*Eco. Env. & Cons. 27 (October Suppl. Issue) : 2021; pp. (S291-S294)*
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*ISSN 0971–765X*

# *In vitro* analysis of antioxidant activity with different polar solvents on crude extracts o*f Rhizophora mucronata* against free radicals Or DPPH (2,*2-diphenyl-1-picrylhydrazyl)*

**Ashari Fahrurrozi[1], Sri Andayani[2], Ating Yuniarti[2] and Nelvan Subayu[1]**

***[1]Universitas Brawijaya, Fisheries and Marine Science Faculty, Magister Program, Aquaculture Science Department, Malang, 65145, East Java, Indonesia***
***[2]Universitas Brawijaya, Fisheries and Marine Science Faculty, Aquatic Resources Management Department, Malang, 65145, East Java, Indonesia***

(Received 16 January, 2021; accepted 20 March, 2021)

**ABSTRACT**

This study aimed to analyze and determine the antioxidant bioactivity of *Rhizhopora mucronata* leaf crude extract against free radical scavenging or DPPH (2,2-*diphenyl*-1-picrylhydrazyl). Antioxidant activity testing was carried out using the DPPH method with a calculation of (%) inhibition for linear regression and inhibition concentration ($IC_{50}$). The research treatment used 3 different polar solvents, namely methanol, ethanol, and distilled water in the maceration method extract process. Also used is a control in the form of 0.1 mM DPPH solution in p.a. ethanol. Positive control comparisons were treated as samples. *R. mucronata* extract is known to contain secondary metabolites which have antioxidant properties. The readings using a UV-Vis spectrophotometer showed that the crude extract of *R. mucronata* with methanol as a solvent had the strongest antioxidant activity with an $IC_{50}$value of 8.5744 ppm. While the crude extract with ethanol and distilled water solvent had a value of 29.2815 ppm and 471.4607 ppm, respectively.

***Key words :*** *Rhizophora mucronata, Antioxidant, Percent inhibition, $IC_{50}$, DPPH*

## Introduction

Free radicals in fish are the result of normal cellular metabolism, and many of these compounds play important roles in various metabolic pathways (Billertakahashi *et al.,* 2015). In cells, there are three main sources of free radical formation, such as oxidative metabolism in mitochondria, the respiratory activity of leukocytes due to pathogen attack, and environmental factors (some drugs, diet, ionizing radiation, ultraviolet light, and visible light). However, free radicals are highly reactive molecules with unpaired electrons in the final electron layer (Ferreira and Matsubara, 1997). So that free radicals can bind nonspecifically with other biological molecules and if excessive, it has the potential to cause damage to tissue proteins, enzymes, DNA, carbohydrates, and membrane lipid peroxidation (Biller and Takahashi, 2018).

The excess of free radicals can be overcome by natural antioxidants in the fish body, such as endogenous sources (enzymes or cofactors) or through exogenous sources (food). Antioxidants are compounds that can give one or more electrons to oxidant compounds and make oxidant compounds stable (Wang *et al.,* 2013). Currently, the commonly

**Corresponding author's email:** ashari.fahrurrozi@gmail.com

used antioxidants are synthetic antioxidants, but these compounds are suspected to have carcinogenic and toxic effects. Therefore, it is important to develop and utilize antioxidants that are safer and of natural origin (Bariyyah *et al.,* 2013). One of the natural ingredients that can be used and are abundant is the mangrove *Rhizophora mucronata.* This is because most of its parts are known to contain bioactive compounds that can potentially act as antioxidants (Dhayanithi *et al.,* 2020). The choice of solvent type becomes important in the extraction process, due to several factors such as selectivity, capability, convenience, and price considerations. The extraction solution used is adjusted to the polarity of the desired compound with the like dissolves like principle (Putri, 2015). Secondary metabolites are known to function as antioxidants in capturing free radicals (DPPH) (Batubara *et al.,* 2020). According to Snehlata *et al.* (2018), extraction to extract secondary metabolites is more effective using polar solvents (ethanol, methanol, and distilled water) than nonpolar solvents. Therefore, this study was conducted to determine which type of polar solvent is best for obtaining the highest content of an antioxidant activity.

## Materials and Methods

### Sample preparation

The samples of *Rhizophora mucronata* leaves that have been obtained are then processed through drying by washing with running water and drying according to the method of Subeki *et al.* (2018).

### Extraction of *Rhizophora mucronata*

At the extraction stage, the method used is the maceration method using volatile organic solvents. Extraction was carried out by maceration and evaporation. The use of solvents with 3 polar solvents such as ethanol, methanol, and distilled water was used to test the bioactive compound *R. mucronata* in capturing free radicals (Verdiana *et al.*, 2018).

### Antioxidant Activity Test with DPPH (1,1-*diphenyl*-2-*picrylhydrazyl*)

Determination of the maximum wavelength and measurement of antioxidant stability were measured based on Bariyyah *et al.* (2013). Then the extract samples (methanol, ethanol, and distilled water) were dissolved in the solvent each with 6 concentrations (ppm) with the addition of 0.1 mM DPPH. After that, it was incubated at 30 ºC and the absorbance was measured using a UV-Vis spectrophotometer at a wavelength of 515 nm. The absorbance data obtained from each concentration of each extract was calculated as a percent (%) of the inhibitory value according to the procedure of Andayani *et al.* (2008). Furthermore, the $IC_{50}$ value is calculated by the formula ((50-B)/A) from the regression equation with the control (DPPH 0.1 mM solution in p.a. ethanol) and the ratio (ascorbic acid) which is used as the sample (Yefrida *et al.,* 2014).

## Results

*Rhizophora mucronata* leaf sample preparation produced dry powder. Then the powder is macerated with a different solvent. After this process, the extract is filtered and evaporated to produce a thick extract with a yield which can be seen in Table 1. The results of determining the wavelength with 0.1 mM DPPH obtained a maximum wavelength of 515 nm. Meanwhile, the measurement of antioxidant stability is in the range of ± 30 minutes.

**Table 1.** *Rhizophora mucronata* extract yield

| Solvent | Yield (%) (w/w) | Concentrated extract color |
|---|---|---|
| Methanol | 6,02 | Deep dark green |
| Ethanol | 5,11 | Deep dark green |
| Distilled water | 2,10 | Chocolate |

The antioxidant activity test of *R. mucronata* crude extract using ethanol methanol and distilled water was carried out at several concentrations. All treatment concentrations including control were measured at an absorption wavelength of 515 nm with the stability time obtained. The results in this process are measured by (%) inhibition and linear regression to obtain A and B values resulting in $R^2$ values close to ≥ 1. The calculation results can be seen in Table 2. Then the inhibition concentration ($IC_{50}$) is obtained which can be seen in Table 3.

## Disscussion

The yield of *Rhizophora mucronata* crude extract produced by methanol solvent was greater with a yield of 6.02% compared to other solvents. This is because methanol has a better polarity than ethanol and dis-

**Table 2.** Determination of linear regression (Y = A + B) with the value of $R^2$

| No | Treatment | Linear regression (Y=A + B) of concentration and (%) inhibition | | $R^2$ |
|---|---|---|---|---|
| | | A | B | |
| 1 | Methanol | 1,6169 | 36,136 | 0,9749 |
| 2 | Ethanol | 0,7516 | 27,992 | 0,9779 |
| 3 | Distilled water | 0,0178 | 41,608 | 0,9801 |
| 4 | Positive control | 9,1099 | 29,919 | 0,9606 |

**Table 3.** Inhibition concentration ($IC_{50}$).

| No | Treatment | $IC_{50}$ (ppm) |
|---|---|---|
| 1 | Methanol | 8,5744 |
| 2 | Ethanol | 29,2815 |
| 3 | Distilled water | 471,4607 |
| 4 | Positive control | 2,2043 |

tilled water. Methanol is a polar solvent that is often used because it penetrates cell walls more efficiently, producing more secondary metabolites. This causes maceration with methanol as a solvent to produce extracts with more variations in secondary metabolites (Bariyyah *et al.*, 2013). Determination of the best solvent, in this case, to produce the highest levels or values of antioxidant activity. The parameter used to see this is using $IC_{50}$. The definition of $IC_{50}$ is the concentration of the sample solution which will cause a decrease in DPPH activity by 50% (Artanti and Lisnasari, 2018).

The results showed that the crude extract of *Rhizophora mucronata* on the leaves, with methanol and ethanol as solvent, had a very high level of antioxidant activity. The antioxidant levels of the two solvents were 8.5744 ppm and 29.2815 ppm. Meanwhile, distilled water solvent extract does not have antioxidant activity, because it produces a value of 471.4607 ppm. This is because antioxidants are very strong if the $IC_{50}$ value is <50 ppm, strong if the $IC_{50}$ value is 50-100 ppm, moderate if the $IC_{50}$ value is 100-150 ppm, weak if the $IC_{50}$ value is 151-200 ppm, and is declared inactive if it has an $IC_{50}$ value> 200 ppm (Nicoli *et al.*, 1999).

This explanation shows that extraction with methanol as a solvent has the potential to be applied to animals (*in vivo*) as a natural remedy to fight oxidative stress due to free radicals. Apart from being a very strong antioxidant, the extract with methanol solvent had an antioxidant value close to the control (ascorbic acid) with a value of 2.2043 ppm compared to the ethanol solvent extract.

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