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Antioxidant Activity of Extract Ethanol from the Leaves *Rhizophora mucronata* and *Rhizophora apiculata*

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Abstract: Mangroves are plants that live a lot on the coast of Indonesia. Mangroves have many benefits in traditional medicine such as medicine for wounds, coughs and others but there is not information about the source of antioxidants. Therefore researchers will test mangroves as antioxidants. There are various types of mangrove plants such as *Rhizophora mucronata* and *Rhizophora apiculata*. *Rhizophora mucronata* and *Rhizophora apiculata* contain flavonoid compounds, phenols and tannins. This research process was carried out antioxidant of *Rhizophora mucronata* and *Rhizophora apiculata* mangrove plants. Antioxidant test used a UV-Vis spectrophotometer the method of inhibiting free radical DPPH. This research used positive control of vitamin E which has antioxidant activity with IC50 1.56 μg/mL. The results of the study of the effectiveness of antioxidants using ethanol solvents. IC50 values of 225.24±1.68 and 135.46±1.75 μg/mL, respectively. IC50 value of ethanol extract *Rhizophora mucronata* and *Rhizophora apiculata* was higher than the positive control of vitamin E, so that, *Rhizophora mucronata* and *Rhizophora apiculata* ethanol extract was not potential as an antioxidant. From these results can be identified compounds found in the *Rhizophora mucronata* and *Rhizophora apiculata* mangrove plants are flavonoid compounds, phenols and tannins.

Key words: Antioxidant, *Rhizophora mucronata*, *Rhizophora apiculata*, mangrove, plants, Indonesia

INTRODUCTION

Indonesia is a country with a coastline reaching 81,000 km and 17,504 Islands, along the coastline of which many mangrove plants grow. Mangroves have the ability to live with extreme weather and are able to withstand water abrasion (Lewis III *et al*., 2016). According to the Mangrove Forests of the World (MFW), the area of mangrove growth reached 23,143 (Hamilton and Casey, 2016). More than 30,000 high-level plant species. Up to now 7000 species of plants have been recorded for their efficacy but <300 plants are used as raw materials for the pharmaceutical industry. In 2008 the World Health Organization (WHO) noted that 68% of the world's population still rely on traditional medicine systems, the majority of which use plants to cure diseases and more than 80% of the world's population use herbal medicines for health. Many things can be used to support the need for the development of traditional medicine through the latest scientific research and modern production, so that, it can be used as medicine for the benefit of health and community welfare.

Indonesian people have long known and used medicinal plants such as soursop leaves, bay leaves koja, mangrove leaves and others (Elfahmi *et al*., 2014). The plant is used as an effort to overcome health problems (Guaadaoui *et al*., 2014). Currently, the use of nutritious plants is only based on experience and skills that have been inherited from one generation to the next. In addition, the side effects caused by medicinal plants are also relatively small, if the use is in the right amount and manner (Kennedy *et al*., 2013; Ekor, 2014). Public awareness of the importance of health has increased such as the food processed by the people they consume must contain sufficient nutrition (Sewell and Rafieian-Kopaei, 2014). The more food consumed, the more complete the acquisition of nutrients to produce optimal health. In the current era of globalization antioxidants are the most fundamental thing in the health science discipline. This is based on the increasing knowledge of major diseases preceded by excessive oxidation reactions in the body. Research on antioxidants is increasing because some of the available antioxidants such as BHA and BHT are thought to be carcinogenic (Zhang *et al*., 2015; Borsato *et al*., 2014), Therefore, it is a challenge for researchers to find natural antioxidants (Akbarirad *et al*., 2016).

One of the plants that will be tested and has the potential as an antioxidant is the type of mangrove plant *Rhizophora mucronata* and *Rhizophora apiculate*. In

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previous studies of skin testing, the isolation of the bark of *Rhizophora apiculata* was triterpenes and diterpenoids and phenolic compounds were found in pyroligneous acid *R. apiculata*. *R. apiculatabark* extract showed antioxidant activity. However, the compounds responsible for antioxidant abilities have never been studied before (Gao and Xiao, 2012). While the results of the research on methanol extract from *R. mucronata* skin as an antioxidant agent reported that the active extract as an antioxidant with IC50 level 438.8349 ppm (Mahmiah *et al*., 2017). Because there have not been many studies regarding antioxidant testing of the leaves of *Rhizophora mucronata* and *Rhizophora apiculata*, the researchers will conduct research on the activity test of the radical catcher *Rhizophora mucronata* and *Rhizophora articulate* using the Diphenylpicrylhydrazyl (DPPH) method.

MATERIALS AND METHODS

Instrument: The tools used during the research process are: analytical scales, blenders, evaporators, vacuum, chambers, incubators, UV-Vis spectrophotometers, filtrate containers, centrifugators, glass beakers, measuring flasks.

Simplicia leaves *Rhizophora mucronata* and *Rhizophora apiculata*, ethanol, DPPH (1,1-Diphenyl-2-Pikrilhidrazil) p.a, ethanol p.a, vitamin E.

Research procedure

Extraction: Simplicia leaves of *Rhizophora mucronata* and *Rhizophora apiculata* obtained from the Cilacap mangrove forest in West Java which have been determined by the plants. The leaves are sorted, wet first and then the leaves are dried in the open air (without direct sunlight). After the leaves are dried then mixers use a tool to obtain a fine powder. Simplicia leaves were then extracted using 96% ethanol solvent for 24 h. After that, the filtrate is filtered and the filter residue is used for the next extraction process. The filtrate results are then combined and evaporated with a rotary evaporator.

Determination of the maximum wavelength: Carefully weighed approximately 1.98 mg DPPH (BM 394.32), then dissolved with ethanol analysis pro in 50 mL (40 ppm), then placed in a dark bottle. Enough the solvent until the boundary mark, then shake until homogeneous. Pipette 2 mL of DPPH solution into a test tube. Then add 2 mL of ethanol. And homogeneous with vortex. The mouth of the tube is covered with aluminum foil, then incubated in a dark room for 30 min. Determine the absorption spectrum using a UV-Vis spectrophotometer at a wavelength of 400-800 nm and determine the maximum wavelength.

Test for antioxidant activity: Weighed approximately 50 mg of extract, then dissolved in 50 mL of ethanol pro analysis (concentration of 1000 ppm). This solution is a mother liquor and then made several concentrations, namely 100-500 ppm. The several concentrations were then pipetted as much as 3 mL into a test tube in each test tube added 1 mL DPPH solution. Wait 30 min at room temperature. Then, it was measured using UV-Vis spectrophotometry at a wavelength of 517 nm. As a positive control for testing antioxidant activity, vitamin E was used. As a control method for testing antioxidant activity, vitamin E was used. Weighed approximately, 50 mg of vitamin E, then dissolved in 50 mL of methanol analysis pro. Several concentrations were made, namely 0.5, 1, 2, 3 and 5 ppm. Of the several concentrations were then pipetted as much as 3 mL into a test tube in each test tube added 1 mL DPPH solution. Wait 30 min at room temperature. Then, it was measured using UV-Vis spectrophotometry at a wavelength of 517 nm.

Data analysis: The absorbance value data from an ethanol extract of *Rhizophora mucronata* and *Rhizophora apiculata* leaves as well as the comparison standard were calculated by the formula:

$$\text{Antioxidant activity}(\%) = \frac{\text{Absorbance control} - \text{Absorbance of the treatment}}{\text{Absorbance control}} \times 100\%$$

The IC50 value is obtained by making a line equation that connects the percent inhibition with the concentration of the test solution. IC50 is obtained by calculating the concentration of the test solution which can produce free radical resistance of 50 based on the regression line equation using the formula:

$$Y = ax+b$$

$$Y = 50$$

The data obtained from UV-Vis spectrophotometry is the observance of DPPH control and DPPH which has been reconciled with a sample test solution with various concentrations. The percent of inhibition is useful to get IC50.

RESULTS AND DISCUSSION

Rhizophora mucronata and *Rhizophora apiculata* mangroves used in this study came from Cilacap, West Java. In the study, the part used is the leaves. Because the leaves contain many flavonoid compounds that can be used as antioxidants. In this research what sorting was done first by separating the impurities. The leaves are then dried or aerated for the drying process, namely by

reducing the water content contained in the sample, so as to prevent decay. After the leaves are dry, then, reduce the size using a blender. After extraction is carried out, the extraction aims to extract the compounds contained in the extract.

The extraction of simplicia *Rhizophora mucronata* and *Rhizophora apiculata* was carried out using 96% ethanol. The extraction uses a maceration method. The maceration process was carried out 3 times, replication using 96% ethanol for 24 h and this process was repeated 3 times. The extract obtained was then concentrated using a rotary evaporator until a thick extract was obtained.

From the extraction results, antioxidant testing is done using the DPPH method using a wavelength of 517 nm. DPPH is a synthetic purple free radical that is widely used in testing antioxidant activity. Data from IC50 values showed that *Rhizophora mucronata* and *Rhizophora apiculata* leaf extract had IC50 225.24±1.68 and 135.46±1.75 μg/mL, respectively, indicating that at this concentration had a 50% inhibition of free radical activity DPPH. Vitamin E as a positive control has an IC50 of 1.56 μg/mL. The antioxidant power of *Rhizophora mucronata* and *Rhizophora apiculata* leaf extract was lower than the antioxidant power of vitamin E as a positive control.

CONCLUSION

The results of this study are the data obtained will be analyzed to determine the inhibitory power of antioxidants for antioxidant effects.

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