Jurnal Gizi dan Dietetik Indonesia
(Indonesian Journal of Nutrition and Dietetics)
Vol 12, Issue 4, 2024: 262-272

Available online at: https://ejournal.almaata.ac.id/index.php/IJND
p-ISSN 2303-3045 e-ISSN 2503-183X
DOI: https://dx.doi.org/10.21927/ijnd.2024.12(4).262-272

Analysis of total phenolic content and antioxidant activity of mahogany seed infusion (*Swietenia mahagoni (L.) Jacq*.)

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ABSTRAK

Latar Belakang: *Tumbuhan mahoni (Swietenia mahagoni) adalah tumbuhan yang digunakan sebagai obat-obatan. Biji mahoni berkhasiat sebagai antiseptik, antioksidan, dan antimikroba. Biji mahoni mempunyai kandungan zat- zat kimia seperti flavonoid, saponin, tannin, minyak atsiri, alkaloid, dan antrakuinon. Penelitian sebelumnya menyatakan bahwa semakin tinggi kadar fenolik maka semakin tinggi aktivitas antioksidan. Menurut penelitian sebelumnya, ekstrak metanol biji mahoni memiliki sifat antioksidan yang baik dan senyawa fenolik yang terkandung dalam biji mahoni mampu menjadi kontributor utama dalam aktivitas antioksidan.*
Tujuan: *Penelitian ini bertujuan untuk melakukan pengukuran kadar fenolik total, pengukuran nilai konsentrasi penghambatan 50% (IC50) serta penentuan aktivitas antioksidan dari sediaan infusa biji mahoni (Swietenia mahagoni).*
Metode: *Jenis penelitian ini adalah eksperimental yang dimulai dengan ekstraksi menggunakan metode Infusa pada suhu 900C selama 15 menit dilanjutkan dengan pengukuran kadar fenolik total dengan cara mereaksikan sediaan infusa dan pembanding asam galat masing-masing dengan reagen Follin-ciocalteu. Uji aktivitas antioksidan dilakukan dengan menggunakan metode penangkapan radikal bebas DPPH (□,□-diphenyl-□-picrylhydrazyl). Masing-masing dilakukan pengukuran absorbansi dengan spektrofotometri UV-Vis. Pengukuran uji fenolik dilakukan dengan membuat regresi linear antara konsentrasi dengan absorbansi yang dilanjutkan dengan perhitungan kadar fenolik total. Sedangkan absorbansi yang diperoleh untuk uji antioksidan dilakukan perhitungan persen inhibisi. Kemudian IC50 ditentukan dengan perhitungan regresi linear hubungan antara konsentrasi dengan persen inhibisi.*
Hasil: *Kadar fenolik total infusa biji mahoni adalah 2,531 ± 0,029 mg GAE/g. Nilai IC50 Asam galat dan infusa biji mahoni masing-masing adalah 5,54 ppm dan 47,04 ppm yang memiliki kemampuan sebagai antioksidan sangat kuat.*
Kesimpulan: *Infusa biji mahoni memiliki memiiki aktivitas antioksidan yang sangat kuat dengan kandungan fenolik total yaitu 2,531 ± 0,029 mg GAE/g.*
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KATA KUNCI*: antioksidan; biji mahoni; fenolik; folin ciocalteu; infusa*

ABSTRACT

Background: *The mahogany plant (Swietenia mahagoni) is a plant used in medicine. Mahogany seeds are also efficacious as antiseptics, antioxidants, and antimicrobials. This plant contains flavonoids, saponins, tannins, essential oils, alkaloids, and anthraquinones. The higher the phenolic content in a plant, the higher the antioxidant activity. According to previous studies, it was stated that the methanol extract of mahogany seeds has good antioxidant properties, and the phenolic compounds contained in mahogany seeds can be the main contributors to antioxidant activity.*

Objectives: *This study aims to analyze the total phenolic content, measure the inhibition concentration 50% (IC50) and determine the antioxidant activity of mahogany (Swietenia mahagoni) seed infusion.*

Methods: *This type of research is experimental which begins with extraction using the infusion method at a temperature of 900C for 15 minutes followed by measuring the total phenolic content by reacting the infusion preparation and the Gallic acid respectively with the Folin-ciocalteu reagent. The antioxidant activity test was carried out using the DPPH (□,□-diphenyl-□-picrylhydrazyl) free radical scavenging method. Each absorbance was measured using UV-Vis Spectrophotometry. The phenolic test was measured by making a linear regression between concentration and absorbance, followed by calculating the total phenolic content. Meanwhile, the absorbance obtained for the antioxidant test was calculated by calculating the percent inhibition. The IC50 is determined by calculating the linear regression of the relationship between concentration and percent inhibition.*

Results: *The results of the phenolic content were 2.531 ± 0.029 mg GAE/g, the IC50 value for gallic acid was 5.54 ppm, and for mahogany seed extract, the IC50 value was 47.04 ppm, which is a very strong antioxidant.*

Conclusions: *This study concluded that the phenolic content of mahogany seed infusion extract contained phenolic compounds equivalent to 2.531 ± 0,029 mg GAE/g of gallic acid and that mahogany seed extract had very strong antioxidant activity.*

KEYWORD*:* *antioxidants; mahagony seeds; phenolics; folin ciocalteu; infusion*

Article info:
Article submitted on October 10, 2023
Articles revised on March 6,, 2024
Articles received on Juny 29, 2024

INTRODUCTION

Free radicals are molecules that are unstable and reactive, due to having one or more unpaired electrons (1). Therefore, our bodies need an important substance, namely antioxidants which can help protect the body from free radical attacks (2). Antioxidants are compounds that can inhibit free radical reactions in the human body. Antioxidants function to neutralize and overcome free radicals antioxidants can prevent damage to the body from the onset of degenerative diseases (2). Many foodstuffs can be sources of natural antioxidants, such as teas, enzymes, proteins, chocolate, spices, leaves and vegetables. Sources of natural antioxidants are mostly from plants spread throughout all parts of the plant in fruit, seeds, wood, fruit, flowers, roots, and pollen. One of the natural sources of antioxidants that have the potential to be antioxidants is mahogany seeds (3). Mahogany seeds contain flavonoids, saponins, tannins, and alkaloids, where flavonoids and tannins are a group of phenolic compounds (4). Previous research stated that phenolic content can be used to determine antioxidant activity. This is because there is a linear relationship between phenolic levels and antioxidant activity (5). Mahogany seed oil contains a large amount of nutrients that are beneficial for health such as oleic acid, linoleic acid and linolenic acid (6). Giving ethanol extract of mahogany seeds have a hepatoprotective effect on rats induced by a high-fat diet by preventing an increase in SGOT and SGPT levels. (7). The nutritional content of

mahogany seeds includes carbohydrates (36.64%), fat (24.03%), protein (14.70%) and fiber (13.03%). The large amount of protein and fat content can help maintain body temperature, absorb nutrients and provide energy. The fiber content in mahogany seeds can lower cholesterol levels, maintain body weight and relieve constipation.(8).

Research by Wahyuni states that the higher the phenolic content, the higher the antioxidant activity (9). This study aims to determine the total phenolic content using the Folin-Ciocalteu method on standardized infusions of mahogany seeds (Swietenia mahagoni), to determine the antioxidant activity of standardized infusions of mahogany seeds (Swietenia mahagoni) using the DPPH method, to determine the IC50 value of the antioxidant activity of standardized infusions of mahogany seeds using the method DPPH. IC50 standardized the antioxidant activity of mahogany seed infusion using the DPPH method. According to previous studies, it was stated that the methanol extract of mahogany seeds has good antioxidant properties and the phenolic compounds contained in mahogany seeds can being a major contributor to antioxidant activity, so mahogany seeds can treat diseases such as rheumatism, hypertension, and blood sugar disorders. Previous research stated that the ethanol extract of mahogany seeds obtained a linearity value on the inhibition curve of 0.7202 with an IC50 value of 6.0 ppm, which means it has a very strong level of antioxidant power (10). Previous research stated that ethanol extract, ethyl acetate fraction, and n-hexane of mahogany seeds, contain alkaloids, flavonoids, triterpenoids, and phenolic compounds. With an IC50 value in the total ethanol extract of 176 ppm, the n-hexane fraction of 189 ppm, and the ethyl acetate fraction of 144 ppm which are moderate (11).

No one has research the determination of phenolic levels and standardized infusion antioxidant activity tests of mahogany seeds (Swietenia mahagoni). The choice of standardized infusion of mahogany seeds is because it is simple, cheap, practical, and it is easy to do. The method used to identify antioxidant activity in this study was DPPH (2,2-diphenyl-1-Pikrylhidrazil). This method was chosen because it is practical, easy, and sensitive in testing antioxidant activity (12). In this method, DPPH acts as a free radical which is inhibited by antioxidants resulting in a color change from purple to yellow. Strong DPPH absorption will be seen at a wavelength of 517 nm (13) and the method chosen for the determination of phenolic content is the Folin-Ciocalteu method. This method is often used to assess phenolic content because it is fast, simple, and expressed as the equivalent mass of gallic acid per mg of sample (14). The oxidation reaction of phenolic compounds using Folin-Ciocalteu reagent in alkaline conditions produces a blue complex with a wavelength of 760 nm (15).

MATERIALS AND METHODS

Research Tools and Materials

The equipment used in this study were electric scales, stirring rods, measuring cups, beakers, stopwatches, filter paper, measuring pipettes, dropping pipettes, measuring flasks, erlenmeyer flasks, test tubes, test tube racks, porcelain cups, blender, aluminum foil, infusion pot, water bath, UV-Vis spectrophotometer, magnetic stirrer, and incubator. The materials used in this study were mahogany seed simplicia, 98% methanol, distilled water, Folin-Ciocalteu solution, DPPH powder, 7.5% sodium carbonate, and gallic acid.

Infusion Method

According to the Indonesian Pharmacopoeia Edition III, liquid preparations are made by extracting (distilling) vegetable simplicia with water at 90 degrees Celsius for 15 minutes (16).

Determination of Total Phenolic Content

The first step is to create a calibration curve using a standard gallic acid solution. As much as 10 mg of gallic acid standard was put into a 25 mL volumetric flask, added methanol to the mark, and shaken until homogeneous (17). Then it was diluted in 5 concentration series, namely 20, 40, 60, 80, and 100 ppm (18). Gallic acid solutions were put into a test tube as much as 1 ml then added 4 ml of 7,5 sodium carbonate. The maximum wavelength was measured using a gallic acid concentration of 60 ppm in the range of 400-800 ppm (17). Then a linear regression is made of the relationship between the concentration of the gallic acid solution vs the absorbance of the gallic acid solution so that the equation y= bx + a is obtained. The next step is the preparation of sample testing. As much as 0.2

g of mahogany seed infusion extract was put into an Erlenmeyer flask, added 25 mL of methanol, and stirred for 30 minutes with a magnetic stirrer. Filtered into a 25 mL volumetric flask, add methanol through a filter up to the mark (17). it were put into a test tube as much as 1 ml then added 5 ml of Folin-Ciocalteu solution and allowed to stand for 8 minutes then added 4 ml of 7.5% sodium carbonate, incubated for 1 hour then measured absorbance at maximum wavelength. Measurements were carried out 3 times. The concentration of mahogany seed infusion is calculated by entering the curve standard regression. Then the concentration is used into the equation for calculate total phenolic content.

Antioxidant Activity Test with DPPH Method

A total of 7.88 mg of DPPH powder was put into a volumetric flask and dissolved in 50 ml of methanol. Wrap it in aluminum foil. Preparation for sample testing: As much as 50 mg of mahogany seed infusion extract was put into a volumetric flask dissolved with 50 methanol, and shaken until homogeneous (19). One mL of DPPH solution respectively added to mahogany seed extract 0,1; 0,2; 0,3; 0,4; and 0,5 ml of 1000 ppm mahogany seed extract. then dissolved in methanol up to 5 mL mark to obtain a concentration of 20, 40, 60, 80, and 100 ppm (19). Preparation for standard testing: As much as 50 mg of gallic acid was put into a volumetric flask dissolved in 50 mL methanol, and shaken until homogeneous (19). One ml DPPH respectively added to gallic acid solutions 0,1; 0,2; 0,3; 0,4 and 0,5 ml of 1000 ppm gallic acid stock solution (19). Measurements were carried out 3 times. The inhibitory ability is shown by calculating the percent inhibition, then continuing with determining the IC50 to calculate the concentration required to inhibit free radicals by calculating the linear regression y=bx + a between concentration vs percent inhibition.

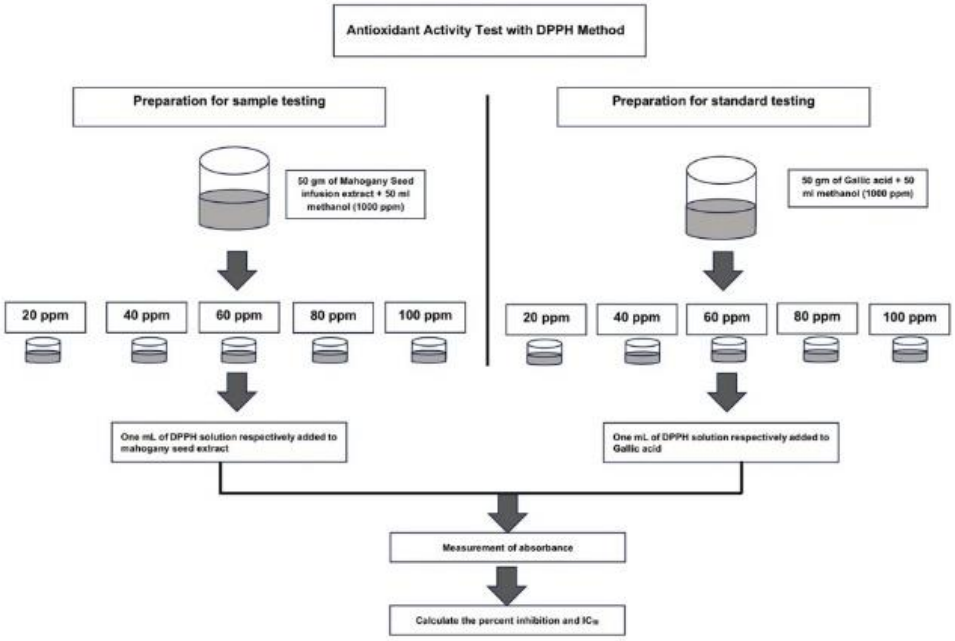

Figure 1. Preparation Sample for Antioxidant Activity

Data Analysis

Determination of Total Phenolic Content (TPC)

Total phenolic content is determined using the formula (20).

$$TPC = \frac{C.V.fp}{g}$$

Note :

TPC : Total Phenolic Content

C : Concentration of mahogany seeds from measurement with

regression
V : Volume Extract
Fp : Factor Dilution
G : Weight of Extract

Figure 1. Determination of Total Phenolic Content (17).

Determination of Percent Inhibition

Antioxidant activity is expressed as a percentage of inhibition using the formula (21):

$$\%\ Inhibition\ = \frac{A\ Blank - A\ Sample}{A\ Blank}\ x\ 100\%$$

Note :
% Inhibition : Percentage of inhibition
A blank : Absorbance of Blank
A sample : Absorbance of Sample (Extract)

Figure 2. Determination of Percent Inhibition (18)

Determination of Inhibition Concentration 50% (IC_{50})

The IC_{50} value is obtained by calculating the concentration needed to inhibit free radicals by 50 based on the equation of the linear regression line using the formula (21):

$$y = bx + a$$

Note :
y : 50
b : Slope
x : Concentration of mahogany seed extract in inhibiting 50% of free radicals
a : intersept

RESULTS AND DISCUSSIONS

The extraction process in this study used the infusion method. The infusion method using aquadest solvent aims to obtain polar active substances, such as flavonoids and polyphenols, which act as antioxidants. Yield is the ratio of the dry weight of the product produced to the weight of the raw material. A high yield value indicates the number of bioactive components contained in it (22). The yield can be seen in **Table 1**.

Table 1. Mahogany seed infusion extract yield results

Sample	Simplisia Powder Weight (kg)	Weight of Viscous Extract (grams)	Extract Yield (b/b) (%)
Mahogany Seeds	2	334	16.7

The yield of a sample is needed to determine the amount of extract obtained during the extraction process and the yield has something to do with the active compounds of a sample so that if the amount of yield increases, the amount of active compounds contained in the sample also increases (21). According to previous research, the high active compound present in a sample is indicated by the high yield. The resulting yield value can be caused by several factors such as particle size of the sample, extraction method, extraction time, comparison of samples and solvents, type of solvent, conditions, and storage time (10). The thick extract of the infusion of mahogany seeds produces a yield of 16.7%, which means that it meets the requirements of the Indonesian Herbal Pharmacopoeia which states that the yield value of the extract of mahogany seeds is not less than 16.0% (17).

Determination of gallic acid wavelength on total phenolic content and antioxidant activity test

The maximum wavelength is the wavelength that can provide the maximum absorbance at the time of measurement. The advantage of using the maximum wavelength is that the measurement is more sensitive because, at the maximum wavelength, a slight change in concentration can result in a large change in absorbance. The determination of the wavelength is carried out to determine the change in absorbance at each concentration unit so that the maximum analytical sensitivity will be obtained to provide maximum absorbance when taking measurements. Maximum wavelength measurement using UV-Vis spectrophotometry at a wavelength of 400-800 nm (23). (6).

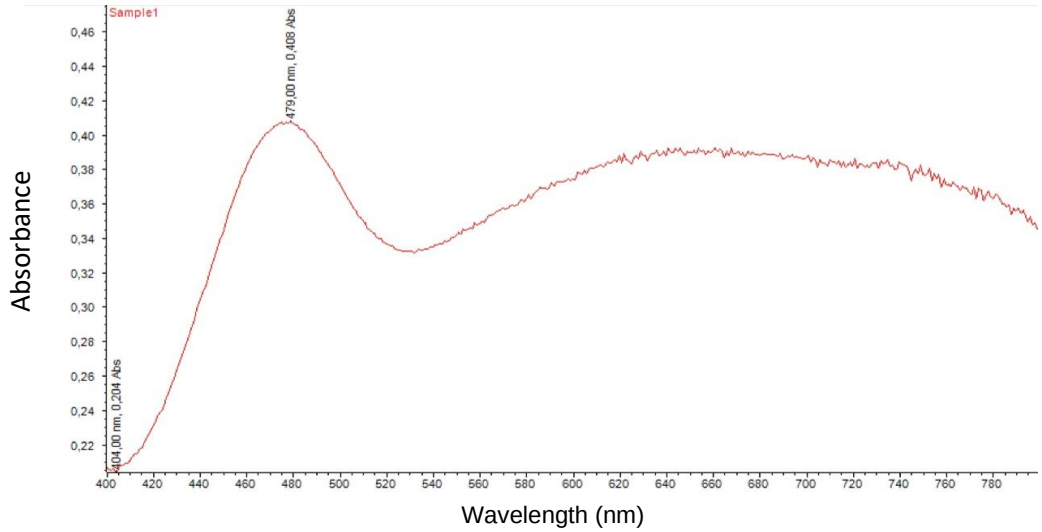

Figure 2. Graph of maximum wavelength determination of gallic acid with follin- ciocalteu reagent

The purpose of determining the maximum wavelength is to determine the maximum absorption wavelength as a result of the reaction between the DPPH radicals and a reference compound or test compound (24). The reaction of gallic acid using Folin-Ciocalteu reagent in alkaline conditions produces a blue complex with a wavelength of 760 nm (15). The difference in wavelength as shown in **Figure 2** can be caused by the dilution of each concentration, namely 1: 10, so that it can affect the scanning wavelength (25). Other factors that can affect the wavelength such as different tools and room temperatures. (27).

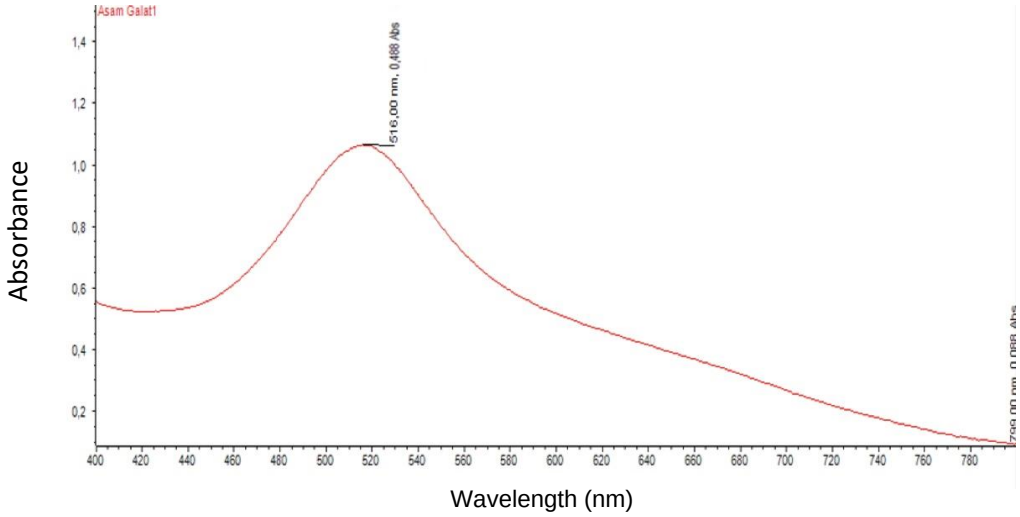

Figure 3. Graph of Maximum Wavelength Determination of Gallic Acid Antioxidant Test with DPPH reagent

The purpose of determining the maximum wavelength is to determine the maximum absorption wavelength as a result of the reaction between the DPPH radicals and a reference

compound or test compound (24). The wavelength obtained in this study is slightly different from the maximum absorption wavelength in theory. The theoretical wavelength for DPPH is 517 nm, while the maximum wavelength from measurement results is 516 nm as shown in Figure 3. Based on the provisions listed in the Indonesian Pharmacopoeia edition IV, the shift limit obtained is ± 10 nm (26). Mean that the maximum absorption wavelength obtained in the study is still within the limits (16).

Determination of Gallic Acid calibration curves and Total Phenolic Content

The calibration curve is the relationship between the response of the instrument and a known amount (concentration) of the analyte. From the calibration curve, a line equation is obtained which states the relationship between concentration and absorbance (27). The results of the gallic acid calibration curve can be seen in **Figure 4**.

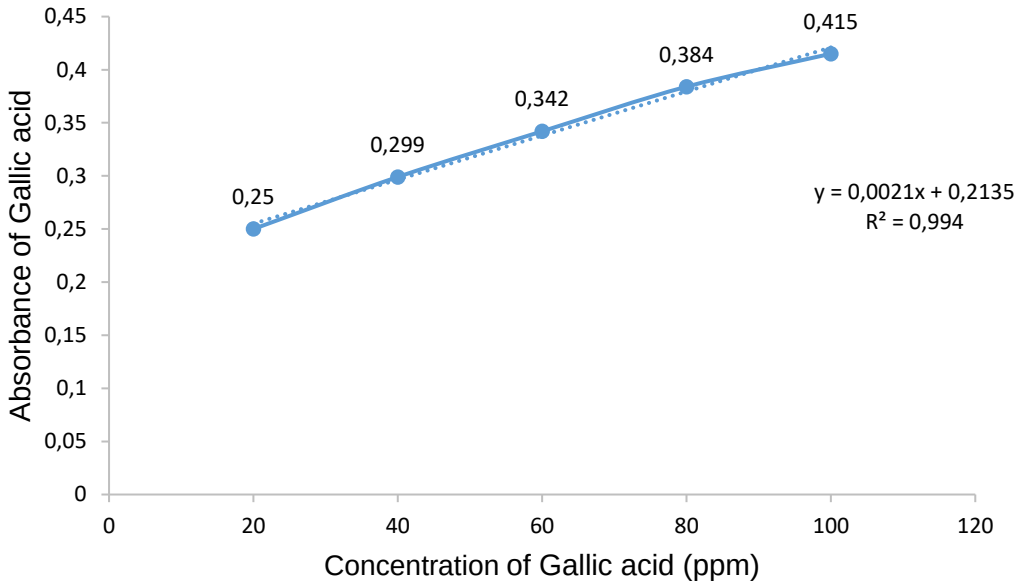

Figure 4. Calibration Curve of Gallic Acid

From the gallic acid calibration curve in **Figure 4** a linear regression equation is obtained by y = 0.0021x + 0.2135 with value of R^2 = 0.994. The results of the correlation coefficient (R^2) state a linear relationship between concentration and the resulting absorption. (23).

Table 2. Results of Determination of Phenolic Levels of Mahogany Seed Infusion Extract

Sample	Replication	Absorbance	Phenolic Levels (mg GAE/g)	Average Phenolic Levels ± SD (mg GAE/g)
Mahogany Seed Infusion Extract	1	0.320	2.535	2.531 ± 0.029
	2	0.321	2.559	
	3	0.320	2.500	

The total phenolic content in the extract depends on the polarity of the solvent used in the extraction. The total phenolic content is expressed as Gallic Acid Equivalent (GAE) because the chemical structure of the phenolic compounds present in the extract is unknown (28).

In **Table 2** it can be seen that the measurement of phenolic compounds was made in 3 replications for the accuracy of the data. Based on the results of the analysis, the phenolic content of mahogany seed infusion extract was 2.531 ± 0.029 mg GAE/gram extract, which means

that in every gram of mahogany seed extract, there is a phenolic equivalent to 2.531 mg of gallic acid. The phenolic compounds contained in the infusion extract of mahogany seeds are the results of secondary metabolites that have the potential as a source of antioxidants. Research conducted by Mongkolsilp et al on five Thai medicinal plants revealed that the higher the total phenolic content, the higher the DPPH free radical scavenging activity (29). Phenolic compounds in several studies have antioxidant activity due to their ability to reduce reactive oxygen, this is because in the aromatic ring, several hydroxy groups act as hydrogen donors (28).

Antioxidant activity test of mahogany seed infusion extract

The antioxidant activity test of mahogany seeds was carried out using the DPPH method. DPPH is a stable free radical compound so that when it is used as a reagent in a free radical scavenging test it is sufficiently dissolved. When the purple DPPH solution encounters an electron donor, the DPPH will be reduced, causing the purple color to fade and be replaced by a yellow color coming from the picryl group (30).

The advantages of DPPH are that the method is simple, fast, easy, accurate, practical, and reliable, the materials used are few, and sensitive to evaluate compounds from natural materials. The DPPH radical is often used because it has high stability and can be applied to both lipophilic and hydrophilic compounds (31). The DPPH method is very sensitive to light and if DPPH is exposed to light it will disturb the instability, so the antioxidant activity test is carried out in a dark place or covered with aluminum foil. The aim of this method is to find out which concentration parameters are equivalent to giving 50% of the effect of antioxidant activity (IC50)(30).

The color change of DPPH which was originally purple after being reacted with gallic acid changed to a yellow color. In the process of testing the antioxidant activity, incubation was carried out at 37℃ for 30 minutes. The goal is to incubate for 30 minutes so that the reaction between the sample solution and the DPPH solution takes place perfectly before measuring the antioxidant activity using a UV-Vis spectrophotometer (19). The use of a UV-Vis spectrophotometer in the antioxidant activity test aims to determine the remaining absorbance of DPPH after adding the sample.

Table 3. Inhibition concentration 50 (IC50) of gallic acid and mahogany seed infusion extract

Sample	IC_{50} (ppm)	Category Antioxidant Activity
Gallic Acid	5,54	Very powerful
Mahogany Seed Extract	47,04	Very powerful

Table 3 shows the IC50 value for gallic acid, which is 5.54 ppm, which means it has very strong antioxidant properties, while for mahogany seed extract, it is 47.04 ppm, which means it has very strong antioxidant properties. So the results of research on extracts of infusion of mahogany seeds and gallic acid as a comparison have very strong antioxidant activity. The smaller the IC50 value the greater the antioxidant power. The IC50 value of mahogany seed infusion is greater than the IC50 value of gallic acid, meaning that it shows higher gallic acid antioxidant potential compared to mahogany seed infusion extract. This is because in the extract of the mahogany seed infusion it is still in the form of a mixture of several compounds that do not have antioxidant activity. Meanwhile, gallic acid is a pure synthetic compound t. Gallic acid also has more hydroxyl groups, so gallic acid can donate more hydrogen atoms to react with the DPPH free radical (32).

CONCLUSIONS AND RECOMMENDATIONS

The phenolic content obtained from the mahogany seed infusion was 2.531 ± 0.029 mg GAE/g. The IC50 values of gallic acid and infusion mahogany seeds was respectively 5.54 ppm and 47.04 ppm. The mahogany seed infusion extract had very strong antioxidant activity that is <50 ppm.

Further research is needed regarding identify the types of phenolic compounds present in mahogany seed infusion and the antioxidant activity of mahogany seed infusion with other methods. Research in vivo is also needed to ensure that mahogany seed infusion is capable of acting as an antioxidant.

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