The potential of mangrove *Avicennia marina* and *A. Alba* from Nguling district, Pasuruan, East Java as an antioxidant

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Abstract. Free radicals are highly reactive molecules due to unpaired electron in their outer orbital. Excess of free radicals inside human body as consequences of environmental exposure such as cigarette smoke may lead to degenerative diseases such as diabetic, cancer etc. This negative effect can be limited by the utilization of natural antioxidant substances, especially produced from plants. *Avicennia alba* dan *A. marina* are mangrove species that widely distributed in Indonesia and are expected potential as antioxidant. The objective of this study is to evaluated *Avicennia alba* dan *A. marina* potency as antioxidant performed with DPPD (1,1-diphenyl-β-picryl hydrazyl) method. Leaf and bark of *Avicennia alba* dan *A. marina* were collected from Nguling District, Pasuruan, East Java. Results shows that based on 50% inhibition Concentration (IC50), *Avicennia alba* leaf were categorized had a very high antioxidant potential (IC50 14,85 ppm) whereas the bark were categorized had a weak antioxidant potential IC50 167,17 ppm). For *A. marina*, the leaf were categorized had a moderate antioxidant (IC50 123,23 ppm) whereas the bark were categorized had a weak antioxidant potential (IC50 198,15 ppm).

1. Introduction

Free radical molecules can originate from environment pollutants such as cigarettes, vehicle smoke and radiation exposure. These molecules affect susceptible compounds such as proteins and lipids and lead to degenerative diseases such as atherosclerosis, heart disease, premature aging and cancer [1]. This could happen due to limited antioxidants in the body, therefore additional antioxidants are needed. Antioxidants are substances that can prevent, delay and eliminate oxidative damage that occur in molecular targets such as DNA, protein and lipid [2]. These substances have a function to regulate the concentration of free radicals so that the level of damage in the body can be minimized. The action mechanism of antioxidants is by breaking free the radical chain, detoxifying and activating oxidative enzymes such as glutathione peroxidase, catalase, and superoxide dismutase [3]. Antioxidants can be found in several forms such as minerals, phytochemicals and vitamins [4]. By source, antioxidants can be derived from natural and synthetic materials. Natural ingredients are often tested for antioxidant content such as plants and fruits.

The mangrove is a potential plant that may contain antioxidant compounds. In some Asian countries such as India and Malaysia, mangrove plants are widely used as traditional medicine for diabetes,
cancer, asthma, cancer, injury and even AIDS. One mangrove species that is used as a drug is *Avicennia* spp. which locals often call as Api api [5,6]. This species is found widely in Indonesia and locals have consumed its fruit as a snack and as medicine to treat diarrhea. Plants that have been used as traditional medicine are potential sources of natural antioxidant substances and can be developed as modern medicines [6]. This study aims to assess the potency of *Avicennia* spp. as an antioxidant.

2. Methodology
Samples of the bark and leaf of *A. alba* and *A. marina* were collected from the location of Nguling Mangrove Ecotourism, Pasuruan District, East Java. Samples were then water dried and grinded to powder. Further laboratory work was performed at the Exploration Laboratory of Fisheries and Marine Faculty of Brawijaya University.

2.1 Extraction
The sample powder was immersed in a methanol solvent (1: 3) for 24 hours. After 24 hours the filtrate was separated (using *Whatman* paper no. 42) and the residual was immersed and filtered again for another three times. Then the whole filtrate was evaporated at a temperature of 42°C for 1 hour to separate the solvent and the sample extract.

2.2 Antioxidant test
The antioxidant activity was tested by the DPPH (1,1-diphenyl-2-picrylhydrazyl) method. The DPPH method was used because the substance is capable of capturing free radicals causing paired electrons which then cause a color repellent proportional to the number of electrons captured. DPPH provides strong absorption at 517 nm wavelength with a dark violet color [7]. The concentration of DPPH solution used for the antioxidant testing was 0.5 mM [8], whereas the four different concentrations of the sample extracts (leaf and bark) used for the testing were 31.25 ppm, 62.5 ppm, 125 ppm, and 250 ppm. Each sample concentration was added with 1 ml of 0.5 mM DPPH and then incubated in a dark room at room temperature for 30 minutes. The positive controls used were vitamin C or ascorbic acid with concentrations of 2 ppm, 4 ppm, 6 ppm, and 8 ppm. According to [8], the classification of antioxidants based on IC50 (50% Inhibitor Concentration) can be seen in Table 1, as follows:

| Concentration | Classification |
|---------------|----------------|
| <50 ppm       | Very Strong    |
| 50 – 100 ppm  | Strong         |
| 100 – 150 ppm | Moderate       |
| 151 – 200 ppm | Weak           |
| >200 ppm      | Very weak      |

The color change (qualitative test) that occurred when the sample was mixed with DPPH (from light purple to yellow) indicated the presence of antioxidant activity in the sample solution [9]. The quantitative test can be evaluated based on percent inhibition value. Percent inhibition is the value of free radical inhibition while IC50 is the concentration of samples needed to reduce 50 % of DPPH. Percent inhibition is calculated using the following formula:

\[
\text{% inhibition} = \left( \frac{\text{blanko absorbance} - \text{sample absorbance}}{\text{sample absorbance}} \right) \times 100\% \tag{1}
\]
The concentration values of the samples as well as vitamin C and the percent of their inhibition were plotted on the x-axis and y-axis in the linear regression equation. The linear regression equation was then used to calculate IC50.

3. Result and Discussion
Antioxidants are substances or molecules that can prevent or restore free radicals by cutting off the chain reaction of free radicals. The color change occurs when the sample extract gives its hydrogen atom to DPPH thus it becomes stable.

The result of the antioxidant activity test of the leaf and bark of A. alba and A. marine showed the color change to yellow, indicating that the leaf and bark extract of Avicennia spp. can reduce DPPH radicals. The results of the qualitative antioxidant activity test on A. alba can be seen in Figure 1, while the results of A. marina can be seen in Figure 2.

![Figure 1. Antioxidant activity of A. alba leaf (left) and bark (right).](image1)

![Figure 2. Antioxidant activity of A. marina leaf (left) and bark (right).](image2)

The subsequent percentage of the inhibition value and IC50 value of Avicennia spp. samples and positive controls were calculated as presented in Table 2 and 3.

| Sample | Concentration (ppm) | Absorbance | % Inhibition | IC50 (ppm) |
|--------|----------------------|------------|--------------|-------------|
| Leaf   | 31.25                | 0.49       | 40.67        | 14.85       |
|        | 62.5                 | 0.27       | 66.88        |             |
|        | 125                  | 0.1        | 87.39        |             |
|        | 250                  | 0.04       | 95.3         |             |
| Bark   | 31.25                | 1          | -21.77       | 167.17      |
|        | 62.5                 | 0.86       | -4.02        |             |
|        | 125                  | 0.49       | 40.74        |             |
|        | 250                  | 0.11       | 86.3         |             |

Table 3. Results of antioxidant activity test on A. marina.
The results of antioxidant activity presented in Tables 2 and 3 show that the different mangrove species and in different organ had different potential of antioxidant. Based on IC50 value, A. marina leaf and bark had a lower potential of antioxidant than A. alba leaf. In addition, A. alba leaf had a higher antioxidant potential than its bark. A. marina leaf can be categorized as a moderate antioxidant whereas the bark can be categorized as a weak antioxidant.

Weak antioxidant activity was also found for A. alba bark whereas a very strong antioxidant activity was found for the leaf. Higher antioxidant activity in the leaf than the bark of A. marina was also found in [10] and [11] whereas the opposite result was found in [12]. A very strong antioxidant potential of A. alba leaf may be the result of the presence of vitamin C in the leaf [13]. The different results of this study compared to previous studies is likely due to different sample collection locations that may result in different bioactive compounds found in the plants. Every plant or organ of plant has a different metabolic seconder, with different polarity and even a different level of concentration [11].

4. Conclusion

Avicennia spp. has a potential as an antioxidant with higher potency found in the leaf than the bark. A. alba had a higher antioxidant potency than A. marina. The highest potency of antioxidant was found in the A. alba leaf.

5. References

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