Antioxidant Activity of Mahogany Parasite Leaves (*Dendrophthu pentandra* (L.) Miq.) Assessed Using the DPPH (2,2-Diphenyl-1-Picrylhydrazyl) Method

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Abstract

Synthetic antioxidants, such as butyl hydroxyanisole (BHA), have carcinogenic (cancer-causing) adverse effects because they can cause type I alveolar damage and damage to lung endothelial genes. The objective of this study was to use the DPPH (2,2 diphenyl-1-picrylhydrazyl) method to investigate the antioxidant activity of natural substances found in the leaves of the mahogany parasite (*Dendrophthu pentandra* (L.) Miq.). Extraction, chromatography, and spectrophotometry are the techniques used. Maceration in stages utilizing n-hexane, ethyl acetate, and ethanol as solvents yielded mahogany parasite leaf extract. Phytochemical screening, thin layer chromatography testing, antioxidant activity testing utilizing a Visible Spectrophotometer, and estimating the IC₅₀ were all performed on mahogany parasite leaf extract. The phytochemical screening results revealed that the samples included four types of secondary metabolites: flavonoids, tannins, saponins, and steroids. A thin layer chromatographic test revealed four well-separated stains using n-hexane and ethyl acetate eluent in a volume ratio of 5:5, showing the RF as the value of the four secondary metabolites mentioned above. Based on the results of the experiments, it is possible to conclude that mahogany parasite leaves have strong antioxidant activity, with an IC₅₀ of 8.47 ppm.

Keywords: Free radicals, Antioxidants, Mahogany parasite leaves

1. Introduction

Reactive oxygen species such as hydrogen peroxide, superoxide, hydroxyl radicals, and other radical chemicals are among the causes of cancer, atherosclerosis, inflammation, and premature aging. This reactive oxygen or radical molecule can oxidize cells in the human body, causing disruption, aberrant growth, and disease [1]. To prevent this oxidation, a molecule called an antioxidant is required, which can protect cells against reactive chemicals [2].

Antioxidants are substances that can slow, postpone, or prevent oxidation or neutralize free radicals [2]. Antioxidants are electron donor molecules, according to the chemical idea. Antioxidants work by donating one electron to oxidants (compounds that easily oxidize) in order to decrease the activity of these oxidants. Antioxidants stabilize free radicals by supplying electrons that free radicals lack and stopping the chain reaction of free radical production [1]. Synthetic antioxidants, such as butyl hydroxyanisole (BHA), have carcinogenic (cancer-causing) adverse effects because they can cause type I alveolar damage and lung endothelial gene damage [3]. According to the World Health Organization (WHO), alternative traditional medicine is used by up to 80% of the population in Asia and Africa. The World Health Organization has also acknowledged that traditional medicine can cure a variety of acute, infectious, and chronic disorders [4]. Fresh guava leaves, for example, can be used to cure diarrhea, ginger can soothe coughs, and betel leaves help reduce nosebleeds [5].

Indonesia is rich in biodiversity, which has the potential to be produced as a medication or medical raw material with antioxidant properties and low cytotoxicity [6]. Plants from the Tabo-tabo forest in South Sulawesi have been used as
traditional medicine in previous investigations. The plant component in the form of leaves is used the most, with 23 species (56.09%), followed by the plant part in the form of fruit (29.26%), while sap is used the least [7]. Other research has used parasite leaves as medications to cure cancer, such as tea parasite leaves (Scurrula oortina), mango parasite (Dendrophthoe pentandra), and jackfruit parasite leaves (Mascrosolenocochinchinensis) [8].

Because it can attack both cultivated and wild plants, parasite is a parasitic plant. The parasite’s bioactivity is determined by the host plant [9]. Parasites are parasitic plants of the Loranthaceae family. Some authorities divide this tribe into two subfamilies, Loranthoideae and Viscoideae, while others break it into two independent tribes, Loranthaceae and Viscaceae. This group includes no fewer than 940 species from 70 families, with around 600 of them belonging to the Loranthus clan [9]. Mistletoe is a damaging plant in commercially significant horticulture crops. Parasite, on the other hand, is renowned as a medicinal plant used in traditional/alternative medicine in Indonesia and other countries, such as coughs, diabetes, hypertension, cancer, and diuretics[10].

Many previous researchers have tested the antioxidant activity of parasite leaves, including research on the antioxidant activity found in coffee parasite leaves in Bener Meniah, which shows that coffee parasite leaf extract has a relatively high level of antioxidants due to an IC₅₀ of 6.063 ppm [11]. Another investigation revealed that antioxidant properties of flavonoid components found in parasite, katuk, johar, and kajahoi leaves. He discovered that parasite leaves had the greatest antioxidant value of the four species of leaves, namely 30.31 ppm [12].

Mahogany plants are frequently encountered on the side of major roads as a road cover that has become overgrown with parasitic leaves. Dendrophthoe pentandra (L.) Miq. parasite leaves are a kind of Dendrophthoe pentandra (L.) Miq. According to research, no studies have been conducted that have resulted in the assessment of antioxidant activity in mahogany parasite leaves. Previous study investigated the metabolite chemicals contained in mahogany seeds, as well as other components of the mahogany plant. He concluded that mahogany seeds can lower MDA (malondialdehyde) levels in the blood[13]. Mahogany fruit can also be used as a malaria treatment, appetite stimulant, and other things. The bark of the mahogany plant can also be used to colour textiles, while the mahogany sap, known as blendok, is a raw ingredient for creating glue (adhesive), and the mahogany leaves are used for animal feed [13].

Saponins, flavonoids, and alkaloids are found in mahogany fruit [14]. Polyphenolic substances found in nature are included in flavonoids. Many oxidation reactions, both enzymatic and non-enzymatic, are inhibited by flavonoids [13]. Parasites, as is generally known, live as parasites on their host plants. Parasites consume all food sources from their hosts. Host plants with high antioxidant levels will be absorbed by the parasite, resulting in them having it as well [15]. As a result, there is a potential that mahogany parasite leaves contain flavonoid chemicals that act as antioxidants against free radicals. In light of the foregoing, the researcher wishes to undertake a study titled antioxidant activity test utilizing the DPPH method (2,2 diphenyl-1-picrylhydrazyl) on mahogany parasite leaves (Dendrophthoe pentandra (L.) Miq).

2. Materials and Methods
2.1. Materials

The materials used in this study were samples of mahogany parasite leaves, ethanol 96% (Technical), methanol 70% (Technical), N-hexane (Technical), Ethyl acetate (Technical), Vitamin C (Merck), DPPH (2,2 diphenyl-1-picrylhydrazyl) (Merck), HCl 37% (Merck), aquadest (Non brand), silica gel F₂₅₄ (Merck), FeCl₃ 1% (Merck), and H₂SO₄ 98% (Merck).

2.2. Methods
2.2.1 Sample Processing

Prior to maceration sample extraction, the leaves of the mahogany parasite were wet sorted and then air-dried at room temperature for seven days. Maceration takes place in a room shielded from direct sunlight to avoid damaging the secondary metabolite chemical compounds found in mahogany parasite leaves. The dried materials were cut into 3-5 cm pieces and crushed with an electronic grinder (blender) before being stored in a sealed container.

2.2.2 Sample Extraction

Extraction A multilayer maceration process was used to extract mahogany parasite leaves. Multilevel maceration is an extraction method that tries to extract all of the chemicals in mahogany parasite leaf samples based on the polarity of the solvent employed in stages [16]. Extraction A multilayer maceration process was used to extract mahogany parasite leaves. Multilevel maceration is an extraction method that tries to extract all of the chemicals in mahogany parasite leaf samples based on the polarity of the solvent employed in stages [16]. N-Hexane, Ethyl Acetate, and Ethanol were the solvents employed in this investigation. The first stage of maceration involves soaking 500 grams of refined simplicia in 1 liter of n-hexane solvent before carefully shutting and wrapping it in black plastic to protect it from light, air, and moisture. Soaking lasted three days, with stirring every 12 hours. The combination was then filtered with filter paper to remove the
dregs and filtrate of mahogany parasite leaves. To obtain concentrated n-hexane extract, the filtrate was concentrated using a rotary evaporator. The dregs from the previous step were re-added to the container for the second stage of maceration with ethyl acetate solvent, with the same soaking and stirring times as the first. A rotary evaporator was used to concentrate the filtrate, yielding a concentrated ethyl acetate extract. Furthermore, the acquired dregs were macerated in the third stage using ethanol solvent, yielding a concentrated ethanol extract.

2.2.3 Fractionation of Ethanol Extract

Using liquid column chromatography, up to 1 gram of sample extract was fractionated. F_{254} was compressed in a column tube filled with 10 grams of silica gel. 1 gram of silica gel F_{254} was added to the extract and agitated until homogenous. The homogenous extract sample was deposited in the liquid column on silica gel F254 and coated with Whatman filter paper. The mobile phase was poured in 50 ml increments of 100% n-hexane (F1), 100% ethyl acetate (F2), and ethanol 96% (F3) [17].

2.2.4 Phytochemical Screening

a. Flavonoids: 2 mL of each extract and fraction were placed in a test tube, followed by 0.1 gram of magnesium powder and 5 drops of strong hydrochloric acid (HCl). The solution is positive for flavonoid compounds if it turns blue, orange yellow, or red [18].

b. Tannins: 2 ml of each extract and fraction were placed in a test tube, followed by 5 drops of 1% FeCl3. If the solution turns greenish brown or blackish blue, it is positive for tannin compounds [18].

c. Saponins: 0.5 gram of sample is placed in a test tube and mixed with 10 ml of hot distilled water, which is then cooled and violently shaken for 10 seconds. If foam or foam is created at heights of 1-10 cm for up to 10 minutes, or when 1 drop of 37% hydrochloric acid (HCl) is applied, the sample is positive for saponins [18].

d. Terpenoid and steroid testing was performed by placing 2 ml of extract and sample fraction into a test tube and adding 3 drops of concentrated hydrochloric acid (HCl) and 1 drop of concentrated sulfuric acid (H2SO4). If the solution is crimson or purple, it contains terpenoids. If the solution turns green, it indicates positive for steroids [18].

2.2.5 Thin Layer Chromatographic Test

The ethanol extract of mahogany parasite leaves was diluted in methanol before being spotted on a thin layer chromatographic silica plate 1 cm from the plate's edge and eluted with n-hexane:ethyl acetate (5:5) as the eluent. Allow to stand for a few moments before viewing in visible and UV light. To establish the class of secondary metabolite chemicals, the Rf value of the stain spots on the TLC silica plate was computed. The Rf value is calculated using the formula stated in Equation (1) [19]:

\[
R_f = \frac{\text{The distance traveled by the substance}}{\text{The distance traveled by the solvent}}
\]  

2.2.6 Antioxidant Activity Test of Mahogany Parasite Ethanol Extract

a. Preparation of DPPH solution DPPH

b. DPPH was weighed to 10 milligrams using an analytical balance and then dissolved in a volumetric flask sealed in aluminum foil with 100 ml of methanol. The DPPH solution obtained had a concentration of 100 ppm, or 0.28 mM. Because light can harm DPPH, it is extremely sensitive to it. As a result, the DPPH solution must be stored in a volumetric flask that is shielded from light with aluminum foil [20].

c. Preparation of Mahogany Mistletoe Leaf Ethanol Extract Solution

d. The mahogany parasite leaf ethanol extract was weighed to 10 milligrams and then placed in a 100 ml volumetric flask with methanol poured to the mark. The sample solution has a concentration of 100 ppm.

e. Preparation of Vitamin C Solution

f. A 100 ppm stock solution was made by dissolving 10 mg of vitamin C in methanol in a 100 ml volumetric flask up to the mark.

2.2.7 Measurement of Antioxidant Activity with the DPPH

a. Wavelength Optimization

DPPH is a purple-colored antioxidant that is stable. Purple has a wavelength of 500-560 nm according to the complimentary color standard [21]. The maximum wavelength of a sample is determined through optimization. The wavelength of DPPH is 517 nm, according to the results.

b. Absorption of DPPH Solution

By taking 10, 20, 30, and 40 ml of 100 ppm solution and then adding methanol to the mark in a 50 ml volumetric flask, a 100 ppm solution was diluted into numerous concentration variations, namely 20, 40, 60, and 80 ppm. Variations in
the concentration of the DPPH solution were also monitored for its absorbance using the Visible Genesys-30 spectrophotometer at 517 nm [22].

c. Measurement of DPPH free radical scavenging activity with samples
The antioxidant activity of samples was tested using five different concentrations of ethanol extract of mahogany parasite leaves, namely 5, 10, 15, 20, and 25 ppm. Concentration series were made by taking 0.5 ml of 100 ppm mahogany parasite leaf ethanol extract solution, then adding 5.5 ml of methanol solvent; 5.0 ml, 4.5 ml, 4.0 ml, and 3.5 ml into each sample concentration series. Finally, each DPPH solution was added up to the 10 ml volumetric flask mark to the ethanol extract solution of mahogany parasite leaves. Every 20 minutes from the 0th to the 120th minute, antioxidant activity in the five concentration variations of the sample solution was measured at a maximum wavelength of 517 nm [22].

d. Measurement of the antioxidant activity of Vitamin C
Vitamin C antioxidant activity was also tested at five different concentrations, namely 5, 10, 15, 20, and 25 ppm. Similarly to the previous sample solution, 0.5 ml; 1.0 ml; 1.5 ml; 2.0 ml; and 2.5 ml of 100 ppm vitamin C solution were created, followed by 5.5 ml of methanol solvent; 5.0 ml; 4.5 ml; 4.0 ml; and 3.5 ml into each series of vitamin C concentrations. Then, up to the 10 ml volumetric flask mark, each DPPH solution was added to the vitamin C solution. Every 20 minutes from the 0th to the 120th minute, antioxidant activity in the five concentration series of vitamin C solutions was measured at a maximum wavelength of 517 nm.

2.3 Measurement of IC₅₀
The inhibitory activity of the data is tallied and determined. The data in this study were gathered from absorbance measurements of an ethanol extract of mahogany parasite leaves (Dendrophthu pentandra (L.) Miq.) using the Visible Genesis-30 spectrophotometer, and the data was analyzed using linear regression. Probit analysis of the data was used to estimate the IC₅₀ (50% Inhibitory Concentration). As stated in Equation (2), log concentration with probit free radical binding percentage [23].

\[
\text{% Inhibisi} = \frac{\text{Abs kontrol} - \text{Abs Sampel}}{\text{Absorbans kontrol}} \times 100\%
\]  

3. Result and Discussion
3.1. Sample Preparation
The materials utilized in this investigation were 4 kg of mahogany parasite leaves collected from mahogany trees in the Ar-Raniry State Islamic University setting. Mahogany parasite leaves are collected in the morning, sorted, and cleaned. Furthermore, the leaves of the mahogany parasite are dried for 7 days using the air drying method by putting them in an open room without direct sunlight. The dried mahogany parasite leaves are then cut into 3-5 cm tiny pieces and blended. A total of 640 grams of finely ground mahogany parasite leaves were collected.

3.2. Preparation of Mahogany Parasite Leaf Extract
In the first step of maceration, 1 liter of non-polar n-hexane solvent was employed. The filtrate was then concentrated using a rotary evaporator at 48°C to get 0.84 grams of concentrated n-hexane extract. The residual pulp from the leaves of the mahogany parasite was macerated with ethyl acetate, a semi-polar solvent, for the same amount of time and length as before. The results of the two-stage maceration and filtrate concentration with a rotary evaporator yielded 14.13 grams of ethyl acetate extract. 1 liter of polar ethanol solvent was employed in the final stage of multilayer maceration. Following the concentration of the filtrate, 43.85 grams of ethanol extract were produced.

<table>
<thead>
<tr>
<th>Table 1. Yield of macerated extract</th>
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<tbody>
<tr>
<td><strong>Extracts</strong></td>
</tr>
<tr>
<td>N-Hexane</td>
</tr>
<tr>
<td>Ethyl Acetate</td>
</tr>
<tr>
<td>Ethanol</td>
</tr>
</tbody>
</table>
3.3 Fractionation

Fractionation is a process of separating the content of one primary component in a simplicia from the content of another main compound [18]. The goal of fractionation is to be able to classify metabolites based on their solubility properties in order to enable chemical group identification. The liquid column chromatography method created three fractions that were consecutively flowed by three eluents/mobile phases from non-polar, semi-polar, and polar solvents. The mobile phases employed were 50 ml each of n-hexane, ethyl acetate, and ethanol. The N-hexane fraction, Ethyl acetate fraction, and Ethanol fraction are the end results.

3.4. Phytochemical Screening

A preliminary test for defining the class of secondary metabolites with biological activity from a plant is phytochemical screening. Previous study study shows only performed four types of secondary metabolite identification tests: flavonoids, saponins, tannins, and steroids/terpenoids [24].

<table>
<thead>
<tr>
<th>Samples</th>
<th>Flavonoid test</th>
<th>Tannin test</th>
<th>Saponin test</th>
<th>Steroid/Terpenoid test</th>
</tr>
</thead>
<tbody>
<tr>
<td>N-Hexane extract</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Ethyl acetate extract</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Ethanol extract</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>N-Hexane fraction</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
<td>Ethyl acetate fraction</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
<td>Ethanol fraction</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Note: + : Positive test; - : Negative test

3.5. Thin Layer Chromatography (TLC)

TLC is a technique for separating compounds based on differences in the distribution of two phases, namely the stationary phase (plate) and the mobile phase (eluent) [19]. The thin layer chromatography test was performed to corroborate the results of the phytochemical screening by comparing the hRf and Rf values standard to the color of the stains that emerged on the spots. The extracts taken were those with the highest concentrations of secondary metabolites, specifically an ethanol extract of mahogany parasite leaves. TLC silica Gel 60 F254 plate was utilized as the stationary phase. This plate was chosen because it is simple to use and has a high monetary value. Before using the TLC silica Gel F254 plate, it must be activated by heating it in an oven at 80°C for 1 hour. Because silica plates contain a lot of water, the TLC test findings may suffer as a result [25].

The try-and-error method is used to select the proper eluent in the TLC test. Where studies must be conducted using specific solvent mixes, such as chloroform: methyl acetate (3:1), chloroform: methanol (3:1), chloroform: ethanol: ethyl acetate (3:1:1), ethanol: ethyl acetate: n-hexane (2:2:6), ethyl acetate: methanol (5:5), and n-hexane: ethyl acetate. The TLC plate results showed that there were four well-separated areas. The Rf values for the four sites are 0.21, 0.27, 0.53, and 0.6, respectively. Figure 1 depicts the results of a thin layer chromatography test on an ethanol extract of mahogany parasite leaves.

Figure 1. Thin layer chromatography test results for ethanol extract

The Rf value for terpenoid compound identification using N-hexane and ethyl acetate as eluents is 0.20-0.25[26]. The Rf value at the first stain in this investigation, 0.21, falls within the normal range of terpenoid chemicals. The Rf of the second stain is 0.27, which is similar to the Rf saponin compound with the eluent N-hexane: Ethyl Acetate, which is 0.29 [27].
Further study revealed that the flavonoid compound had an Rf of 0.54 with the eluent N-hexane: Ethyl Acetate [27]. This number is nearly identical to the value Rf at the third node. In addition, the latest stain has an Rf of 0.6. Spotting is included in the standard Rf of tannin compounds and has a value of 0.29-0.85 [19]. As a result, the stain must be present in the Rf tannin compounds.

Table 3 Literature of Rf Value

<table>
<thead>
<tr>
<th>Sample</th>
<th>Rf Value</th>
<th>Compounds</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethanol extract</td>
<td>0.21</td>
<td>Terpenoid</td>
<td>[26]</td>
</tr>
<tr>
<td></td>
<td>0.22</td>
<td>Terpenoid</td>
<td>[26]</td>
</tr>
<tr>
<td></td>
<td>0.23</td>
<td>Terpenoid</td>
<td>[26]</td>
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<tr>
<td></td>
<td>0.24</td>
<td>Terpenoid</td>
<td>[26]</td>
</tr>
<tr>
<td></td>
<td>0.25</td>
<td>Terpenoid</td>
<td>[26]</td>
</tr>
<tr>
<td></td>
<td>0.29</td>
<td>Saponin</td>
<td>[27]</td>
</tr>
<tr>
<td></td>
<td>0.54</td>
<td>Flavonoid</td>
<td>[27]</td>
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<tr>
<td></td>
<td>0.60</td>
<td>Tannin</td>
<td>[19]</td>
</tr>
<tr>
<td></td>
<td>0.85</td>
<td>Tannin</td>
<td>[19]</td>
</tr>
</tbody>
</table>

3.6. Antioxidant Activity

Absorbance was determined for solutions with 5 concentration variations to generate a calibration curve of the DPPH solution as a reference for the DPPH concentration used in assessing antioxidant activity in samples of ethanol extract of mahogany parasite leaves and vitamin C. Linear regression obtained is equal to 1 and the value is acceptable for standard calibration curve [28].

Antioxidant activity evaluation The DPPH method was used to analyze plant extract profiles because it is effective and simple to apply, and it can reveal the potential of a sample [17]. The basic idea behind this molecule is to give a hydrogen atom to a free radical, causing DPPH to reduce and become a non-radical DPPH compound. The conversion of DPPH radical molecules to non-radicals is shown by a decrease in the purple color of the DPPH solution, which is accompanied by a drop in absorbance as measured with the Visible Genesis-30 Spectrophotometer at a wavelength of 517 nm.

The IC50 value is used to calculate a compound’s antioxidant activity. The IC50 concentration is an inhibitory concentration capable of reducing 50% of DPPH radicals [23]. The lower the IC50 power of a compound, the larger its free radical scavenging capacity; conversely, the higher the IC50, the stronger its free radical scavenging power. The graph of the linear regression equation between the concentration of the test solution and the percent reduction of DPPH was used to calculate the IC50. The percentage of damping, also known as percentage of inhibition, is determined by measuring the absorbance of the test solution.

The ethanol extract of mahogany parasite leaves contains the most metabolites. The secondary metabolite components found in the ethanol extract of mahogany parasite leaves are flavonoids, tannins, saponins, and steroids, according to the results of phytochemical screening. As a result, antioxidant activity tests were performed on samples of ethanol extract of mahogany parasite leaves at five different concentrations: 5 ppm, 10 ppm, 15 ppm, 20 ppm, and 25 ppm. Figure 3 depicts the absorbance measurement findings in the mahogany parasite leaf ethanol extract test solution, whereas Figure 4 depicts the calculation results of the DPPH radical reduction by the mahogany parasite leaf ethanol extract compound.
Figure 3. Relationship between concentration and absorbance of ethanol extract of mahogany parasite leaves

Figure 3 depicts the connection between ethanol extract concentration and absorbance/absorption of mahogany parasite leaves. It can be seen that the absorbance value of the ethanol extract drops as the concentration value increases. This is because a high concentration of ethanol extract of mahogany parasite leaves has stronger antioxidant action, transferring more hydrogen atoms and making the DPPH molecule stable. When DPPH becomes non-radical, it affects the purple to yellow hue of the ethanol extract sample, lowering the absorbance of the test solution.

Figure 4. Relationship between concentration and percent inhibition of DPPH radical compounds from ethanol extract of mahogany parasite leaves

Figure 4, on the other hand, explains the relationship between the concentration of the sample solution of the ethanol extract of the mahogany parasite leaves and the percentage of radical scavenging for DPPH and shows that the higher the concentration of the ethanol extract sample of mahogany parasite leaves, the higher the percentage of DPPH radical inhibition. This suggests that a high concentration of ethanol extract of mahogany parasite leaves has a stronger DPPH radical scavenging power than a low concentration of ethanol extract. A high concentration of ethanol extract of mahogany parasite leaves has molecules that are rich in more antioxidant chemicals, allowing them to donate hydrogen atoms more effectively to block free radical processes.

The absorbance of the test solution was measured once every 20 minutes for each concentration fluctuation. The length of the interaction between the ethanol extract and the DPPH molecule is used to determine the DPPH radical scavenging activity. The test solution’s first absorbance was measured from 0 to 120 minutes. Figure 5 shows a graph showing percent inhibition depending on variation in measurement time for ethanol extracts of mahogany parasite leaves with concentrations of 5 ppm, 10 ppm, 15 ppm, 20 ppm, and 25 ppm.
The five graphs above depicting the link between measurement time and percent inhibition of the ethanol extract of mahogany parasite leaves at five concentration changes, indicating that time has a significant influence on the antioxidant activity of the ethanol extract samples studied. Whereas the fraction of free radical inhibition increases with measurement time. This suggests that the chemicals in the ethanol extract sample of mahogany parasite leaves are still interacting.

The linear regression equation between the concentration of the test solution and the percentage decrease of DPPH radicals was used to calculate the IC$_{50}$ of an ethanol extract of mahogany parasite leaves. $y = 0.92x + 42.2$ is the linear regression equation. The IC$_{50}$ is calculated by inputting 50 as y and x as the IC$_{50}$. The ethanol extract of mahogany parasite leaves showed an IC$_{50}$ of 8.47 ppm, according to the data. These findings suggest that the ethanol extract of mahogany parasite leaves has high antioxidant activity, because the lower a compound's IC$_{50}$, the greater its free radical scavenging power. This suggests that at a concentration of 8.47 ppm, an ethanol extract of mahogany parasite leaves may neutralize 50% of free radicals.

Pure vitamin C was evaluated to compare the IC$_{50}$ of a good antioxidant molecule. Figure 6 depicts the findings of determining the percentage of antioxidant inhibition of vitamin C.
The higher the concentration of vitamin C, the greater the antioxidant action. The IC$_{50}$ of pure vitamin C, which serves as a good antioxidant reference, is 5.5 ppm. This suggests that at a concentration of 5.5 ppm, pure vitamin C may catch 50% of the DPPH radicals. When compared to samples of ethanol extract of mahogany parasite leaves, vitamin C absorbs slightly better. This research is only limited to determination of secondary metabolites compounds and their antioxidant activities without further application for medicine formulation.

4. Conclusion

According to the study’s findings, the ethanol extract of mahogany parasite leaves has excellent antioxidant activity, with an IC$_{50}$ of 8.47 ppm. The IC$_{50}$ is not significantly different from the IC$_{50}$ of pure vitamin C, which is 5.5 ppm. The recommendation for further studies is to purify flavonoids contained in ethanol extract. Due to the prediction of huge concentration of secondary metabolites of flavonoids and their high antioxidant activities.

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