Antioxidant Activity Test Of Methanol Extract Of Gaharu (*Aquilaria Malaccensis* Lam.) Bark With Dpph (1,1 Diphenyl-2-Picrylhydrazyl) Method

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Abstract.
*Gaharu (*Aquilaria malaccensis *Lam.) is one of the natural ingredients that can be used as an antioxidant. Antioxidants are compounds that can inhibit free radical reactions in the body. Free radicals have an impact on the pathogenesis of several diseases in humans due to oxidative stress in cells. The purpose of this study was to determine the class of secondary metabolites of agarwood bark and antioxidant activity and to compare the value of IC_{50} (Inhibitory concentrations) of the methanol extract of agarwood bark with the IC_{50} of vitamin C in the DPPH method using UV-Vis spectrophotometry. The methanol extract of agarwood bark was macerated using methanol, then determined the content of secondary metabolites of Simplicia. The determination of antioxidant activity was carried out on the methanol extract of agarwood bark with the addition of DPPH at various concentrations. The sample concentrations were 20, 40, 60, 80, and 100 µg/mL.. As a comparison, vitamin C was used with concentrations of 4, 8, 12, 16 and 20 µg/mL.. Then they calculated percent attenuation (% inhibition) and the value of IC_{50}. The results of the screening on the methanol extract of agarwood bark contained chemical compounds such as flavonoids, saponins, and tannins. The determination of antioxidant activity was carried out using a UV-Vis spectrophotometer with the DDPH method. The results were obtained from the methanol extract of the bark of agarwood, which has antioxidant activity in the strong category with an IC_{50} value of 94.59 µg/mL.and vitamin C in the very strong category with an IC_{50} value of 22,11 µg/mL.*

Keywords*: agarwood bark, methanol extract, antioxidant activity*

I. INTRODUCTION

Agarwood (*Aquilaria malaccensis*) is a plant that grows in Sumatera and Kalimantan. Aquilaria malaccensis is also one of the natural ingredients that has been studied and has antioxidant activity, especially in the bark and leaves (Mukti, 2016). Antioxidants are compounds that donate electrons (electron donors) or reductants. This compound has a small molecular weight, preventing the formation of radicals. Antioxidants are compounds that can inhibit oxidation reactions by binding to free radicals and highly reactive molecules. As a result, cell damage can be inhibited (Winarsi et al., 2011).Decreasing air quality has the potential to increase the formation of free radicals in the body. Free radicals have an impact on the pathogenesis of several diseases in humans due to oxidative stress in cells (Sitorus et al., 2017). The body's natural defense mechanism will counteract the free radicals formed by producing compounds that can counteract them, one of which is antioxidants. Therefore, the ability of antioxidants and lipid peroxidation naturally present in the body is very important to protect the body from exposure to various diseases due to increased free radicals (Khani et al., 2017). Currently, there are many synthetic antioxidants available on the market. Inappropriate use of synthetic antioxidants has been reported to cause side effects, so new natural sources of antioxidants are needed (Rico et al., 2013). One of the plants that has antioxidant activity is agarwood. This plant is a large woody tree with many benefits (Taher et al., 2007). Efforts to prevent or reduce the risk posed by free radical activity are to consume foods or supplements that contain antioxidants. Antioxidants can neutralize free radicals by donating one proton atom so that free radicals become stable and unreactive (Lusiana, 2010).

Many studies have been carried out on this agarwood extract, but the most researched is on the leaves while the bark of the stem is still small. In the field of traditional medicine, it is reported that agarwood contains chemical compounds from the flavonoid group and is used by the community as a brewed

drink (Silaban, 2014). Research conducted by Amalina (2015), shows that the ethanol extract of agarwood leaves contains flavonoid compounds, tannins, and phenols. Based on the results of research by Mega and Swastini (2010) on the antioxidant activity of methanol extract of agarwood leaf (Gyrinops versteegii) with the 1,1-diphenyl-2-picrylhydrazyl (DPPH) method, it was concluded that the antioxidant activity was quite high because it had a % reduction of 106.32% (at 5 minutes) and 111.31% (at 60 minutes). According to Norhasima (2019) that the most antioxidants were shown in polar solvents (MeOH) in either the air-dry or oven-dry methods with insignificant differences. To analyze the glucosidase inhibitory activity, the methanol extract of *Aquilaria malaccensis* in the air-dry method showed the highest activity with an IC_{50} value of 196.31 µg/mL.From several research results and descriptions above, researchers are interested in testing the antioxidant activity of the bark of the agarwood plant (Aquilaria malaccensis Lam.) using the DPPH method. This research is expected to provide information on secondary metabolites found in agarwood bark and the antioxidant activity of methanol extract of agarwood bark.

II. METHODS

2.1 Tools

The tools used consisted of a UV-Vis spectrophotometer (Shimadzu UV-1800), glass funnel, filter paper, aluminum foil, rotary evaporator R-3 (Buchi), reaction tube rack, drip plate, beaker glass, measuring cup, dropper pipette, stirring rods, volumetric flasks, and analytical balances.

2.2 Materials

The samples used in this study were methanol extract of agarwood bark (Aquilaria malaccensis Lam), methanol, aquadest, chloroform ($CHCl_3$), norite, concentrated hydrochloric acid (HCl), magnesium metal (Mg), ferric (III) chloride ($FeCl_3$) reagent, anhydrous acetic acid $(CH_3CO)_2O$, concentrated sulfuric acid (H_2SO_4), chloroform- ammonia 0,05 N, Mayer's reagent, DPPH (1,1-diphenyl-2-picrylhydrazyl) (ALDRICH) and ascorbic acid (vitamin C).

2.2 Sample

The bark samples of agarwood (*Aquilaria malaccensis* Lam.) used in this study were obtained in Pekanbaru, Riau. Sampling was carried out on one tree and was carried out by barking at a very large tree, and it was estimated that the tree was approximately 20 years old.Processing of bark samples of agarwood (Aquilaria malaccensis Lam.) which have been collected, washed with running water, weighed wet, and dried in a drying cabinet at 50˚C The bark is considered dry if it can be broken into brittle pieces and crushed, then weighed at a dry weight. Then it is powdered using a blender and then stored in a dry container protected from sunlight.

2.4 Making Agarwood Bark Methanol Extract

Maceration extraction was carried out by weighing 300 g of Simplicia powder and soaking it in methanol solvent with a ratio of 1:5 sample to methanol so that 300 g of sample was dissolved in 1.5 L of methanol. Maceration was carried out for 3x24 hours, where every 1x24 hours the residue and filtrate had to be separated, and the same solvent was changed. After the filtering process, the filtrate obtained was then allowed to stand for 2 days, and the solvent was evaporated using a rotary vacuum evaporator at a temperature of 50˚C. The liquid extract obtained was then stored in a porcelain dish, which was then concentrated using a water bath at a temperature of 60˚C.

2.5 Simplified Characterization Examination

Examination of Simplicia characterization includes macroscopic and microscopic examination; determination of water content by the azeotropic method; determination of water-soluble extract content; determination of ethanol-soluble extract content; determination of total ash content; and determination of acid-insoluble ash content (Ditjen POM, 1995).

2.6 Phytochemical Screening Test

1. Glycoside Examination

A total of 1 gram of Simplicia powder and the extract were extracted with 30 mL of a mixture of 96% ethanol and distilled water (7:3), then added 10 mL of 2N HCl, refluxed for 10 minutes, cooled and filtered. 20 mL of the filtrate was taken, plus 25 mL of distilled water and 25 mL of 0.4 M lead (II) acetate,

shaken, allowed to stand for 5 minutes, and then filtered. The filtrate was extracted 3 times, each time with 20 mL of the chloroform-isopropanol mixture (3:2). In the collection of juices, added anhydrous sodium was tasted. Filtered and evaporated at a temperature of not more than 50℃. The remainder was dissolved with 2 mL of ethanol, and then 0.1 mL of the experimental solution was taken into a test tube and evaporated over a water bath. 2 mL of water and 5 drops of Molisch reagent were added to the residue. 2 mL of concentrated sulfuric acid was added carefully. A purple ring was formed at the boundary of the two liquids, indicating the presence of glycosides (Ditjen POM, 1995).

2. Alkaloid Examination

Simplicia powder and extract weighed 0.5 g, added 1 mL HCl 2 N, added 9 mL distilled water, then heated in a water bath for 2 minutes, cooled and filtered the filtrate used for alkaloid examination:

1. When three drops of filtrate are mixed with two drops of Mayer reagent, a white or yellow lumpy residue is formed.
2. When three drops of filtrate are mixed with two drops of Bouchardat reagent, a brown to black residue is formed.
3. To form brown or orange, 3 drops of filtrate are mixed with 2 drops of Dragendroff reagent.

If there is a residue or turbidity in at least 2 of the test tubes in the above experiment, the alkaloid is positive (Ditjen POM, 1995).

3. Flavonoid Examination

The Simplicia powder was weighed at 10 g, then added to 100 mL of hot water, boiled for 5 minutes, and filtered in hot conditions; the filtrate was then transferred to 5 mL and mixed with 0.1 gram of Mg powder, 1 mL of concentrated HCl, and 2 mL of amyl alcohol, shaken, and allowed to separate. Flavonoids are positive if they occur in red or yellow-orange colors on the amyl alcohol layer (Fransworth, 1996).

1. Tannin Examination

The Simplicia powder weighed 0,5 grams of the sample and was extracted with 10 mL of distilled water, then the filtrate was diluted with distilled water until it was colorless. Take 2 mL of the solution and add 1 to 2 drops of iron (III) chloride reagent. The occurrence of a blue or green-black color indicates the presence of tannins. (Frarnsworth, 1996).

2. Saponin Examination

The powdered Simplicia was weighed as much as 0,5 grams of the sample was put in a test tube and added to 10 mL of hot distilled water, cooled, and then shaken vigorously for 10 seconds, a steady foam appeared no less than 10 minutes as high as 1-10 cm. Added 1 drop of 2 N hydrochloric acid solution, if the foam does not disappear, it indicates the presence of saponins (Ditjen POM, 1995).

3. Steroid/Triterpenoid Examination

The Simplicia powder weighed as much as 1 gram of the sample, macerated with 20 mL of n-hexane for 2 hours, then filtered. The filtrate evaporated in a vaporizer cup. Add 2 drops of anhydrous acetic acid and 1 drop of concentrated sulfuric acid to the remainder. A purple-red color appears indicate the presence of triterpenoids or green color indicates the presence of steroids (Fansworth, 1996).

2.7 Preparation of the Test Solution

1. Preparation of a Blank Solution

DPPH solution of 0,5 mM (concentration 500 µg/mL) was pipetted as much as 1 mL, then put into a 10 mL measuring flask, filled with methanol to the marked line (concentration 50 µg/mL)

2. DPPH Maximum Wavelength Determination

DPPH solution with a concentration of 500 µg/mL, 1 mL was pipetted and put into a 10 mL measuring flask, filled with methanol until the marking line, obtained a DPPH solution with a concentration of 50 µg/mL, the absorbance was measured at a wavelength of 400-800 nm, so that the absorbance was obtained. maximum as the maximum wavelength of the DPPH.

3. Measurement of DPPH operating time

A total of 1 mL of DPPH solution (from a solution with a concentration of 500 µg/mL), was put in a 10 mL volumetric flask the volume made up to the marked line, and a DPPH solution of 50 µg/mL was obtained. Then the absorbance was measured using a visible spectrophotometer at the maximum wavelength

obtained, starting from the first minute until a stable absorbance was obtained, as operating time (good working time).

4. DPPH Absorbance Measurement Without Test Material

2.5 mL of DPPH solution with a concentration of 500 µg/mL is pipetted into a 25 mL volumetric flask, the volume is filled with methanol until the marking line is obtained, a DPPH solution with a concentration of 50 µg/mL is obtained, allowed to stand for a few minutes according to the stable time, the absorbance is measured at the maximum wavelength obtained. The process was repeated 3 times, to obtain absorbance data from the DPPH solution without the test material.

5. Absorbance Measurement of Mixture of DPPH and Vitamin C

5 mL of vitamin C solution (from a solution with a concentration of 1000 µg/mL) was pipetted, diluted in a volumetric flask to 25 mL, and the volume was filled with methanol to the marked line, so a vitamin C solution with a concentration of 100 µg/mL was obtained. Furthermore, this solution is used to measure the absorbance of each pipette as much as 1; 2 ; 3 ; 4 , and 5 mL, diluted with methanol in a volumetric flask to 25 mL, then added to 2 mL of 0,5 mM DPPH solution (50 ppm concentration), to obtain a vitamin C solution with a concentration of 4; 8; 12; 16; 20 µg/mL. Then it was allowed to stand for a few minutes according to the stable time, the absorbance was measured at the maximum wavelength obtained. The treatment was repeated up to 3 times, to obtain absorbance data from a mixture of DPPH and vitamin C at various concentrations.

6. Measurement of DPPH Absorbance and Agarwood Bark

Pipette a solution of methanol extract (from a concentration of 1000 µg/mL) 0.5; 1; 1.5; 2; and 2,5 mL;, each was put into a 25 mL volumetric flask, and each was added with 2 mL of DPPH solution (from a concentration of 50 µg/mL), then the volume was made up with methanol solution to the mark line, then obtained extract solution concentration of 20; 40; 60; 80 and 100 µg/mL.

Then it was allowed to stand for a few minutes according to the stable time, the absorbance was measured at the maximum wavelength. The treatment was repeated up to 3 times, to obtain absorbance data from a mixture of DPPH and agarwood bark extract with various concentrations.

7. Validation of Antioxidant Activity Test Method

The results of the procedure were validated for accuracy (% recovery), specificity (control spectra), and linearity (r-value) % *Recovery*

$$\%\,Recovery = \frac{\text{measured concentration}}{\text{theoretical concentration}} \text{ x } 100\%$$

8. Data analysis

1) Determination of Percent Attenuation (% inhibition)

Antioxidant ability is calculated from the decrease in the absorption of DPPH solution (decrease/attenuation of the purple color of DPPH) due to the addition of fraction extract solution as a test material and vitamin C solution as comparison material. The difference in the absorption value of the DPPH solution before and after the addition of the test solution was calculated as the percentage of inhibition.

$$\%\text{ inhibition} = \frac{\text{DPPH Absorbance} - \text{Sample Absorbance}}{\text{DPPH Absorbance}} \times 100\%$$

2) IC_{50} Value Determination

The IC50 value is a number that indicates the concentration of the test sample (µg/mL) which provides 50% DPPH reduction (capable of inhibiting or reducing the oxidation process by 50%). A value of 0% means that it does not have antioxidant activity, while a value of 100% means that the total attenuation with the test needs to be continued with a dilution of the test solution to see the limit of its activity concentration.

The results of the calculations are entered into the regression equation with the extract concentration (µg/mL) as the abscissa (X-axis) and the value of % absorption (antioxidant) as the ordinate (Y). Then from this equation, the IC_{50} value is calculated to get the antioxidant value.

$$y = ax \pm b$$

III. RESULT

3.1 Agarwood Bark Extract

The results of the processing of agarwood bark with a base weight of 3 kg, were dried in a drying cabinet at a temperature of 40°C. The dry weight was obtained at 1.2 kg, crushed to become a powder of as much as 1 kg. Weighed 300 g of agarwood bark Simplicia powder, extracted by maceration method using 5 liters of methanol solvent, then evaporated with a rotary evaporator and concentrated to obtain a thick extract of 3.53 grams, the yield was 1.17%.

3.2 Simplicia Characterization of Agarwood Bark

A Simplicia is said to be of good quality if it meets the quality requirements stated in the Simplicia monograph contained in the Indonesian Herbal Pharmacopoeia. This quality requirement applies to Simplicia used for treatment and health maintenance. Based on the results of Simplicia characterization, macroscopic testing of the physical form of the bark of agarwood (Aquilaria malacensis Lam.) is brown, contains fine fibers, has an aromatic odor, and is tasteless. In the observation of agarwood bark powder, identification fragments were observed in the form of groups of stone cells with thick cell walls, fibers with fingers, long fibers similar to longitudinal lines with blunt ends, tangential cork tissue, and wooden vessels.

The results of the examination of water content, water-soluble extract content, ethanol-soluble extract content, total ash content, and acid-insoluble ash content of agarwood bark Simplicia can be seen in Table 3.1

Tabel 3.1. The results of the Simplicia characterization of Agarwood Bark

No.	Parameter	Result (%)
1.	Moisture content	6%
2.	Water-Soluble Extract Content	5.33%
3.	Ash content	2.13%
4.	Acid-Insoluble Ash content	0.63%
5.	Ethanol-Soluble Extract Content	0.6%

The results above indicate the determination of the water content of the bark Simplicia of agarwood (*Aquilaria malacensis* Lam.). This test is carried out by determining the water content obtained is 6%. These results indicate that the water content in the sample still meets the requirements of < 10%. The results of water content below 10% can prevent hydrolysis reactions and microbial growth in Simplicia powder. Determination of ash content includes total ash content and acid insoluble ash content. The ash content obtained was 2.13% and for the determination of the acid-insoluble ash content, it indicated the presence of silica compounds originating from the sand. This silica compound is not acid soluble so it is a constituent component of acid-insoluble ash, in this examination the result is 0.63%. Determination of ethanol-soluble extract content and water-soluble extract content, the results obtained from ethanol-soluble extract content was 0.6%. As for the examination of the water-soluble extract, content obtained results of 5.33%.

3.3 Phytochemical Screening of Agarwood Bark

Phytochemical screening was carried out to obtain information on the class of secondary metabolites contained in the Simplicia methanol extract of agarwood bark. Phytochemical screening was carried out on Simplicia, a methanol extract of agarwood bark, including examination of alkaloids, flavonoids, saponins, tannins, steroids, triterpenoids, and glycosides. According to Suryana's research (2017) and in this study, the results of phytochemical screening of agarwood roots and twigs were negative alkaloid compounds, positive flavonoid group compounds, and negative saponins. While in this research sample the results were positive, then for tannin compounds, the results were both negative. The results of phytochemical screening in this study showed that Simplicia, methanol extract of agarwood bark contains secondary metabolites, namely flavonoids, which are characterized by the occurrence of orange color in the amyl alcohol layer with the addition of magnesium metal and concentrated hydrochloric acid. Saponins are characterized by foam formation and do not disappear with the addition of 2N hydrochloric acid. Tannins are characterized by the occurrence of blue-black or green-black color with the addition of an iron (III) reagent.The results of phytochemical screening that have been obtained can provide important information about the chemical compounds contained in agarwood bark. After knowing the chemical compounds contained, it will be easier to determine their use, especially in the field of medicine. Phytopharmacology, namely the initial selection of

the examination of the plant, can prove the presence of certain chemical compounds in the plant that can be related to its biological activity in the pharmaceutical field.

3.4 Antioxidant Ability Test

An antioxidant activity test on methanol extract of agarwood bark was carried out using the DPPH (1,1-diphenyl-2-picrylhydrazyl) method. DPPH as a free radical will react with antioxidants and the sample forms DPPH with a colored solution. Antioxidants will donate hydrogen atoms to DPPH radicals to complete the electron deficiency and form more stable antioxidant radicals (Prakash, 2001).The DPPH radical which has unpaired electrons have a purple-violet complementary color with a maximum absorbance at a wavelength of 515-520 nm with methanol as solvent (Rohmaniyah, 2016). DPPH solution with a concentration of 50 µg/mL which had been incubated in the dark for 30 minutes at 37ºC was measured and obtained a maximum wavelength of 515 nm (Figure a).The determination of operating time (working time) aims to determine the exact time required by the DPPH radical to obtain a stable solution. Working time is indicated by the constant absorbance value obtained at a certain time range of 0–30 minutes. The results of determining the operating time of the DPPH solution with a concentration of 50 g/mL obtained an absorbance of 0.502 in the 3rd to 5th minute (Figure b). So at that minute, it is a good working time for measuring samples of various concentrations.

Measurement of the absorbance of the DPPH solution without the addition of the test material at the maximum wavelength obtained, namely 515 nm with a concentration of 50 µg/mL obtained an average absorbance result of 0.421, the result is declared good because it lies in the range of 0.2-0.8 nm, where this absorbance was used to calculate the percent attenuation (% inhibition).Measurement of DPPH absorbance after addition of agarwood bark methanol extract with a concentration of 20; 40; 60; 80 and 100 µg/mL were carried out at the maximum wavelength obtained, namely 515 nm. The principle of this method is that DPPH compounds that do not react with antioxidants (remaining) will be read as absorbance values at a wavelength of 515 nm in methanol solvent and can be seen organoleptically through a color change from purple to light purple, or light yellow. The DPPH absorbance after the addition of methanol extract of agarwood bark at various concentrations is shown in Table 3.2 and Figure c.

Table 3.2. The result of the measurement of DPPH absorbance after the addition of a methanol extract of Agarwood bark

No	Sample	Concentration	Absorbance
1	Agarwood Bark Methanol Extract	20 µg/mL	0,390
2		40 µg/mL	0,342
3		60 µg/mL	0,291
4		80 µg/mL	0,235
5		100 µg/mL	0,201

Table 3.2 shows the higher the concentration, the lower the absorbance, and the more DPPH is attenuated. The results obtained show that at a concentration of 100 µg/mL lower than the concentration of 80 µg/mL as well as the others.Measurement of DPPH absorbance after the addition of vitamin C raw material was carried out at a maximum wavelength of 515 nm, with a concentration of 4 µg/mL; 8 µg/mL; 12 µg/mL; 16 µg/mL; and 20 µg/mL. Then the results of the absorbance of DPPH after the addition of vitamin C in various concentrations were obtained in Table 3.3 and Figure d.

Table 3.3. DPPH absorbance measurement results after the addition of vitamin C

No	Standard	Concentration	Absorbance
1	Vitamin C	4 µg/mL	0,301
2		8 µg/mL	0,261
3		12 µg/mL	0,223
4		16 µg/mL	0,215

5		20 µg/mL	0,202

Table 3.3 shows that the higher the concentration, the lower the absorbance and the more DPPH attenuated.The DPPH solution after being added with methanol extract, agarwood bark, and standard vitamin C changed color, from purple to light purple and light yellow, thereby reducing the absorbance of DPPH (attenuation). The color change occurs because the radical electron acceptor from secondary metabolite compounds in the sample is against the DPPH compound so that it becomes a non-radical compound, indicating that the higher the sample concentration, the greater the sample's ability to ward off free radicals. This is due to the increasing number of hydrogen atoms donated by secondary metabolites in DPPH compounds (Rahayu, 2010). The results of determining the % damping can be seen in Table 3.4

Table 3.4. Percent Yield of Reduction of Methanol Extract and Vitamin C

Sample	% Inhibition of various concentrations				
	20 µg/mL	40 µg/mL	60 µg/mL	80 µg/mL	100 µg/mL
Methanol Extract	7.28	18.84	30.88	44.18	52.18
	% Inhibition of various concentrations				
	4 µg/mL	8 µg/mL	12 µg/mL	16 µg/mL	20 µg/mL
Vitamin C	12.08	23.68	34.79	37.13	41.03

The parameter used to determine the ability of the compound as an antioxidant is IC_{50}. The IC_{50} value is the concentration of antioxidant compounds needed to reduce DPPH radicals by 50%. The IC_{50} value is obtained from the linear regression equation, which states the relationship between the concentration of the extract or test fraction as the x-axis and the % reduction as the y-axis. The smaller the IC_{50} value, the more active the extract is as a DPPH radical scavenging compound or antioxidant compound. The measurement results of the antioxidant activity of the methanol extract of agarwood bark and vitamin C showed that standard vitamin C was four times stronger as an antioxidant than the methanol extract of agarwood bark.

The methanol extract of agarwood bark obtained an IC_{50} value of 94.59 µg/mL in the category of strong antioxidant activity, while the IC_{50} value of vitamin C was 22.11 µg/mL in the very strong category.Compounds that are classified as natural antioxidants are also extracted in the phenol or polyphenol test compounds, which can be in the form of flavonoids, cinnamic acid derivatives, and tocopherols. The flavonoid group that has antioxidant activity includes flavonols, isoflavones, flavones, catechins, flavanones, and chalcones (Kumalaningsih, 2006). These flavonoid compounds act as free radical scavengers because the hydroxyl groups they contain donate hydrogen to radicals. These compounds can neutralize free radicals by donating electrons to free radicals so that atoms with unpaired electrons get electron pairs and are no longer radicals (Silalahi, 2006).

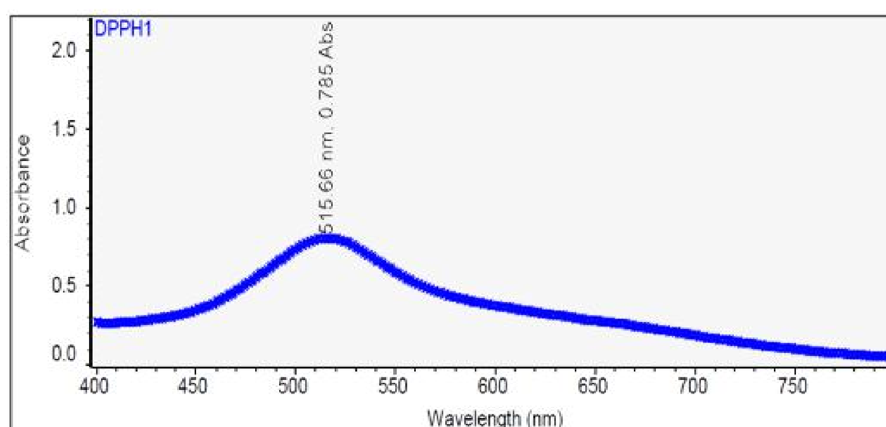

Fig A. Result of Determination of Maximum Absorption Curve of DPPH Solution in Methanol by Visible Spectrophotometry

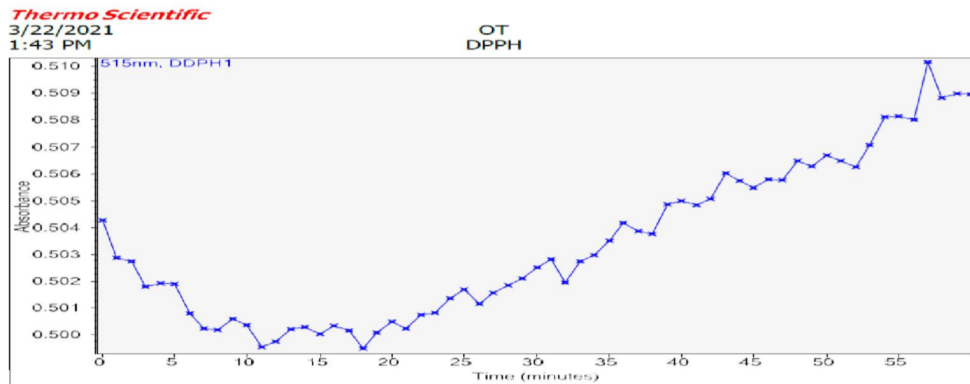

Fig B. Results of Operating Time of DPPH Solution

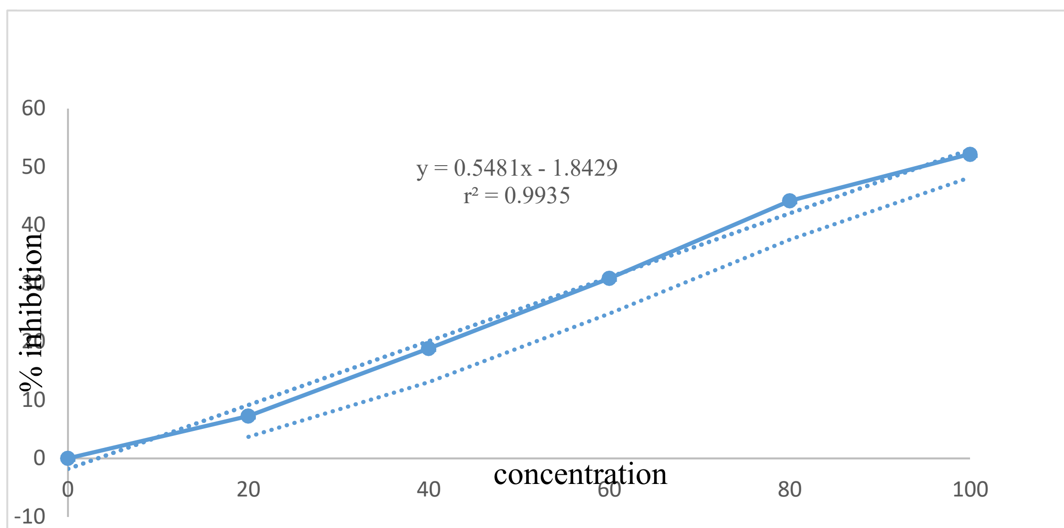

Fig C. Result of Absorbance Measurement of 50 µg/mL DPPH Solution in addition of Agarwood Bark Extract Solution in Various Concentrations

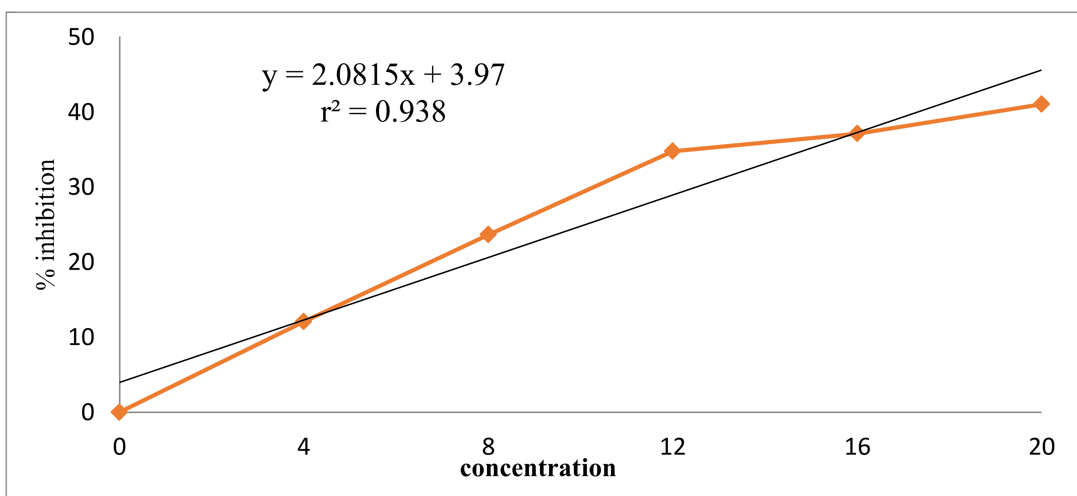

Fig D. Result of Absorbance Measurement of 50µg/mL DPPH Solution in addition of Vitamin C in Various Concentrations

IV. CONCLUSION

The results of the examination of agarwood bark extract showed the presence of flavonoids, saponins, and tannin compounds. The methanol extract of agarwood bark has a strong category of antioxidant activity with an IC_{50} value of 94.59 μg/mL and vitamin C in a very strong category with an IC_{50} value of 22.11 μg/mL.

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