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Antioxidant Activity of Mangrove *Sonneratia caseolaris L* using the FRAP Method

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Abstract

Antioxidants are compounds that have an important role in maintaining health because they can ward off free radicals that inhibit oxidation reactions in the body that cause various diseases. Antioxidant compounds are many flavonoid compounds found in mangrove plants. The purpose of this study was to determine the antioxidant activity of red pidada leaves (*Sonneratia caseolaris L*) using the Ferric Reduction Antioxidant Power (FRAP) method with vitamin C as a comparison. The research process begins with the extraction of red pidada (*Sonneratia caseolaris L*) leaves with ethanol. The extraction process was carried out by maceration method for 3 x 24 hours @ 24 hours and continued with fractionation. The maserate obtained was then evaporated with a rotary evaporator at a temperature of 40 - 60^{O}C obtained a thick extract, then the fractions obtained were non-polar, semi-polar and polar fractions. The results showed antioxidant activity; ethanol extract, non-polar, semi-polar and polar fractions were 6.37; 5,35; 6.59 and 5.96 mgAAE / g.

Keywords : *Sonneratia caseolaris L, extract, fraction, Antioxidants, FRAP*

INTRODUCTION

Today the world of medicine and health talks a lot about free radicals and antioxidants. Free radicals are atoms or groups that have unpaired electrons in their outer shells and are reactive (Winarsi, 2007). Free radicals are the cause of various diseases, especially degenerative diseases such as coronary heart disease, stroke, cancer, kidney failure, and premature aging (Maryam *et al.*, 2017). Free radicals we often encounter in the environment, cigarette smoke, vehicle smoke, food in addictive packaging are examples of free radicals (Stevi, G, 2012).

In counteracting the reaction of free radicals in nature the body produces anti-oxidant compounds. Antioxidants are compounds that can inhibit oxidation reactions, by binding to free radicals and molecules that are very reactive so that they can protect cells from the danger of free radicals generated from the body's metabolism and other external factors (Maesaroh, *et al.*, 2018). The human body produces antioxidant compounds such as the enzyme SOD (superoxide dismutase), glutathione, and catalase, but the amount is often insufficient so it requires exogenous antioxidants to counteract the effects of free radicals. Based on the source of the intake, exogenous antioxidants consist of natural antioxidants and synthetic antioxidants (Sukmawati *et al.*, 2015).

Antioxidants are compounds at low concentrations that can prevent or slow the oxidation of easily oxidized biomolecules such as lipids, proteins or DNA. Antioxidants can ward off free radicals, so they can prevent oxidative damage. There are two categories of antioxidants, namely synthetic and natural. Natural antioxidants, especially those found in fruits and vegetables, can reduce the risk of heart disease and cancer. The defensive effects of natural antioxidants in fruits and vegetables are related to three main groups: vitamins, flavonoids, and carotene. Ascorbic acid and flavonoids are known as hydrophilic antioxidants, while carotenoids are known as lipophilic antioxidants (Labiad *et al.*, 2017). One class of compounds that are antioxidants is the flavonoid

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group. Flavonoid compounds have the potential as an antioxidant because they can donate electrons to stabilize radicals in the presence of the -OH group attached to the aromatic ring carbon, free radical products are resonant stabilized and not reactive with most other free radicals (Apak *et al.*, 2013).

One of the plants that have the potential as an antioxidant is the red pidada plant (*Sonneratia caseolaris*). This plant contains alkaloids, flavonoids, glycosides, saponins and phenols (Avenido, P. and Serrano, A.E. 2012). Varghese et al (2010) reported that Pidada has 24 components including 8 steroids, 9 triterpenoids, and 3 flavonoids, and 4 benzene carboxyl derivatives. Wu et al (2009) reported that pedada contained triterpenoids and sterols. Pidada rind contains flavonoids which function as antioxidants. Flavonoid compounds found in plant extracts can also be used as antioxidants and anti-rheumatism (Sadhu *et al.*, 2006).

During its development a variety of measurement methods have been developed to measure the characteristics of total antioxidants, but none are truly ideal. The method of measuring antioxidant activity will detect different characteristics of antioxidants in the sample, this explains why different methods of measuring activity will refer to observing the mechanism of action of different antioxidants (Hasannbaglou, *et al.*, 2012). Some of the methods used are DPPH, CUPRAC, FRAP.

According to Benzie & Strain (1996) that the Ferric Reduction Antioxidant Power (FRAP) method is a method used to test antioxidants in plants. The advantage of the FRAP method is that the method is inexpensive, the reagents are easy to prepare and are quite simple and fast. The FRAP method can be used to determine the total antioxidant content of an ingredient. The principle of the FRAP method is based on the ability of antioxidant compounds to reduce Fe3 + ions to Fe2 + so that the antioxidant power of a compound is analogous to the ability to reduce it (Halvorsen, *et al.*, 2002).

Based on research that has been done previously in this study will be tested for antioxidant activity from ethanol extract and fraction of red pepada mangrove leaves (*Sonneratia caseolaris*) using FRAP (Ferric Reducing Antioxidant Power) method. The principle of the FRAP test is the electron transfer reaction from antioxidants to Fe3 + - TPTZ compounds. Fe3 + - TPTZ compounds themselves represent oxidizing compounds that may be present in the body and can damage cells.

METHODS

Processing Samples and Extraction

Sampling of red pidada mangrove leaves (*Sonneratia caseolaris*) is cleaned of dirt attached to the leaves using flowing water and then dried by aerating. After drying, blend and then it is ready to be extracted by maceration method. Red pidada mangrove leaf powder was extracted by maceration using 96% ethanol solvent for 1x24 hours. The waste is remarried 3 times. Maserat is concentrated with a rotary evaporator at a temperature of 40 – 60^{O}C.

Test Solution

1. Phosphate buffer solution 0.2 M pH 6.6

   The solution is prepared by weighing 2 grams of NaOH and dissolved with CO_2-free aquades to exactly 250 mL in a measuring flask. Then as much as 6.8 grams of KH_2PO_4 were dissolved with 250 mL CO_2-free aquades in a measuring flask. Then pipetted as much as 16.4 mL NaOH was put into a measuring flask and mixed with 50 mL KH_2PO_4, then measured to pH 6.6 and sufficient with CO2-free aquades to 200 mL.

2. Oxalate 1%

   The solution is prepared by dissolving 1 gram of oxalic acid in CO_2 free water and diluting it in a 100 mL measuring flask.

3. Potassium Ferrisianide 1 %

   The solution is prepared by dissolving 1 gram of potassium ferrisianide in distilled water and diluted in a 100 mL measuring flask.

4. $FeCl_3$ 0.1 %

   The solution is prepared by dissolving 0.1 gram of FeCl3 in distilled water and diluted in a 100 mL measuring flask.

5. Trichloroacetic acid (TCA) 10%

   The solution is prepared by dissolving 10 grams of TCA in aquades and diluted in a 100 mL measuring flask.

6. Preparation of Ethanolic Extract Samples

   Ethanol extract and fraction were weighed with 3 replications, each of 10 mg. Each extract and fraction was dissolved with 96% ethanol as much as 10 mL. then homogenized.

7. Standard Curve Solution

   Stock solution is made by dissolving 25 mg of ascorbic acid dissolved with 1% oxalic acid to the limit of 25 mL measuring flask. Furthermore, from the 1000 ppm stock solution each taken 0.6; 0.7; 0.8; 0.9; and 1.0 mL and placed in a different 10 mL volumetric flask and diluted with 1% to 10 mL oxalic acid and homogenized to obtain ascorbic acid concentration series of 60, 70, 80, 90, 100 ppm.

Antioxidant Activity with the FRAP Method

1 mL of sample was taken, added 1 mL of 0.2 M phosphate buffer (pH 6.6) and 1 mL of $K_3Fe(CN)$ 6% after that, incubated for 20 minutes at 50 °C. After incubation, 1 mL of TCA was added and centrifuged at 3000 rpm for 10 minutes. After disentifuge 1 mL pipette top layer into the test tube, and added 1 mL aquades and 0.5 mL $FeCl_3$ 0.1%. The solution was allowed to stand for 10 minutes and the absorbance was measured at 720 nm. As a blank, a mixture of oxalate solution is used. 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

Table 1. The measurement results of absorbance of the ascorbic acid

Concentration (ppm)	Absorbance
60	0,281
70	0,393
80	0,481
90	0,583
100	0,675

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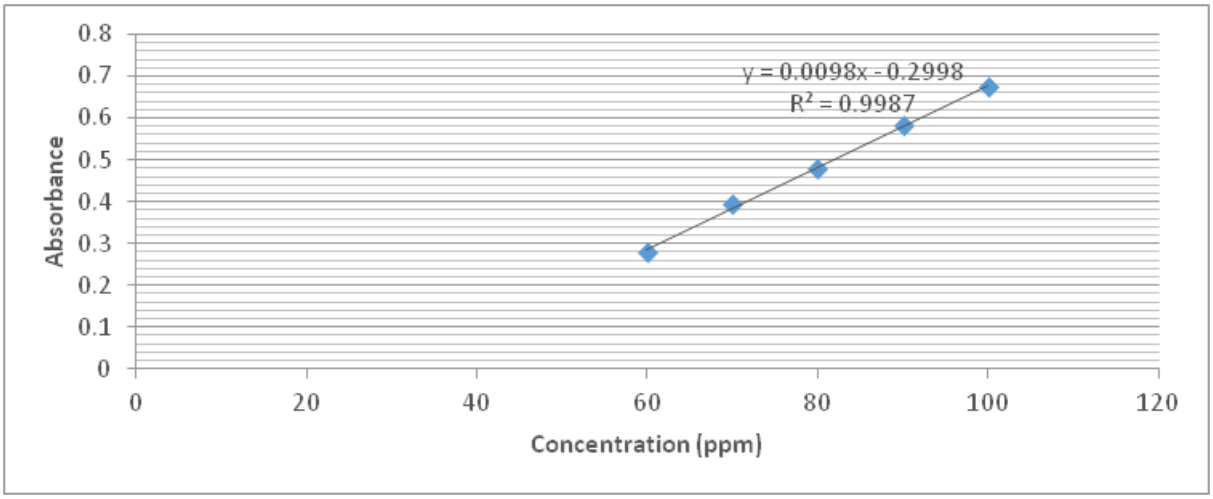

Figure 1. Graph of Standard Curve of Ascorbic Acid

Table 2. The measurement results of absorbance and antioxidant activity value of extracts of red pidada mangrove leaves (*Sonneratia caseolaris*)

Sample	Replication	Absorbance	Antioxidant Activity (mgAAE/g exstract)	Average
Ethanol Extract	1	0,325	6,38	6,37
	2	0,318	6,30	
	3	0,33	6,43	
Non-Polar Fraction	1	0,226	5,37	5,34
	2	0,228	5,39	
	3	0,218	5,28	
Semi-Polar Fraction	1	0,34	6,53	6,59
	2	0,347	6,60	
	3	0,351	6,64	
Polar Fraction	1	0,28	5,92	5,95
	2	0,283	5,95	
	3	0,288	6,00	

To reduce the activity of free radicals needed compounds that can delay or prevent oxidation or called antioxidants. One class of compounds that are antioxidants is the flavonoid group. Flavonoid compounds have the potential as an antioxidant because they can donate electrons to stabilize radicals in the presence of the -OH group attached to the aromatic ring carbon, free radical products are resonant stabilized and not reactive with most other free radicals (Apak *et al.*, 2013).

One of the plants that have the potential as an antioxidant is the red pidada plant (*Sonneratia caseolaris*). This plant contains alkaloids, flavonoids, glycosides, saponins and phenols (Avenido, P. and Serrano, A.E. 2012). Wu *et al* (2009) reported that pedada contained triterpenoids and sterols. Pidada rind contains flavonoids which function as antioxidants. Flavonoid compounds found in plant extracts can also be used as antioxidants and anti-rheumatism (Sadhu *et al.*, 2006).

The purpose of this study was to determine the total antioxidant capacity present in the extract sample and the Pidada red leaf fraction. In this study antioxidant activity was measured using the Ferric Reduction Antioxidant Power (FRAP) method. This FRAP testing method was chosen because the procedure is simple, the method is cheap, fast and the reagents used are quite simple and do not use special tools to calculate total antioxidants. In addition to the advantages above the FRAP method also has several disadvantages in this method which does not react quickly with some antioxidants such as gluthathion (Rabeta & Faranisa, 2013).

Ascorbic acid is used as a comparison because it functions as a secondary antioxidant that captures free radicals and prevents chain reactions. Ascorbic acid is a secondary antioxidant group that is able to ward off various extracellular free radicals. That is because ascorbic acid has a free hydroxy group that acts as a free radical scavenger and if it has a polyhydroxy group will increase antioxidant activity (Kim, 2005).

The principle of the FRAP method is based on the ability of antioxidant compounds to reduce Fe3 + ions to Fe2 + so that the antioxidant power of a compound is analogous to the ability to reduce it. The addition of TCA solution serves to precipitate the potassium ferrosianide complex. While the addition of $FeCl_3$ serves to form a green to blue complex (berlin blue). So the reduction ability can be determined by measuring the color complex at 720 nm. Compounds that have the power to reduce are likely to act as antioxidants because they can stabilize radicals by donating electrons or hydrogen atoms so that radical compounds turn out to be more stable.

From the measurement of ascorbic acid absorbance as a comparison, the regression results obtained y = 0.0098x - 0.2998 with the value R^2 = 0.9987 where the concentration (x) and absorbance absorbance (y) (table 1). To calculate the value of antioxidant activity carried out by entering the absorbance value of the sample into the equation. The FRAP value is expressed in mg equivalent of ascorbic acid/g extract (AAE). Ascorbic Acid Equivalent (AAE) is a standard for measuring the amount of ascorbic acid contained in an ingredient. The results of absorbance measurements and the antioxidant activity value of ethanol extract and red pidada leaf fraction can be seen in table 2. The results showed the antioxidant activity of ethanol extract, non-polar, semi-polar and polar fractions was 6.37; 5,35; 6.59 and 5.96 mgAAE/g extract (equivalent mg of ascorbic acid/g extract).

The ability of extracts to reduce iron (FRAP) shows that they contain compounds that are electron donors, which can react with free radicals to turn them into more stable products and stop radical chain reactions. The FRAP test showed a positive correlation between power reduction and phenolic content in red pidada extract. Reported by Rice-Evans *et al.*, 1997 that phenolic compounds have redox properties, which enable them to act as reducing agents, hydrogen donors, and singlet oxygen coolers. The redox potential of phenolic compounds plays an important role in determining antioxidant potential.

CONCLUSION

From the results of testing the antioxidant activity of ethanol extract and fraction of red pidada leaves (*Sonneratia caseolaris*) using the FRAP method with a comparative solution of ascorbic acid, the antioxidant activity of the ethanol extract antioxidant activity, non-polar, semi-polar and polar fractions was 6.37; 5,35; 6.59 and 5.96 mgAAE / g extract (equivalent mg of ascorbic acid/g extract).

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