

Analysis of the Antioxidant Activity of Young and Mature Bidara Leaves Using 2,2-Diphenyl-1-Picrylhydrazyl (DPPH) and Phytochemical Screening Test

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Abstract

This study aimed to compare the antioxidant activity of young and mature *bidara* leaves using the DPPH method and to identify the content of secondary metabolites through phytochemical testing. Antioxidant activity was measured using the IC₅₀ parameter, which represents the concentration of the extract that can capture 50% of DPPH free radicals. The test results showed that mature *bidara* leaves had an IC₅₀ value of 34.984 ppm, indicating vigorous antioxidant activity, while young leaves had an IC₅₀ value of 100.327 ppm, classified as moderate activity. Phytochemical testing revealed that both types of leaves contained alkaloids, saponins, and tannins, while flavonoids and triterpenoids were only found in old leaves. These results suggest that variations in secondary metabolite content, influenced by leaf age, contribute to differences in antioxidant activity. Thus, older *bidara* leaves are more potent as an antioxidant source compared to younger leaves. This study supports the utilization of *bidara* plants in the pharmaceutical industry and the development of traditional medicine based on natural ingredients.

Keywords: Antioxidant; Bidara (*Ziziphus mauritiana*); DPPH; Phytochemical; Secondary metabolite.

INTRODUCTION

Indonesia has thousands of plant species spread across various regions. This biodiversity can be utilized as raw materials for modern and traditional medicines. Indonesian society has long been familiar with and used traditional medicine to treat various illnesses. The rising cost of modern medicine in the market is one reason for revisiting the use of conventional medicine. Many medicinal plants in Indonesia have been utilized as raw materials for medicinal purposes, and some of these plant species have undergone clinical testing to assess their phytochemical content, efficacy, and safety of use (Kusumawati, 2018).

Bidara leaves (*Ziziphus mauritiana*) have long been used in traditional medicine due to their various bioactive compounds. The bidara plant is more widely known for its health benefits, including anti-inflammatory, antibacterial, and antioxidant properties. The phytochemicals present in bidara leaves, including flavonoids, alkaloids, saponins, and tannins, play a crucial role in producing their pharmacological effects. Some phytochemical compounds that contribute to

pharmacological effects include flavonoids, which are believed to have antibacterial activity by disrupting protein bonds in bacterial cell membranes, and saponins, which can form complexes with bacterial proteins and cell walls, leading to cell wall damage (Ardinimia et al., 2023). Additionally, triterpenoids function as antioxidants due to their ability to scavenge DPPH radicals (Samirana et al., 2017).

The natural complex compounds in these plants can be used as free radical scavengers. These complex compounds are used without isolating specific components to obtain a single compound. All components in the plant are used to form complex compounds. According to Jayanti and Rahayu (2023), complex antioxidants have more electrons than single antioxidants, making them more efficient.

Antioxidants are compounds that, in specific amounts, can inhibit or counteract the adverse effects of oxidation. Natural antioxidants are often preferred over synthetic ones because some synthetic antioxidants, such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT), have recently been suspected of being carcinogenic (Kesuma, 2015).

The leaves and branches of the jujube (*Ziziphus jujuba*), synonymous with *Ziziphus mauritiana* Lamk, are known to contain various active compounds. Among others, 14 compounds, including coumarin, rutin, saponin, tannin, and tartaric acid, are found in the leaves. In comparison, three main compounds, namely betulinic acid, ceanothic acid, and leucopelargonidine, are found in the branches. Based on analysis using Duke's Phytochemical and Ethnobotanical Database, these compounds have antioxidant potential because their Pa values are higher than their Pi values, with coumarin and rutin from the leaves and alpha from the branches showing Pa values >0.7, which indicates vigorous antioxidant activity and is estimated to be not much different from laboratory test results. Additionally, the DPPH test also demonstrated antioxidant activity, as indicated by a change in the colour of the solution, with the combination of leaves and branches showing higher activity than the single samples (Nisa et al., 2023).

This study tested antioxidant activity using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method by spectrophotometry. DPPH is a relatively stable free radical compound. This compound is suitable for use as a reagent for testing compounds that have a free radical scavenging effect (antioxidant compounds). The parameter used for the DPPH radical scavenging test is IC₅₀, which is the concentration of the extract or test fraction required to capture 50% of the DPPH radicals (Taufik, 2016). A comparative test was also conducted, namely phytochemical screening, to determine the compound content in the sample.

Based on several studies conducted, *bidara* leaves are one of the plants tested for their effectiveness as an antibacterial agent. This is due to the content of several secondary metabolites in *bidara* leaves that can function as antibacterial agents. According to Muharrami et al. (2019), the results of phytochemical screening of *bidara* leaves extracted using the maceration method with 96% ethanol solvent revealed the presence of several secondary compounds, including phenolic compounds, tannins, and saponins. On the other hand, research by Ardinimia et al. (2023) explains that the flavonoids and saponins present in *Bidara* leaves exhibit significant antibacterial activity. However, this study did not further investigate the antioxidant activity or the potential for development as a raw material for phytopharmaceuticals.

The purpose of this study was to compare the antioxidant activity of young and mature *bidara* leaves using the DPPH method and to identify their secondary metabolites through phytochemical testing. Therefore, this study was conducted to support the updating of data that can be used for further studies related to the antioxidant activity found in old and young *bidara* leaves.

MATERIALS AND METHODS

Study area

This research was conducted from June to August 2025 at the Biochemistry Laboratory, Terpadu Laboratory, Universitas Islam Malang, East Java, Indonesia.

Procedures

Sample Preparation

Mature and young *bidara* leaves were obtained from the Al-Qur'an Garden of the Universitas Islam Malang. The initial process involved sorting old and young leaves, washing them, drying them in the sun, and then sorting them by hand. Then, the leaves were dried in an oven at 60°C until they were scorched. After drying, the samples were blended into a powder and sieved using a 40-mesh sieve to increase the surface area, thereby facilitating the extraction process (Wijaya & Noviana, 2022). Subsequently, the dry weight of the sample was measured, and the moisture content was calculated to ensure it was <10%.

Extraction

The *bidara* leaves were extracted using the maceration method. A total of 100 grams of crude drug was weighed and placed in a dark glass bottle (reagent bottle), then 700 mL of 96% pro-analytical ethanol was added. The mixture was homogenized repeatedly and left to stand for 3 days and 2 nights. The first maceration filtrate was then filtered. Next, 300 mL of 96% pro-analytical ethanol was added to the residue in the dark glass bottle from the first maceration, homogenized, and filtered again into an Erlenmeyer flask. The filtrates from the first and second macerations were combined and evaporated using a rotary evaporator at a speed of 30 rpm and a temperature of 65°C to produce a concentrated extract or paste (Salamah et al., 2024).

Yield Calculation

Yield is the ratio between the dry weight obtained and the weight of the raw material used. In extraction, yield is calculated by comparing the final weight of the extract obtained with the initial weight of the cell biomass used, then multiplying by 100% (i) (Mahyantika et al., 2025):

$$\text{Yield (\%)} = \frac{\text{weight of extract (g)}}{\text{weight of raw material (g)}} \times 100\% \quad (\text{i})$$

Antioxidant Testing Using the DPPH Method

- Determination of the Maximum Wavelength of 0.1 mM DPPH Solution

A 0.1 mM DPPH solution was prepared by weighing 2 mg of DPPH powder and dissolving it in pro-analysis ethanol in a 50 mL volumetric flask, bringing it up to the mark. Then, 3 mL of the DPPH solution was pipetted into a cuvette and measured using a UV-Vis spectrophotometer in the wavelength range of 400–600 nm. After the maximum wavelength was

determined, absorbance measurements were performed by mixing 3 mL of DPPH solution with 1 mL of analytical-grade ethanol, and absorbance was measured at the determined wavelength (Theafelicia & Narsito Wulan, 2023).

- Preparation of Standard Solution as Reference

The standard reference solution was prepared by weighing 1 mg of quercetin and dissolving it in pro-analysis ethanol in a 10 mL volumetric flask, bringing it up to the mark. The solution was homogenized using a vortex and then divided into several concentrations (3.13 ppm, 6.25 ppm, 12.5 ppm, 25 ppm, and 50 ppm). Each concentration is pipetted into a reaction tube and mixed with 3 mL of 0.1 mM DPPH solution. The mixture is vortexed again and incubated in the dark for 30 minutes, then measured using a UV-Visible spectrophotometer at a wavelength of 517 nm (Theafelicia & Narsito Wulan, 2023).

- Preparation of Test Solutions from Old and Young Bidara Leaf Extracts

Samples of old and young Bidara leaf extracts were prepared by weighing 10 mg of extract and dissolving it in pro-analysis ethanol in a 10 mL measuring flask, then bringing the volume up to the mark. The sample solution was homogenized using a vortex and then divided into several concentrations (20 ppm, 40 ppm, 60 ppm, 80 ppm, and 100 ppm). Each concentration was pipetted into a reaction tube and mixed with 3 mL of 0.1 mM DPPH solution. The mixture was vortexed again and incubated for 30 minutes, then measured using a UV-Vis spectrophotometer at a wavelength of 517 nm (Theafelicia & Wulan, 2023).

- DPPH Free Radical Scavenging Activity

Antioxidant activity was determined through inhibition values using a UV-Vis spectrophotometer. The absorbance data obtained were used to calculate the percentage of DPPH free radical inhibition. Antioxidant capacity in capturing free radicals was expressed using the following formula (ii) (Khalil et al., 2020):

$$\text{DPPH Free Radical Inhibition (\%)} = \frac{A_0 - A_1}{A_0} \times 100\% \quad (\text{ii})$$

Information:

A_0 = Control Absorbance

A_1 = Sample/Standard Absorbance

The control absorbance is the absorbance data of DPPH mixed with ethanol for analysis. In contrast, the sample/standard absorbance is the absorbance data of the standard/sample solution mixed with DPPH (Khalil et al., 2020).

Antioxidant Testing Using Phytochemical Screening Methods

The phytochemical screening test method refers to Ramadhan et al. (2019) conducted qualitatively with color tests using reagents to determine the secondary metabolites contained in plants:

- ***Alkaloid Test***

- a) Mayer's Test

The liquid extract of mature and young *bidara* leaves was placed in a test tube. Then, 4 drops of Mayer's reagent were added drop by drop into the test tube, and the tube was shaken gently. The formation of a white precipitate accompanied by a yellowish color change indicated a positive result for alkaloids.

- b) Wagner's Test

The liquid extract of mature and young *bidara* leaves was placed in a test tube. Then, 2 drops of Wagner's reagent were added drop by drop into the test tube, and the tube was shaken gently. The formation of a reddish-brown precipitate indicated a positive result for alkaloids.

- c) Dragendorff's Test

The liquid extract of mature and young *bidara* leaves was placed in a test tube. Then, 2 drops of Dragendorff's reagent were added drop by drop into the test tube, and the tube was shaken gently. The formation of an orange or yellow precipitate indicated a positive result for alkaloids.

- d) Bouchardat Test

The liquid extract of mature and young *bidara* leaves was placed in a test tube. Then, 2 drops of Bouchardat reagent were added drop by drop into the test tube, and the tube was shaken gently. The formation of a brown precipitate indicated a positive result for alkaloids.

- ***Flavonoid Test***

- a) Shinoda Test

The liquid extract was placed in a test tube. Then, 4 drops of absolute ethanol and 2 drops of concentrated hydrochloric acid were added to the test tube, and the mixture was shaken gently. A color change to red indicated the presence of aurone and chalcone. If no colour change occurred, a small spatula of magnesium was added to the test tube containing the extract, and the mixture was gently shaken. A color change from pink to red, such as orange, red, or magenta, indicated the presence of flavones and flavonols.

- b) 10% Sodium Hydroxide Test

The liquid extract of mature and young *bidara* leaves was placed in a test tube. Then, 2 drops of 10% NaOH solution were added into the test tube, and the mixture was shaken gently. The color changes to reddish yellow, dark orange, reddish purple, or blue indicate the presence of anthocyanins, flavones, flavonols, or chalcones.

▪ Saponin Test

a) Foam Test

The liquid extract of mature and young *bidara* leaves was placed in a test tube. Then, 10 mL of distilled water was added to the test tube, and the mixture was shaken continuously and gently. The formation of a stable foam layer about 1 cm high indicated a positive result for saponins.

▪ Tanin Test

a) Braymer's Test

The liquid extract of mature and young *bidara* leaves was placed in a test tube. Then, 2 drops of Braymer's reagent were added drop by drop into the test tube, and the tube was shaken gently. The formation of a greenish-black precipitate indicated a positive result for tannins.

b) Base Solution Test

The liquid extract of old and young *bidara* leaves was placed in a test tube. Then, 2 drops of 10% ammonium hydroxide solution were added to the test tube, and the mixture was shaken gently. The formation of a fluorescent yellow color indicated a positive result for tannins.

▪ Steroid/Triterpenoid Test

a) Salkowski Test

The liquid extract of mature and young *bidara* leaves was placed in a test tube. Then, two drops of Salkowski reagent were added drop by drop into the test tube, and the mixture was shaken gently. The formation of a brown ring in the center of the solution indicated the presence of steroids.

b) Lieberman Bourchard Test

The liquid extract of mature and young *bidara* leaves was placed in a test tube. Then, two drops of Lieberman-Bourchard reagent were added slowly to the test tube, and the mixture was shaken gently. After incubation for 5 minutes, the appearance of a blue-green color indicated the presence of sterols, while a pink to purplish-red color indicated the presence of terpenoids.

Data analysis

The research data were analyzed both descriptively and quantitatively, as well as qualitatively. Phytochemical screening activity was analyzed using tables and figures. In contrast, antioxidant activity was analyzed using Microsoft Excel with data presented in graphs and expressed as IC₅₀ values obtained from the linear regression equation $y = ax + b$ (Werdyani et al., 2019). The higher the IC₅₀ value, the lower the antioxidant activity of the sample; conversely, the lower the IC₅₀ value, the higher the antioxidant activity of the sample. Meanwhile, to compare the differences between the two treatment group means, a T-test was conducted using PAST 4.03.

RESULTS AND DISCUSSION

Quercetin Reference Solution Test

Table 1. Results of Quercetin Antioxidant Activity Measurements.

Concentration (ppm)	Absorbance	% Inhibition	IC ₅₀
3,125	0,731	28,655	
6,25	0,601	41,343	2,283
12,5	0,381	62,815	(Very Strong)
25	0,159	84,482	
50	0,023	97,755	

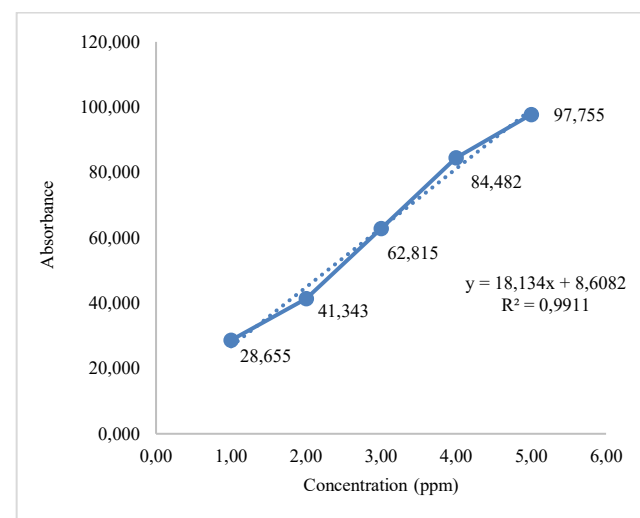


Figure 1. Linear regression curve of the antioxidant activity of quercetin reference solution.

Antioxidant Activity Test Using the DPPH Method

Table 2. Results of Antioxidant Activity Measurements of mature *Bidara* Leaf Samples.

Concentration (ppm)	Absorbance	% Inhibition	IC ₅₀
20	0,365	36,65	
40	0,268	53,52	34,984
60	0,161	72,02	(Very Strong)
80	0,092	84,06	
100	0,048	91,60	

Table 3. Results of Antioxidant Activity Measurements of Young *Bidara* Leaf Samples

Concentration (ppm)	Absorbance	% Inhibition	IC ₅₀
20	0,130	55,90	
40	0,076	74,22	
60	0,097	67,21	100,327
80	0,122	58,50	(Currently)
100	0,148	49,80	

T Test

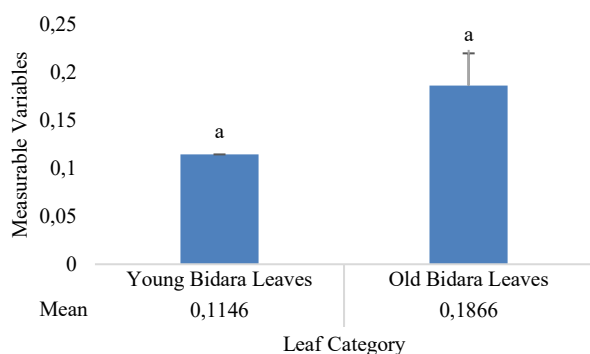


Figure 2. Results of statistical analysis comparing young bidara leaves (YBL) and mature bidara leaves (OBL).

Phytochemical Screening Test

Table 4. Chemical Compounds in Mature and Young Bidara Leaves.

Test	Reagents	mature Bidara Leaves	Young Bidara Leaves
Alkaloid	Mayer's	+	+
	Wagner's	+	+
	Dragendorff's	+	+
	Bouchardat	+	+
Flavonoid	Shinoda	-	-
	NaOH 10%	+	+
Saponin	Busa	+	+
Tanin	Braymer's	+	+
	Larutan Basa	+	+
Triterpenoid	Salkowski	-	-
	Lieberman bouchad	-	-

Discussion

The determination of antioxidant activity began with the preparation of extracts from mature and young jujube leaves (*Ziziphus mauritiana* L.) using pro-analytical ethanol as a solvent in the maceration method. Ethanol was chosen because it can extract both polar and non-polar soluble components, enabling the extraction of all chemical compounds present in jujube leaves (Dianda & Suharti, 2023). The extracts of mature and young jujube leaves obtained were then tested using the (DPPH) method with quercetin as the standard solution. The DPPH method was used because it is simple, fast, sensitive, and widely used to evaluate the ability of chemical compounds in samples to scavenge free radicals (Putri, 2023). Additionally, a phytochemical screening test was conducted to determine the content of compounds present in the old and young bidara leaves.

The antioxidant activity test of the reference solution was conducted at concentrations of 3.13 ppm, 6.25 ppm, 12.5 ppm, 25 ppm, and 50 ppm. Quercetin was reacted with the DPPH reagent and then measured for absorbance using a UV-Vis spectrophotometer at a wavelength of 517 nm. The absorbance values and

inhibition percentages of the standard solutions are presented in Table 1, demonstrating extreme free radical scavenging activity with an IC_{50} value of 2.283 ppm, which falls into the powerful category (<50 ppm). This is evidenced by the increase in inhibition percentage with increasing concentration, where at the lowest concentration of 3.125 ppm, it was already able to inhibit by 28.655%, and continued to increase to 97.755% at a concentration of 50 ppm. The inhibition percentage is a parameter reflecting the effectiveness of antioxidants in inhibiting free radicals. The parameter used to measure the antioxidant capacity of a compound is IC_{50} . The IC_{50} value indicates the concentration of the antioxidant compound required to capture 50% of DPPH free radicals (Pratiwi et al., 2023). The inhibition concentration was calculated using a linear regression equation to determine the relationship between concentration (ppm) (x) and absorbance (y) (Figure 1). From the curve, a linear regression equation, $y = 18.134x + 8.6082$, with an R^2 value of 0.9911, was obtained, indicating a robust and linear relationship between concentration and free radical inhibition activity.

Antioxidant activity testing of mature and young *bidara* leaves using the DPPH method yielded IC_{50} values of 34.984 for mature *bidara* leaves, indicating extreme antioxidant activity. In contrast, young *bidara* leaves had an IC_{50} value of 100.327, categorized as moderate. The smaller the IC_{50} value obtained, the higher the antioxidant activity of a compound, and conversely, the larger the IC_{50} value, the weaker the antioxidant activity (Putri, 2023). This is presented in Tables 2 and 3, which show a significant difference in antioxidant activity between mature and young *bidara* leaves, where mature *bidara* leaves exhibit a clear and consistent inhibitory pattern that increases with rising concentration.

Based on the graph, the average value of the variable measured in young bidara leaves (YBL) was 0.1146, and in old bidara leaves (OBL), it was 0.1866. Although the mean value of DBT is higher than that of DBM, the statistical test results indicate that there is no significant difference between the two ($p > 0.05$), as indicated by the same letter ("a") on both treatments. This indicates that the measured activity levels are relatively comparable between young and mature leaves. Variations may influence this difference in mean values in the content of secondary metabolites such as flavonoids, tannins, and saponins, which play a role in biological activity (Wulansari, 2022).

The phytochemical testing process in this study was conducted to determine the chemical compounds present in the water extract samples of *bidara* leaves. The phytochemical tests used included tests for alkaloids, flavonoids, tannins, saponins, and triterpenoids. The results of the phytochemical tests, as shown in Table 4, indicate that both young and mature *bidara* leaf extracts contain alkaloids, saponins, and tannins. However, flavonoids and triterpenoids were only detected in mature

leaves. This suggests that leaf maturity influences the content of secondary metabolites. These results are consistent with Wulansari's (2022) study, which reported that ethanol extracts of *bidara* leaves contain alkaloids, saponins, tannins, and flavonoids, but no triterpenoids were detected. These differences may be due to differences in leaf age, solvent, or extraction method.

The results of this study align with those of Hafiz et al. (2025) and Javed et al. (2022), which demonstrate that *bidara* leaf extract exhibits high antioxidant activity and contains several active compounds, including tannins, saponins, alkaloids, and flavonoids. Hafiz et al. (2025) explored three species of *Ziziphus* (*Ziziphus mauritiana*, *Ziziphus spina-christi*, and *Ziziphus jujuba*). They found that *Ziziphus mauritiana* leaves had the highest antioxidant capacity, as determined by DPPH assays, consistent with their high total phenolic content (6.534 g GAE/100 g) and total flavonoid content (2.025 g QE/100 g). This present study also identified the main flavonoid compounds, including rutin, quercetin, kaempferol, and apigenin, via HPLC, and demonstrated a strong correlation between flavonoid content and antioxidant activity. However, a fundamental difference from the study by Hafiz et al. (2025) is that their study compared antioxidant activity between species and plant parts (leaves, fruits, seeds), while this study focused on comparing antioxidant activity between old and young *bidara* leaves within a single species, *Ziziphus mauritiana*.

Meanwhile, the study conducted by Javed et al. (2022) on the phytochemistry and antioxidant activity of *Ziziphus mauritiana*, a species within the genus *Ziziphus*, revealed the presence of compounds such as tannins, saponins, alkaloids, and flavonoids in the methanol extracts of its leaves and fruits. Both studies support the idea that plants of the *Ziziphus* genus, including *Bidara*, are rich in secondary metabolites that function as both antioxidants and antimicrobials. Differences in antioxidant activity between different parts of the plant also indicate that the age and part of the plant used significantly influence their chemical composition and bioactive potential.

CONCLUSIONS

The extract of mature *bidara* leaves showed more potent antioxidant activity ($IC_{50} = 34.984$ ppm, powerful category) compared to young leaves ($IC_{50} = 100.327$ ppm, moderate category). This indicates that the maturity level of the leaves affects their antioxidant capacity. Phytochemical screening results also confirm that both young and old leaves contain alkaloids, tannins, and saponins, but flavonoids are only detected in old leaves. These differences in secondary metabolite content contribute to the higher antioxidant activity of old *bidara* leaves. Therefore, mature *bidara* leaves have greater

potential to be developed as a natural source of antioxidants compared to young leaves.

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