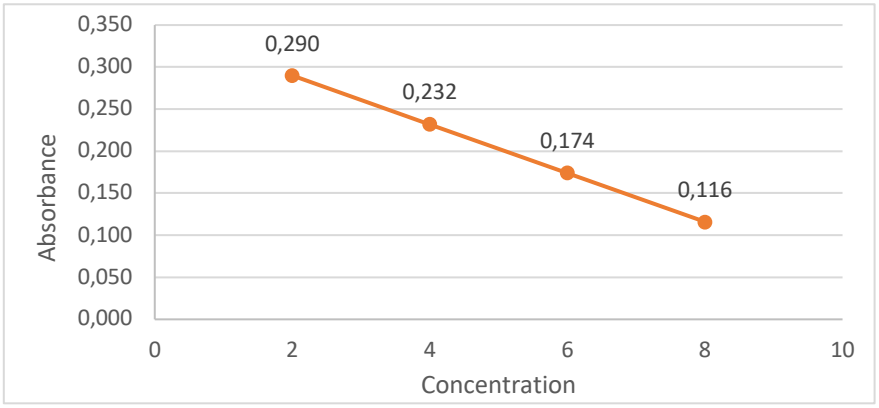

Figure 5. Graph of the relationship between concentration and absorbance of vitamin C

Based on Figure 4, the absorbance value of young butterfly pea leaf extract decreased from 0.300 at 20 µg/mL to 0.184 at 80 µg/mL. In mature butterfly pea leaf extract, the absorbance decreased from 0.270 at 20 µg/mL to 0.134 at 80 µg/mL. This decrease in absorbance indicates that higher extract concentrations provide more antioxidant compounds that can react with DPPH radicals.

In Figure 5, vitamin C also showed a decrease in absorbance with increasing concentration. The absorbance value of vitamin C decreased from 0.290 at 2 µg/mL to 0.116 at 8 µg/mL. The sharper decrease in vitamin C absorbance indicates that vitamin C has stronger DPPH radical scavenging ability than the butterfly pea leaf extracts.

The DPPH method has several limitations, including the sensitivity of DPPH to light and temperature; therefore, storage and testing should be conducted under light-protected conditions. In addition, DPPH is more soluble in organic solvents such as methanol or ethanol.[31] For this reason, in this study, the DPPH solution was kept under minimal light exposure to maintain its stability during the assay.

3.3.5. Inhibition Percentage and IC_{50} Value

Antioxidant activity was determined based on the percentage inhibition of DPPH radicals. The inhibition percentage represents the ability of the sample to scavenge free radicals. A higher inhibition percentage indicates a greater ability of the sample to neutralize DPPH radicals. The absorbance and inhibition percentage data of young butterfly pea leaf extract, mature butterfly pea leaf extract, and vitamin C are presented in Table 3.

e-ISSN : 2622-4968, p-ISSN : 2622-1349

Table 3. Percentage inhibition of DPPH radicals by young butterfly pea leaf extract, mature butterfly pea leaf extract, and vitamin C

Sample	Concentration (µg/mL)	absorbance			Mean absorbance	Blank absorbance	Inhibition (%)
		1	2	3			
Young butterfly pea leaves	20	0,301	0,298	0,300	0,300	0,368	18,478
	40	0,263	0,265	0,264	0,264		28,261
	60	0,225	0,222	0,224	0,224		39,130
	80	0,183	0,186	0,184	0,184		50,000
Mature butterfly pea leaves	20	0,271	0,269	0,270	0,270	0,368	26,630
	40	0,225	0,228	0,226	0,226		38,587
	60	0,180	0,178	0,179	0,179		51,359
	80	0,133	0,135	0,134	0,134		63,587
Vitamin C	2	0,291	0,289	0,290	0,290	0,368	21,195
	4	0,232	0,234	0,231	0,232		36,957
	6	0,173	0,176	0,174	0,174		52,717
	8	0,117	0,114	0,116	0,116		68,478

Based on Table 3, young butterfly pea leaf extract showed an increase in inhibition percentage from 18.478% at 20 µg/mL to 50.000% at 80 µg/mL. Mature butterfly pea leaf extract showed an increase from 26.630% at 20 µg/mL to 63.587% at 80 µg/mL. Meanwhile, vitamin C showed an increase in inhibition percentage from 21.195% at 2 µg/mL to 68.478% at 8 µg/mL.

The increase in inhibition percentage with increasing concentration indicates that antioxidant activity is concentration-dependent. As extract concentration increases, more antioxidant compounds are available to donate electrons or hydrogen atoms to DPPH radicals. As a result, the amount of remaining DPPH radicals decreases, leading to lower absorbance and higher inhibition percentage.[33]

Mature butterfly pea leaf extract produced higher inhibition percentages than young butterfly pea leaf extract at all tested concentrations. This indicates that mature leaf extract has stronger DPPH radical scavenging ability than young leaf extract. This difference is thought to be related to leaf maturity, which may affect the accumulation of bioactive compounds, particularly phenolics, flavonoids, and tannins.[35]

The inhibition percentage data were then used to construct linear regression graphs between concentration and inhibition percentage. The linear regression graph of antioxidant activity of young and mature butterfly pea leaf extracts is shown in Figure 6, while the linear regression graph of vitamin C antioxidant activity is shown in Figure 7.

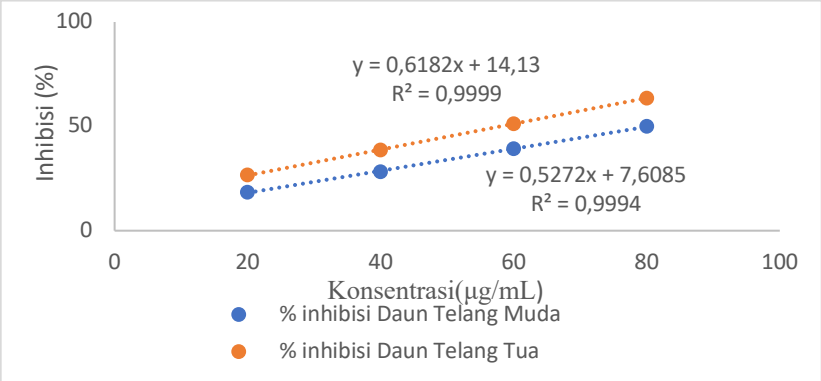

Figure 6. Linear regression graph of antioxidant activity of young and mature butterfly pea leaf extracts

e-ISSN : 2622-4968, p-ISSN : 2622-1349

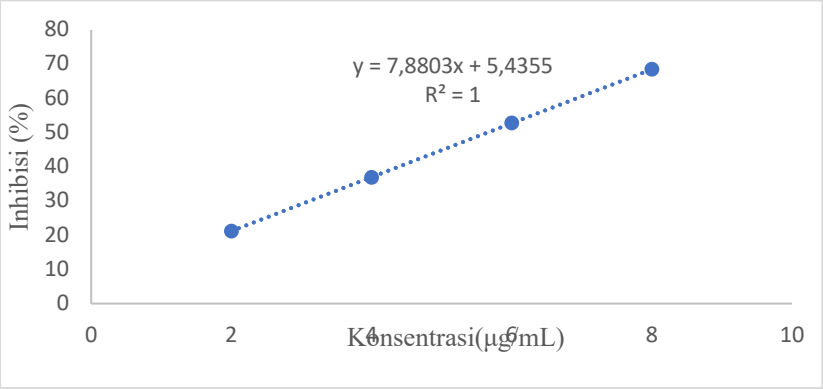

Figure 7. Linear regression graph of antioxidant activity of vitamin C

Based on Figure 6, the linear regression equation of mature butterfly pea leaf extract was y = 0.618215x + 14.13 with an R^2 value of 0.999855, while the young butterfly pea leaf extract showed the equation y = 0.527175x + 7.6085 with an R^2 value of 0.999363. The R^2 values close to 1 indicate a very strong linear relationship between sample concentration and inhibition percentage. This shows that increasing extract concentration contributes to increased DPPH radical scavenging activity.

Based on Figure 7, vitamin C showed the linear regression equation y = 7.8803x + 5.4355 with an R^2 value of 0.999999. This R^2 value indicates a very strong linear relationship between vitamin C concentration and inhibition percentage. The linear regression equation was used to determine the IC_{50} value, which represents the concentration required to inhibit 50% of DPPH radicals.

The IC_{50} values of young butterfly pea leaf extract, mature butterfly pea leaf extract, and vitamin C are presented in Table 4.

Table 4. IC_{50} values and antioxidant strength classification

Sample	Linear Regression Equation	IC_{50} (µg/mL)	Antioxidant strength
Young butterfly pea leaf extract	y = 0,5272x + 7,6085 R^2 = 0,9994	80,408	Strong
Mature butterfly pea leaf extract	y = 0,6182x + 14,13 R^2 = 0,9999	58,023	Strong
Vitamin C	y = 7,8803x + 5,4355 R^2 = 1	5,655	Very Strong

Antioxidant activity can be classified based on IC_{50} values. Samples with IC_{50} values below 50 µg/mL are classified as very strong antioxidants, 50–100 µg/mL as strong, 100–150 µg/mL as moderate, 150–200 µg/mL as weak, and above 200 µg/mL as very weak.[36] Based on Table 4, mature butterfly pea leaf extract had an IC_{50} value of 58.023 µg/mL, while young butterfly pea leaf extract had an IC_{50} value of 80.408 µg/mL. Both extracts were classified as strong antioxidants. Vitamin C as the reference standard had an IC_{50} value of 5.655 µg/mL and was classified as a very strong antioxidant.

Mature butterfly pea leaf extract showed higher antioxidant activity than young butterfly pea leaf extract because it had a lower IC_{50} value. A lower IC_{50} value indicates stronger antioxidant activity because a smaller concentration is required to inhibit 50% of free radicals.[37] These results show that mature butterfly pea leaves are more effective in scavenging DPPH free radicals than young leaves.

The ability of butterfly pea leaf extracts to scavenge free radicals is supported by the secondary metabolites identified in the phytochemical screening. Flavonoids act as antioxidants by releasing hydrogen atoms from phenolic hydroxyl groups to neutralize free radicals. After donating a hydrogen atom, flavonoids form more stable phenoxyl radicals due to electron delocalization in the aromatic ring.[38] This mechanism supports the ability of butterfly pea leaf extracts to reduce DPPH radicals.

e-ISSN : 2622-4968, p-ISSN : 2622-1349

In addition to flavonoids, tannins may also contribute to antioxidant activity because they are polyphenolic compounds containing hydroxyl groups on aromatic rings. These hydroxyl groups can donate hydrogen atoms to free radicals, converting them into more stable forms. The presence of phenolics, flavonoids, and tannins in butterfly pea leaf extracts is therefore considered a major factor supporting their antioxidant activity.

The difference in antioxidant activity between young and mature butterfly pea leaves indicates that leaf maturity can influence antioxidant potential. Mature leaves are thought to accumulate higher levels of bioactive compounds, particularly phenolics and flavonoids, because physiological maturity may represent an important stage for plants to store secondary metabolites as a defense mechanism against environmental stress.[39] Therefore, although young leaf extract produced a higher extraction yield, mature butterfly pea leaf extract showed stronger antioxidant activity based on its IC_{50} value.

Overall, the results show that the ethanolic extracts of young and mature butterfly pea leaves have strong antioxidant activity. Mature butterfly pea leaf extract showed higher antioxidant potential than young butterfly pea leaf extract, as indicated by its higher inhibition percentage and lower IC_{50} value. These findings indicate that leaf maturity influences the antioxidant potential of butterfly pea leaf extract.

4. CONCLUSION

Ethanolic extracts of young and mature butterfly pea (*Clitoria ternatea*) leaves contained phenolics, flavonoids, tannins, alkaloids, saponins, and steroids, but did not contain triterpenoids. Both extracts showed strong DPPH radical scavenging activity, with IC_{50} values of 80.408 µg/mL for young leaves and 58.023 µg/mL for mature leaves. Vitamin C showed very strong antioxidant activity with an IC_{50} value of 5.655 µg/mL. Mature leaves exhibited higher antioxidant activity than young leaves, indicating that leaf maturity influences the antioxidant potential of *C. ternatea* leaves.

ACKNOWLEDGEMENT

The authors would like to thank the Chemistry Laboratory, Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Negeri Medan, for providing laboratory facilities and technical support during the research. The authors also thank the laboratory staff and academic supervisors for their guidance and assistance throughout this study.

REFERENCES

1. Chaudhary, P., Janmeda, P., Docea, A. O., Yeskaliyeva, B., Abdull Razis, A. F., Modu, B., et al. (2023). Oxidative stress, free radicals and antioxidants: Potential crosstalk in the pathophysiology of human diseases. *Frontiers in Chemistry*, 11, 1158198.
2. Chandimali, N., Bak, S. G., Park, E. H., Lim, H. J., Won, Y. S., Kim, E. K., et al. (2025). Free radicals and their impact on health and antioxidant defenses: A review. *Cell Death Discovery*, 11(1), 19.
3. Pruteanu, L. L., Bailey, D. S., Grădinaru, A. C., & Jäntschi, L. (2023). The biochemistry and effectiveness of antioxidants in food, fruits, and marine algae. *Antioxidants*, 12(4), 860.
4. Shoker, R. M., Al-Shammery, W. H., & Al-Aidy, S. (2023). A review article: Free radical and replacement synthetic antioxidant by natural antioxidant. *Journal of Research in Applied Sciences and Biotechnology*, 2, 206–211.
5. Oguis, G. K., Gilding, E. K., Jackson, M. A., & Craik, D. J. (2019). Butterfly pea (*Clitoria ternatea*), a cyclotide-bearing plant with applications in agriculture and medicine. *Frontiers in Plant Science*, 10, 645.
6. Sholihatunnisa, K. F., & Ramadhania, Z. M. (2024). *Clitoria ternatea* L.: Senyawa bioaktif, aktivitas antioksidan, dan potensinya sebagai agen UV filter alami. *Indonesian Journal of Biological Pharmacy*, 4(2), 125–135.

e-ISSN : 2622-4968, p-ISSN : 2622-1349

7. Kavitha, R., & Premalakshmi, V. (2013). Phytochemical analysis of ethanolic extract of leaves of *Clitoria ternatea* L. *International Journal of Pharma and Bio Sciences*, 4(4), 236–242.
8. Purwanto, U. M. S., & Aprilia, K. (2022). Antioxidant activity of telang (*Clitoria ternatea* L.) extract in inhibiting lipid peroxidation. *Current Biochemistry*, 9(1), 26–37.
9. Singh, S., Singh, M., Bisht, S., & Sharma, J. G. (2022). Leaf senescence and its regulation with phytohormones and essential elements: An overview. *Journal of Applied Biology and Biotechnology*, 10(2), 185–197.
10. Devitria, R., Satria, D., & Anwar, H. (2020). Uji aktivitas antioksidan ekstrak metanol daun ciplukan (*Physalis angulata* L.) dengan metode DPPH. *Jurnal Pengkajian dan Penerapan Ilmu Farmasi Indonesia*, 2(1), 12–20.
11. Pambudi, D. B., Raharjo, D., & Fajriyah, N. N. (2021). Aktivitas antioksidan ekstrak daun kersen (*Muntingia calabura* L.) dengan menggunakan metode DPPH. In *Prosiding University Research Colloquium* (pp. 979–985).
12. Wijaya, A., dan Noviana, N. (2022). Penetapan Kadar Air Simplisia Daun Kemangi (*Ocimum basilicum* L.) Berdasarkan Perbedaan Metode Pengeringan. *Jurnal Riset Kefarmasian Indonesia*, 4(2), 185–194.
13. Nurjanah, F., Vifta, R. L., Suyudi, S. D., dan Aunila, F. T. (2025). Penentuan Kadar Fenolik Total Ekstrak Terpurifikasi Kopi Hijau Arabika (*Coffea arabica* L.) Asal Kabupaten Temanggung. *JurnalTech: Jurnal Ilmiah Multidisiplin Ilmu Pengetahuan*, 1(01), 23–31.
14. Qomariyah, A. A., dan Indrayudha, P. (2021). Antibacterial activity of combination of ethanol extract of peppermint leaves (*Mentha piperita*) and amikacin against *Klebsiella pneumoniae*, *Staphylococcus aureus*, and *Escherichia coli*. *Journal of Nutraceuticals and Herbal Medicine*, 4(1), 12–29.
15. Yahya, M. A., dan Nurrosyidah, I. H. (2020). Aktivitas antioksidan ekstrak etanol herba pegagan (*Centella asiatica* (L.) Urban) dengan metode DPPH (2,2-Difenil-1-Pikrilhidrazil). *Journal of Halal Product and Research*, 3(2), 106–112.
16. Kiromah, N. Z. W., Husein, S., dan Rahayu, T. P. (2021). Uji aktivitas antioksidan ekstrak etanol daun ganitri (*Elaeocarpus ganitrus* Roxb.) dengan metode DPPH (2,2-difenil-1-pikrilhidrazil). *Pharmacon: Jurnal Farmasi Indonesia*, 18(1), 60–66.
17. Wahyuni, R. S., Arel, A., dan Widya, N. (2024). Uji Aktivitas Antioksidan Ekstrak Akar Kayu Manis *Cinnamomum burmanii* (Nees dan T. Nees) Blume) Berdasarkan Tingkat Polaritas Pelarut. *Jurnal Penelitian Farmasi Indonesia*, 4(2), 1–7.
18. Rahmadhani, A., dan Hanwar, D. (2024). Optimasi ekstraksi antosianin daun jati (*Tectona grandis* Linn.) dan aktivitas antioksidannya. *BIOEDUSAINS: Jurnal Pendidikan Biologi dan Sains*, 7(2), 549–565.
19. Yusasrini, N. L. A., & Darmayanti, L. P. T. (2024). Karakteristik Kimia Ekstrak Etanol Daun Tenggulun (*Protium javanicum*) Berdasarkan Tingkat Ketuaan Daun. *Jurnal Teknologi Pertanian*, 25(1), 67–76.
20. Herawati, E., Wijayanti, E. T., & Budiretnani, D. A. (2025). Optimasi suhu dan waktu pengeringan simplisia daun telang sebagai kandidat antibakteri alami. *Judika (Jurnal Nusantara Medika)*, 9(1), 37–45.
21. Asworo, R. Y., & Widwiastuti, H. (2023). Pengaruh ukuran serbuk simplisia dan waktu maserasi terhadap aktivitas antioksidan ekstrak kulit sirsak. *Indonesian Journal of Pharmaceutical Education*, 3(2).
22. Vifta, R. L., & Susmayanti, W. (2023). Potensi antioksidan kombinasi ekstrak jahe merah (*Zingiber officinale* var. *rubrum*) dan bunga telang (*Clitoria ternatea* L.) dengan metode DPPH. *Journal of Holistics and Health Sciences*, 5(2), 385–394.
23. Krisyanella, K., Bestari, W. D., Irnameria, D., Susilo, A. I., & Meinisasti, R. (2025). Pengaruh Tingkat Kematangan Daun Terhadap Potensi Antioksidan Dari Ekstrak Etanol Daun Kersen (*Muntingia calabura* L.) Menggunakan Metoda DPPH (2,2-diphenyl-1-picrylhydrazlyl). *JAFP (Jurnal Akademi Farmasi Prayoga)*, 10(1), 1–10.
24. Wardani, A. D. (2022). Validasi metode dan penentuan kadar alkaloid total fraksi etil asetat daun sirsak (*Annona muricata* L.) secara spektrofotometri UV-Vis di Desa Kemiri Kabupaten Jember. Doctoral dissertation, Universitas dr. Soebandi.
25. Kameliani, D., Salamah, N., & Guntarti, A. (2020). Uji aktivitas antioksidan ekstrak ganggang hijau (*Ulva lactuca* L.) dengan variasi konsentrasi pelarut etanol 60%, 75%, dan 96% menggunakan metode DPPH (1,1-difenil-2-pikrilhidrazil). *JIIS (Jurnal Ilmiah Ibnu Sina): Ilmu Farmasi dan Kesehatan*, 5(2), 387–396.
26. Ramadhani, P. N., Rahmah, F., Salim, N. L., Rismawati, S., Mas'adah, N., Rina, R., & Ramadhani, P. A. (2025). Review Metode Uji Aktivitas Antioksidan dalam Riset Bahan Alam. *Journal of Literature Review*, 1(2), 501–519.
27. Suharyanto, S., & 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).

e-ISSN : 2622-4968, p-ISSN : 2622-1349

28. Harahap, A. T. A., & Ridwanto, R. (2024). Uji Aktivitas Antioksidan Ekstrak Etanol Daun Gandarusa (*Justicia gendarussa* Burm. F.) di Daerah Sibolga, Sumatera Utara dengan Metode DPPH (1,1-Diphenyl-2-Picrylhydrazil). *Journal of Health and Medical Science*, 3(1).
29. Hidayati, S., & Masykuroh, A. (2023). Uji Aktivitas Antioksidan Ekstrak Etanol Bunga Pulutan (*Urena lobata* L.) Menggunakan Metode DPPH. *Jurnal Komunitas Farmasi Nasional*, 3(1), 494–508.
30. Gani, A. P., Murwanti, R., Putri, D. D. P., & Sa'adah, M. (2022). Uji aktivitas penangkapan radikal 2,2-difenil-1-pikrilhidrazil (DPPH) ekstrak kering meniran (*Phyllanthus niruri* L.). *Majalah Farmaseutik*, 18(3), 300–306.
31. Gulcin, İ., & Alwasel, S. H. (2023). DPPH radical scavenging assay. *Processes*, 11(8), 2248.
32. Devitria, R. (2020). Uji Aktivitas Antioksidan Ekstrak Metanol Daun Ciplukan menggunakan Metode 2,2-Diphenyl-1-Picrylhydrazyl (DPPH). *Jurnal Penelitian Farmasi Indonesia*, 9(1), 31–36.
33. Ansyori, A. K., Tamrin, M., & Sa'adah, H. (2024). Uji aktivitas antioksidan ekstrak etanol biji buah nyirih (*Xylocarpus granatum*) dengan metode DPPH secara spektrofotometri UV-Vis. *Jurnal Riset Kefarmasian Indonesia*, 6(2), 233–248.
34. Telaumbanua, D. P. S., Londok, J. J. M. R., & Regar, M. N. (2026). Aktivitas antioksidan dan antibakteri daun kumis kucing (*Orthosiphon stamineus* Benth) sebagai kandidat feed additive pada ayam pedaging. *Zootec*, 46(1), 206–218.
35. Fernando, J., Yanuarto, T., & Herlina, H. (2022). Uji aktivitas antioksidan sediaan sirup bunga telang (*Clitoria ternatea* L.) dengan metode DPPH (1,1-diphenyl-2-picrylhydrazil). Doctoral dissertation, STIKES Al-Fatah Bengkulu.
36. Putri, A. L., Lubis, A. R., Dewi, A. F., & Octaviani, A. (2025). Perbedaan jenis pelarut terhadap aktivitas antioksidan ekstrak daun lamtoro (*Leucaena leucocephala* (Lam.) de Wit). *Lumbung Farmasi*, 6(1), 34–40.
37. Pratiwi, A., Razoki, R., Yunus, M., & Harahap, D. W. S. (2025). Uji aktivitas antioksidan ekstrak etanol daun pepaya (*Carica papaya* L.) dengan metode DPPH. *Jurnal Ilmu Kedokteran dan Kesehatan Indonesia*, 5(2), 257–264.
38. Kurniawati, I. F., & Sutoyo, S. (2021). Review Artikel: Potensi Bunga Tanaman Sukun (*Artocarpus altilis* [Park. I] Fosberg) Sebagai Bahan Antioksidan Alami. *Unesa Journal of Chemistry*, 10(1), 1–11.
39. Lekjing, S., Venkatachalam, K., Charoenphun, N., & Noonim, P. (2024). Effect of different drying methods on the phytochemical and antioxidant properties of soursop leaves at two stages of maturity. *ACS Omega*, 9(38), 40095–40109.

e-ISSN : 2622-4968, p-ISSN : 2622-1349