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ANTIOXIDANT ACTIVITY OF METHANOL EXTRACTS FROM THE STEM BARK OF MANGROVE PLANT *Rhizophora mucronata*

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INTRODUCTION

Numbers of diseases such as stroke, diabetes, gout, and even cancer are caused by the reaction of free radicals (oxidants) found in the body. Those diseases currently have not yet controlled. Deleterious lifestyle, lack of exercise, or genetics can be the trigger to this oxidant. Based on Chemotaxonomic, drugs that have been used to inhibit the oxidation process or termination stage of free radicals contain the active ingredient in the form of secondary metabolites of alkaloids, phenolics, terpenoids and steroids. For example, phenolic compounds such as flavonoids, xanthones, antioxidants and polyphenols is a good agent because it has a structure with a high degree of oxidation (Suares, et al, 2010).

Researches related to the exploration of antioxidant active ingredient has been more focused on secondary metabolites found in terrestrial plants. The development of natural compounds potential of marine plant material such as mangrove are still not received much attention. Spalding *et al*. in 2001 explained that mangroves plant in Indonesia is the highest in the world, both in terms of quantity area (± 42 550 km2) as well as the number of species (± 45 species). This basic natural resource of Indonesia mangrove is certainly valuable promising opportunities to be expanded as a biological drug.

Mangroves plant that commonly used as medicine discovered from various species i.e: Acanthus ilicifolius, Avicennia alba, Avicennia marina, Avicennia offinalis, Bruguiera cylindrical, Bruguiera exaristata, Bruguiera gymnorrhiza, Ceriops tagal, Hisbiscus tiliaceus, Ipomoea pes-capre, Lumnitzera racemosa, Nypa fructicans, Pluchea indica Rhizophora apiculata, Rhizophora mucronata and Sonneratia alba. Those plants are usually utilized as antiasma, antidiuretic, antidiabetic, reliever itching, and others. (Purnobasuki, 2004).

The potential of mangroves as a drug is very important to be developed considering the need for drugs is increasing deals with the growing of population and many kinds of diseases such as cancer, hypertension, tumor diseases and diseases caused by chemical or biological waste pollution from viruses and bacteria. People are more likely to choose drugs that are natural because relatively take few side effects or even none at all.

Several studies of mangrove plants from genus *Rhizophora* that have antioxidant bioactivity are shown in the crude butanol extract of mangrove *R. apiculata* with IC_{50} 33.34 pg / mL (Gao, 2012). The methanol extract of *R. mangle*'s stem were also documented to have antioxidant activity (Palacio, et al, 2014).

One of mangroves found in Surabaya East Coastal (Pamurbaya), East Java, Indonesia is the mangrove *Rhizophora mucronata*. This mangrove species is indigenous mangroves that ethno-botanically popular used as a pain reliever and dyes natural wood. Secondary metabolites contained in the leaves, bark, stems, roots, and fruit are different in quantity. The content of secondary metabolites in plant commonly used as a medicine is from general part of the bark. Therefore, on the basis of chemotaxonomic and ethno-botany of mangroves *R. mucronata*, this study aims to explore the bioactivity of antioxidant from the stem bark of *R. mucronata*.

Figure 1. Mangrove *Rhizophora mucronata*

METHOD

Sample Preparation

Samples of mangrove's bark, *R. mucronata* were obtained in Pamurbaya, Surabaya, East Java. Samples were then dried at room temperature and ground into powder. The total sample that has been obtained was 3 kg of *R. mocronata* powder.

Figure 2. Map of the sampling site of mangrove *R. mucronata* in Pamurbaya

The chemicals used in the extraction process include organic solvents that are pro analysis (p.a) that has been technically distilled such as methanol, ethyl acetate, ethanol, and n-hexane. Chemicals used in the in vitro test of antioxidant bioactivity are thin-layer plates of chromatography (TLC), and reagent DPPH (2,2-diphenil-1-picrilhidrazil).

Extraction

Methanol was used in the extraction of the stem bark of mangrove *R. mucronata*. This solvent has been chosen because it is the universal solvent that can dissolve all the active ingredients of secondary metabolites.

TLC-Autograph test

TLC plate was spotted using methanol extract, and then sprayed with DPPH reagent. This test is performed as a preliminary test of antioxidant. The extract is determined to be active as an antioxidant if only after spraying, the DPPH turns color from purple to yellow.

Bioactivity Invitro Test of Antioxidant

DPPH method was used in bioactivity invitro test of antioxidant of the methanol extract of the stem bark of mangrove *R. mucronata* (Kristanti, et al, 2008). The methanol extract samples were prepared in various concentrations i.e: 50, 100,200, 400, 800, and 1000 ppm; and then added 0.0004% DPPH reagent in ethanol. DPPH solution was added as much as 3.9 ml to 0.1 ml of ethanol accounted for its absorbance with UV-Vis spectrophotometer at a wavelength of 497 nm, 517 nm and 537 nm (as a standard solution). Analysis of inhibition IC_{50} (Inhibitor Concentration of 50%) is determined by linear regression analysis of the concentration DPPH percentage and isolated compounds. If IC_{50} < 1000 ppm, these compounds have antioxidant activity (Cos, 1998).

RESULT

Extraction and TLC-autograph

Maceration technique was treated in the extraction process of 3 kg sample powder of mangrove *R. mucronata*'s stem bark by solid-liquid extraction method under room temperature. The extraction process was done for 3 days using methanol. Extraction product from the previous process was evaporated to separate the organic solvent. As much as 191.2513 g of solid brown methanol extract was obtained in the final maceration process.

TLC-autograph was performed to qualitatively determine whether the extract is active or not as an antioxidant. Qualitative test of antioxidants on the methanol extract of the stem bark of *R. mucronata* showed that after TLC plate spotted with the methanol brown-extract then sprayed with DPPH reagent, the DPPH turned color from purple to yellow. This color change indicates that the methanol extract can inhibit the activity of free radicals of DPPH reagent. The qualitative test results then became the basis for bioactivity invitro test to determine the IC_{50} of the extract.

Bioactivity invitro test of Antioxidant

The antioxidant activity was quantitatively demonstrated by measuring the absorbance of the test solution with varying concentrations using UV-Vis spectrophotometer. The measurement was performed to determine the concentrations level of potensial inhibition of the compound as an active antioxidant by calculating the IC_{50}. The data of antioxidant invitro test analysis of the methanol extract of *R. mucronata*'s stem bark are shown in Table 1.

Based on data in Table 1, a linear regression analysis was performed to obtain the linear regression equation. The linear regression equation was used to calculate inhibition level of test samples against free radical DPPH by observing IC_{50} of methanol extract.

Table 1. Antioxidant invitro tes analysis of methanol extract of *Rhizophora mucronata*'s stem bark using UV-Vis spectrophotometer at various concentrations.

Concentration (ppm)	Absorbance at λ (nm)			% Reduction of DPPH
	497	517	537	
1000	0.438	0.319	0.215	-6.138107417
800	0.368	0.269	0.181	10.23017903
400	0.192	0.142	0.097	51.66240409
200	0.104	0.078	0.052	73.40153453
100	0.06	0.043	0.029	85.93350384
50	0.036	0.026	0.017	91.5601023
standar	0.146	0.181	0.175	

The results of % DPPH reduction calculations of samples at various concentrations obtained a linear regression y = -0,103x + 95.20. The regression equation was then used to calculate the IC_{50} values in order to determine antioxidant activity by observing the level of extract inhibition against DPPH free radicals. The IC_{50} of methanol extract of the stem bark of mangrove *R. mucronata* was obtained at 438.8349 ppm. LC_{50} < 1000 ppm showed that the extract is active as an antioxidant (Cos, et al., 1998).

Based on research by Gao and Xiao (2012) to the species *Rhizophora apiculata*, it is reported that the antioxidant activity of mangroves can be influenced by the content of secondary metabolites compounds contained in crude extracts such as class phenolics, terpenoids, alkaloids, and lignans. The study of antioxidant activity conducted by Palacio, et al (2014) of the mangrove *Rhizophora mangle* in Mexico also showed that the methanol extract obtained have high antioxidant activity due to compounds which contain phenolic groups.

Reviews from the genus *Rhizophora,* antioxidant activity of mangrove plant *R. mucronata* found in Pamurbaya is influenced by the content of secondary metabolites compounds contained in the methanol extract. This research can be used as a basis for phytochemical test and the isolation of active antioxidants compounds so that the potential of natural materials from the mangroves *R. mucronata* can be used as an alternative to drug ingredients.

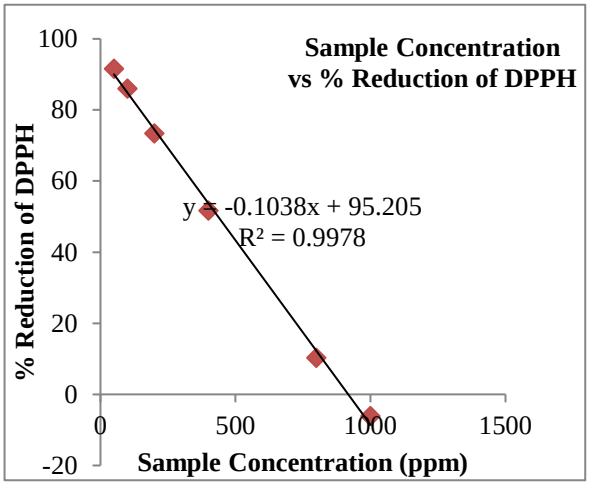

Figure 3. Linear regression curve of bioactivity invitro test of methanol extracts of *Rhizophora mucronata*'s stem bark.

CONCLUSION

Results of the study of the methanol extract from bark of *R. mucronata* originated in Pamurbaya as an antioxidant agent reported that the extract is active as an antioxidant with IC_{50} level 438.8349 ppm.

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