

Antioxidant Activity Etanolic Extract from White Mangrove Leaf (*Avicennia marina*) by FRAP (Ferric Reducing Antioxidant Power) Method

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Abstract

White mangrove (*Avicennia marina*) is one type of mangrove that has potential as a natural antioxidant. Antioxidants are compounds that can eliminate and withstand the effects of negative reactions from free radicals. This aim study to determine the antioxidants of the ethanolic extracts of the mangrove leaves of white mangrove by using the FRAP (Ferric Reducing Antioxidant Power) method. White mangrove leaves (*Avicennia marina*) were extracted by maceration with 96% ethanol solvent. Then fractionation was carried out, resulting in three fractions : n-hexane, ethyl acetate and ethanol. These three fractions were then tested for antioxidant activity using the FRAP (Ferric Reducing Antioxidant Power) method. This method use a UV-Vis spectrophotometer with a wavelength of 720 nm and the total value of antioxidant activity is calculated based on the absorbance of the data obtained. Based on the calculation results show that ethanol extract, hexane fraction, ethyl acetate and ethanol of white mangrove leaves (*Avicennia marina*) have antioxidant activity respectively 2.7906; 2.71808; 2.78178 and 2.7854 mgAAE/g extract.

Keywords: *Avicennia marina*, Antioxidants, FRAP, Extracts, Fractions

INTRODUCTION

Antioxidants are important compounds that protect the body from free radicals. Free radicals can be caused by the body's metabolism (endogenous) or from the surrounding environment (exogenous). The formation of free radicals produced by the body is the result of oxidation from the process of transformation of metabolic energy. Free radicals are the main cause of various chronic and degenerative diseases, such as coronary heart disease, inflammation, stroke, diabetes mellitus and cancer (Mohan & Anand, 2019). Then the free radicals produced by the surrounding environment occur due to exposure to ROS (Reactive Oxygen Species) (Jatmika, Maggadani, & Hayun, 2015). Excessive exposure to ROS results in oxidative damage to macro-molecules. Antioxidants have ability to neutralize harmful ROS by inhibiting oxidative chain reactions, preventing lipid peroxidation, reducing the concentration of free radicals and metal ion chelating (Qasim et al., 2017).

Based on the source, antioxidants are classified into two types namely synthetic and natural. Examples of synthetic antioxidants that are often used by people include butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), tert-butylhydroquinone (TBHQ) and propyl gallate (PG). Synthetic antioxidants have the advantage that their anti-radical activity is very strong. But the weakness of this synthetic material is also very dangerous, which has potentially carcinogenic properties (Mardiono Jacob, Purwaningsih, & Rinto, 2011).

Avicennia marina mangrove is a type of mangrove that is spread throughout Indonesia and has many benefits because it contains compounds with potential medicinal abilities. Organic compounds from *Avicennia marina* mangrove plants have antioxidant, antimalarial, antisitotoxic, antibacterial activities, and so on (Mardiono Jacob et al., 2011). Based on previous research, this

study aims to determine the presence of antioxidant activity with the FRAP (Ferric Reducing Antioxidant Power) method in *Avicennia marina* extract.

METHOD

Research design

This research is an experimental research to determine the antioxidant effect in *Avicennia marina* mangrove extract with FRAP method.

Tools

The tools used in this research are UV-Vis spectrophotometer, rotary evaporator, digital weighing scale, blender, glass bottle, oven, watch glass, measuring flask, chemical beaker, micropipette, clamp, centrifuge, test tube, centrifuge tube, separating funnel, mouthpiece, spatula, pH meter and measuring cup.

Materials

Aquades, ascorbic acid, methanol, oxalic acid 1%, ethanol 96%, trichloroacetic acid 10%, FeCl₃ 0.1%, phosphate buffer, white mangrove leaf extract (*Avicennia marina*), potassium ferrisianida 1%, filter paper.

Sample processing

White mangrove leaf samples (*Avicennia marina*) were taken in the province Batang. The leaves are cleaned of dirt attached to the leaves using running water and then dried in the oven. After dry, simplicia is blended to a small size (powder) and then extracted by maceration method.

Making Ethanol Extract from White Mangrove Leaves (*Avicennia marina*)

Five hundred grams simplicia leaf powder from mangrove was put into a glass jar then added 96% ethanol as much as 1.5 mL. Keep the simplicia in jar and left for 1x24 hours and then stirred repeatedly. Filtering is done 3 times using a new liquid filter in the form of 96% ethanol. The results obtained were evaporated using *rotary evaporatory* until thick extracts were obtained.

Fractionation

Fractionation is done using separating funnels with non-polar, semi-polar, and polar fractions. Viscous extract was taken as much as 5 mg and then added as much as 1 pipette methanol then dissolved with ethanol up to 50 mL. Because the extract is in the form of leaves, it is possible that the extract still contains chlorophyll, so chlorophyll removal is carried out by using aquades non CO₂ with a separating funnel and then the waste is discarded. Separation was carried out using N-hexane 50 mL in chlorophyll free extract with 3 repetitions. Fractionation was carried out again using 50 ml of ethyl acetate with 3 repetitions. The results of fractionation of N-hexane and ethyl acetate are evaporated with *rotary evaporatory* until they become thick.

Preparation of Standard Curve Solution

For a standard 1000 ppm curve solution made by dissolving 25 mg of ascorbic acid dissolved with 1% to 25 mL oxalic acid in a measuring flask. From the standard stock curve of 1000 ppm, each taken 0.6; 0.7; 0.8; 0.9 and 1.0 mL. Each was diluted with 1% to 10 mL oxalic acid and homogenized. So that the concentration of standard solutions obtained 60, 70, 80, 90, 100 ppm.

Preparation of Phosphate buffer solution

NaOH was weighed as much as 2 grams and then dissolved with aquadest non CO₂ up to 250 mL in a measuring flask. Then KH₂PO₄ weighed as much as 6.8 grams and then dissolved with aquadest non CO₂ up to 250 mL in a measuring flask. NaOH solution that has been made, taken as much as 16.4 mL and added KH₂PO₄ to 50 mL in a measuring flask. Then the solution is measured with a pH meter to get a pH of 6.6 and given aquadest non CO₂ up to 200 mL.

Oxalic acid weighed as much as 1 gram and then dissolved with aquadest non CO₂ in a measuring flask up to 100 mL. Potassium Ferrisianide is weighed as much as 1 gram then diluted with aquadest non CO₂ in a measuring flask up to 100 mL. FeCl₃ was weighed as much as 0.1 gram then diluted with distilled water in a measuring flask up to 100 mL. Trichloroacetic acid (TCA) is taken as much as 10 grams and then diluted with distilled water in a measuring flask up to 100 mL.

Antioxidant Activity with FRAP Method

Mangrove leaf extract was taken as much as 5 mg then dissolved in 5 mL 96% ethanol then taken as much as 1 mL, added 1 mL of phosphate buffer 0.2 M (pH 6.6) and 1 mL K₃Fe (CN)₆ 1% and incubated for 20 minutes at 50°C. after incubation, 1 mL of TCA was given and then centrifuged at 3000 rpm for 10 minutes. After centrifuge, 1 mL of the upper layer was taken into the test tube and then added 1 mL of distilled water and 0.5 mL of 0.1% FeCl₃. The solution was allowed to stand for 10 minutes and the absorbance was measured at 720 nm. The blank used is a mixture of oxalate solution. Calibration curves are made using ascorbic acid solutions with various concentrations. The FRAP value is expressed in mg equivalent of ascorbic acid / gr extract.

N-hexane and ethyl acetate fractions were taken as much as 5 mg then dissolved in 5 mL 96% ethanol then taken as much as 1 mL, added 1 mL of phosphate buffer 0.2 M (pH 6.6) and 1 mL K₃Fe (CN)₆ 1% and incubated for 20 minutes at 50°C. after incubation, 1 mL of TCA was given and then centrifuged at 3000 rpm for 10 minutes. After centrifuge, 1 mL of the upper layer was taken into the test tube and then added 1 mL of distilled water and 0.5 mL of 0.1% FeCl₃. The solution was allowed to stand for 10 minutes and the absorbance was measured at 720 nm. The blank used is a mixture of oxalate solution. Calibration curves are made using ascorbic acid solutions with various concentrations. The FRAP value is expressed in mg equivalent of ascorbic acid / gr extract.

RESULTS AND DISCUSSION

From the results of measurements using a UV-Vis spectrophotometer obtained the absorbance value of the comparative solution of ascorbic acid as follows in Table 1. Then from the measurement of absorbance and antioxidant activity of mangrove leaves with ethanol extract and 3 non-polar, semi-polar and polar fractions, as follows in Table 2.

Table 1. Results of ascorbic acid Absorbance

| Concentration (ppm) | Absorbance Value |
|---------------------|------------------|
| 60 | 0,358 |
| 70 | 0,454 |
| 80 | 0,672 |
| 90 | 0,760 |
| 100 | 0,810 |

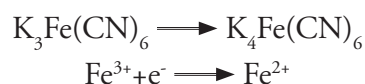
Table 2. Results of antioxidant activity of mangrove leaves

| Solution | Absorbance (720 nm) | Antioxidant Activity (mgAAE / g extract) |
|-----------------------|---------------------|--|
| N-Hexan Faction | 1,5958 | 2,7180 |
| Ethyl Acetate Faction | 2,2328 | 2,7818 |
| Ethanol Faction | 2,3110 | 2,7896 |
| Ethanol Extract | 2,3218 | 2,7906 |

The bioactive compounds from the mangrove leaves of the fires are flavonoids, steroids and reducing sugars. Fire leaves have antioxidant activity and able to inhibit the oxidation process in oil emulsions (Mardiono Jacob et al., 2011). This research was conducted with the aim of testing to measure antioxidant activity in mangrove leaf samples from white fires (*Avicennia marina*). Antioxidant testing is done by the FRAP test method, this test is carried out because this method has a simple, inexpensive, fast procedure and the reagents used are quite simple.

The standard solution used is ascorbic acid, used as a comparison because it has a function as a secondary antioxidant that is capturing free radicals and preventing chain reactions. Vitamin C is a secondary antioxidant group that is able to ward off various extracellular free radicals. This is because vitamin C has a free hydroxy group that acts as a catcher of free radicals and polyhydroxy groups will increase antioxidant activity (Maryam, Baits, & Nadia, 2015).

Measurement of antioxidant activity by the FRAP method with ascorbic acid solution as a standard solution. The addition of TCA in this study aims to make potassium ferrosianide settle. The addition of $FeCl_3$ also aims to form green to blue complexes. Reducing power is an indicator of the potential of an antioxidant compound. The reducing power is measured by the ability of an antioxidant to convert Fe^{3+} to Fe^{2+} . Compounds that have a reducing power that acts as antioxidants because they can stabilize radicals by donating electrons or hydrogen atoms so that radical compounds change to be more stable. Reactions that occur:



The results of linear regression of concentration (x) with absorbance values (y) of the comparative solution of ascorbic acid obtained an equation that is $y = -0,28732 + 0.01123x$ with a value of $R^2 = 0.81734$ and to calculate the antioxidant activity the absorbance value of the sample is entered into the equation. The FRAP value is expressed in mg equivalent of ascorbic acid / gr extract (AAE). The content of vitamin C in each replication is expressed as an ascorbic acid equivalent or *Ascorbic Acid Equivalent* (AAE). AAE is a general reference for measuring the amount of vitamin C contained in an ingredient. The results of absorbance measurements and antioxidant activity value of ethanol extract of mangrove leaves of white fires (*Avicennia marina*) are listed in the results of the study so that the average value of the antioxidant samples of ethanol extract of mangrove leaves of white fires (*Avicennia marina*) is 2.77 mgAAE / g extract, meaning that in every gram is equivalent to 2.77 mg of ascorbic acid.

CONCLUSION

From the results of testing the antioxidant activity of white mangrove extract (*Avicennia marina*) using the FRAP method with a comparative solution of ascorbic acid, the antioxidant activity of white fire mangrove extract (*Avicennia marina*) was 2.77 mgAAE / g extract.

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