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ANTIOXIDANT ACTIVITY OF Rhizophora mucronata MANGROVE FRUIT IN LANGGE VILLAGE, ANGGREK DISTRICT, GORONTALO REGENCY NORTH

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Abstract

Rhizophora mucronata is one of the many mangrove plants found on the coasts of countries in Asia, including Indonesia. The fruit of this plant is widely used as medicine and food and is believed to have potential as a source of antioxidants. The purpose of this study was to study the antioxidant activity of R. mucronata fruit with a diameter of 2–3 cm using different solvents, namely ethanol (food grade) and water (Aquades). The antioxidant activity of mangrove fruit was tested using the DPPH (1,1-diphenyl-2-picrylhydrazil) method. The results of the analysis showed that R. mucronata fruit extracted using water (aquades) had an IC50 value of 40.54 ppm while using ethanol (food grade) the IC50 value was 18.94 ppm.

PRELIMINARY

Mangroves have many benefits that are directly related to human life on land, ranging from ecological benefits to as a food source where extracts and raw materials from mangrove plants have been used by coastal communities for natural medicinal purposes. People use mangroves as traditional medicine because they have a very high potential for bioactive content, one of which can be used as an antioxidant (Spalding*et al.*, 2010).

The coastal area of Gorontalo Province, especially Langge Village, Anggrek District, North Gorontalo Regency, there is an area known as the "tracking mangrove" area where there is a variety of mangrove vegetation. According to Baderan and Kumaji (2017) that true mangrove plant species found in mangrove areas in Langge Village, Anggrek District, North Gorontalo Regency at the tree, sapling and seedling levels are

Soneratia alba, Soneratia ovata, Avicennia alba, Avicennia marina, Ceriops tagal, Ceriops decandra, Rhizophora mucronata, Rhizopora apiculata, Rhizophora stylosa, Xylocarpus granatum, Burgueira gymnorhiza and Burgueira parviflora.

Given the importance of the function of the mangrove area, it is necessary to apply or promote the principles of protecting, studying and utilizing. All of this requires coordination between *stakeholders* and communities around the area and environmentalists, especially academics (Arief, 2003).

Purnomobasuki, (2004) stated that traditionally in several areas in Indonesia such as Java, Sulawesi and Maluku, mangrove plants have been used as medicine, drinks and as raw materials for various kinds of cakes. However, this cannot be developed because there is not much knowledge about the potential and benefits of mangrove plants as a source of functional food and as food ingredients.

According to Noor *et al*., (2006), mangrove forests are plants that live along coastal areas that are influenced by the highest tides to areas close to the average height of sea water that grow in tropical and sub-tropical areas. Mangrove forest is a plant community that grows in the tropics and is dominated by plants that have breath roots (pneumatophores) and have the ability to grow in salty waters or the outermost zone (Indriyanto, 2006).

With these environmental conditions, mangroves can produce compounds to protect themselves from damage in the form of antioxidants. According to Percival (1998), phenolic compounds such as flavonoids can be found in almost all types of plants. Flavonoids in plants act as a protector against stresses that come from the environment.

Based on the statement above, the authors feel it is necessary to determine the antioxidant activity of fruit flour *R. mucronata* which is in Langge Village, Anggrek District, North Gorontalo Regency, Gorontalo Province.

RESEARCH METHODOLOGY

Tools and materials

The equipment used are knives, plastic bags, glass jars, *freezer*. Analytical equipment, namely oven, magnetic stirrer centrifuge, vortex, spectrophotometer, analytical balance, glassware, *vacuum rotary evaporator*.

The research raw material is mangrove fruit *R. mucronata* taken from Langge Village, Anggrek District, North Gorontalo Regency, Gorontalo Province. Chemicals used for analysis are methanol 90%, distilled water, distilled water, DPPH, Mg, $H_2SO_4$2N, NaOH, 2N HCl, chloroform, sulfuric acid, anhydrous acetic acid.

Preparation

Fruit *R. mucronata* raw/fresh with fruit size 25–32 cm, brought to the UNG Fisheries Laboratory and then the fruit petals are removed, washed and drained, after that the fruit is thinly sliced, cut into small pieces and then dried by airing in an open space for 3-5 days until dry, after drying then blended into flour.

Extraction

a. Using water solvent: fruit flour *R. mucronata* weighed 1 kg and put in

jar, then added with water solvent (aquades) in a ratio of 1:5 (w/v). Then heated to a temperature of 70-85^{0}C for 30 minutes. When finished heating then cooled and ready to use

b. Using Ethanol solvent (Food grade): Fruit flour *R. mucronata* weighed 1 kg and put into a jar, then added ethanol solvent in a ratio of 1:3 (w/v). Then macerated for 3x24 hours and every 24 hours the ethanol solvent was replaced.

c. The maceration results were then filtered using Wharman filter paper no. 42 to produce the filtrate.

d. The filtrate was extracted with ethanol solvent and then dried with *vacuum rotary evaporator* with a temperature of 40^{o}to obtain a thick extract.

e. The viscous extract obtained was then tested for antioxidant activity using the DPPH method.

Antioxidant Activity Test with DPPH . Method

1. Preparation of 0.1mM . DPPH solution

0.39432 gram of DPPH powder (BM 394.32) was dissolved in 10 ml of methanol pa. The 0.1M DPPH solution was pipetted 100 l into a 100 ml volumetric flask filled with methanol pa to the mark (DPPH 0.1mM).

2. Determination of Maximum Wavelength DPPH

2 ml of 0.1 mM DPPH solution was put into a test tube and then added with 2 ml of methanol, vortexed until homogeneous and then poured into a cuvette and measured at a wavelength of 400-800 nm using a UV-Vis spectrophotometer (Musfiroh and Syarief, 2009). maximum wavelength is 517 nm.

3. Preparation of blank solution

2 ml of 0.1 mM DPPH solution was put into a test tube, added 2 ml of ethanol pa, vortexed until homogeneous, incubated in a dark room for 30 minutes, then the absorption was measured at a wavelength of 517 nm.

4. Preparation of the mother liquor with a concentration of 1000 ppm

A total of 50 mg of the sample was dissolved with ethanol pa and then put into a 50 ml volumetric flask, the volume was made up with ethanol pa to the limit mark.

Table 1.Preparation of concentration series test solution

Concentration	Solution stock (ml)	Ethanol p. a (ml)
25ppm	0.25	10
50 ppm	0.5	10
75 ppm	0.75	10
100 ppm	1	10
125 ppm	1.25	10

5. Measurement of absorption using a UV-Vis spectrophotometer

A total of 2 ml of each concentration of the test solution was put into a reaction tube, added 2 ml of 0.15 mM DPPH solution, vortexed until homogeneous, incubated in a dark room for 30 minutes. Next, the absorption was measured at a wavelength of 517 nm.

6. Determination of Percent inhibition

The radical scavenging activity was expressed as the percentage of inhibition which could be calculated using the following formula:

$$\% \text{ inhibition} = \frac{\text{Control absorbance} - \text{Absorbance of the test material}}{\text{Control absorption}} \times 100\%$$

7. Determination of IC . Value$_{50}$(*Inhibitory Concentration)*

The sample concentration and the percent inhibition were plotted on the x and y axes of the linear regression equation, respectively. The equation is used to determine the value of $IC_{50}$of each sample is expressed as a y value of 50 and the x value to be obtained as IC_{50}(Nurjanah, Izzati & Abdullah, 2011)

Data analysis

The results of observations made were obtained in 2 ways, namely qualitative and quantitative observations. The study was conducted with 3 repetitions so that the average value was obtained.

The data that has been obtained from the absorbance value to be tested for antioxidant activity is then calculated by the formula: % antioxidant activity = absorbance of the blank (absorbance of DPPH)-absorbance of the sample (absorbance of extract) divided by absorbance of the blank (absorbance of DPPH) multiplied by 100%. After getting the percent inhibition, a curve was made between the concentration (x) and the % inhibition (y) and obtained the linear regression equation and the table of antioxidant activity test results.

RESULTS AND DISCUSSION

Fruit DPPH Antioxidant Activity *R. mucronata* with water solvent (Aquades)

The results of the analysis of the antioxidant activity of DPPH from fruit *R. mucronata* can be seen in Table 2 below:

Table 2. Antioxidant activity with water solvent (Aquades)

Cons (ppm)	Test			Flat-flat	% Inhibition	IC50
	U1	U2	U3			
25	0.455	0.496	0.473	0.474	45.39	40.54
50	0.418	0.414	0.426	0.419	51.72	
75	0.354	0.356	0.320	0.343	60.48	
100	0.305	0.303	0.318	0.308	64.51	
125	0.267	0.283	0.277	0.275	68.31	
DPPH Control	0.874	0.865	0.865	0.868	-	

The relationship between the sample concentration and the percentage of inhibition obtained a linear equation, as in Figure 1.

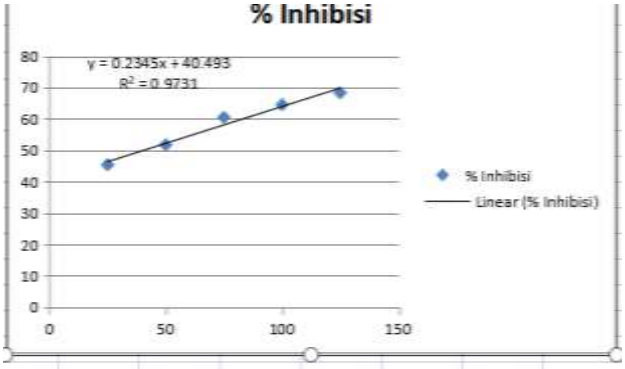

Figure 1. Antioxidant Activity Testing Curve Water Extract by Boiling Fruit Flour *R. mucronata* Using the DPPH Method

After getting the % inhibition data, a graph is made between concentration (x) and % inhibition (y) and the linear regression equation is Y=50, so IC_{50}= 40.54 ppm.

Table 3. Antioxidant activity with ethanol solvent (*food grade*)

Cons (ppm)	Test			Average	% Inhibition	IC50
	U1	U2	U3			
25	0.466	0.421	0.439	0.442	49.07	18.93
50	0.326	0.318	0.310	0.318	63.36	
75	0.293	0.288	0.289	0.29	66.56	
100	0.242	0.235	0.223	0.233	73.15	
125	0.150	0.176	0.157	0.161	81.45	
Control DPPH	0.874	0.865	0.865	0.868	-	

The relationship between the sample concentration and the percentage of inhibition obtained a linear equation, as in Figure 2 below:

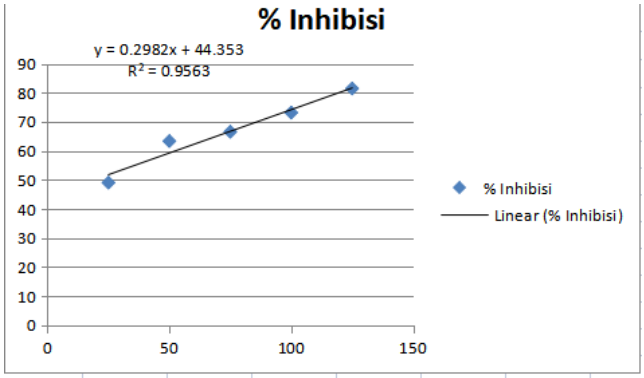

Figure 2. Antioxidant Activity Testing Curve of Ethanol Extract (Food grade) Fruit Flour *R. mucronata* Using the DPPH Method

After getting the % inhibition data, a graph is made between concentration (x) and % inhibition (y) and the linear regression equation is Y=50, so IC_{50}= 18.93 ppm.

Based on these results, it shows that IC_{50} from fruit *Rhizophora mucronata* which were extracted using water (aquades) and ethanol (food grade) solvents had antioxidant properties. From the data above shows the value of IC_{50} using two types of solvents categorized as very strong. This is in accordance with the statement of Molyneux (2004) *in* Purwaningsih *et al.*, (2013) that the value of IC_{50}<50

ppm is a very strong antioxidant, IC_{50}=50–100 ppm strong, 100–150 ppm moderate, 150–200 ppm weak and IC_{50}>200 ppm is categorized as very weak.

The antioxidant activity of each mangrove is different in each place of its habitat. This is because mangroves have characteristics and species composition of each mangrove forest influenced by weather factors, coastal landforms, distance between tides, water availability, oxygen and soil type (LPP mangrove, 2006).

Flavonoids are one of the bioactive compounds that can act as antioxidants. According to Purwaningsih et al., (2013) that one of the fruits that contain high anti-oxidants from mangrove plants is black mangrove fruit (*R. mucronata*). Furthermore, according to Kumar et al., (2009) flavonoids can act as antioxidants because of their properties as good acceptors of free radicals, namely a species that has one or more unpaired electrons in its orbital such as hydroxy radicals and superoxide which are commonly referred to as ROS.*Reactive Oxygen Species*).

CONCLUSIONS AND SUGGESTIONS

Conclusion

1. Mangroves*Rhizophora mucronata*taken around the coast of Langge Village, Anggrek District, contains antioxidants

2 pieces*R. mucronata*extracted using water as a solvent (aquades) has an IC . value$_{50}$is 40.54 ppm while using ethanol solvent (food grade) IC . value$_{50}$is 18.94 ppm

3. IC_{50}fruit*Rhizophora mucronata*measuring 25–32 cm which was taken around the coast of Langge Village, Anggrek District, has an IC . value$_{50}$which is classified as very strong **Suggestion**

Further research is needed to determine the antioxidant activity of mangrove fruit*R. mucronata*with a diameter smaller than 25 cm by comparison using different solvents (polar, semi-polar and non-polar) in order to determine their antioxidant activity.

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