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## Antioxidant Activity of *Rhizophora apiculata* Methanol Leaves Extract

### Aktivitas Antioksidan Ekstrak Metanol Daun *Rhizophora apiculata*

Husnul Khatimah Ramli<sup>1\*</sup>, Riris Roiska<sup>2</sup>, Muh Aksa Azis<sup>1</sup>, Eka Aprianti<sup>1</sup>, Putinur<sup>2</sup>

<sup>1</sup>Fisheries Resource Utilization, Khairun University, Jl. Raya Pertamina, Gambesi, Ternate 97719, Indonesia

<sup>2</sup>Fisheries Resource Utilization, Faculty of Animal Husbandry, Universitas Jambi, Jl. Jambi – Muara Bulian KM. 15, Mendalo Darat, Jambi 36361, Indonesia

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\*Corresponding author: [husnul.khatimah@unkhair.ac.id](mailto:husnul.khatimah@unkhair.ac.id)

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#### ABSTRACT

Mangroves are well-known for their diverse benefits, with all plant parts containing beneficial bioactive compounds. This study aims to evaluate the antioxidant activity of the methanolic extract of *Rhizophora apiculata* mangrove leaves. Mature mangrove leaves were extracted using 70% methanol through a maceration method, and antioxidant activity was assessed using the DPPH radical scavenging assay. The results indicated that the extract exhibited inhibition percentages ranging from 56% to 91% at concentrations of 50–1000 ppm, with an IC<sub>50</sub> value of 30.86 µg/mL. These findings suggest that the methanolic extract of mature *R. apiculata* leaves possesses powerful antioxidant potential (IC<sub>50</sub> < 50 µg/mL).

**Keywords:** bioactive compound, DPPH, mangrove

#### ABSTRAK

Mangrove merupakan salah satu tanaman yang dikenal kaya akan manfaat. Seluruh bagian tubuh mangrove dapat dimanfaatkan dan memiliki komponen bioaktif. Penelitian ini bertujuan untuk mengidentifikasi aktivitas antioksidan ekstrak metanol daun mangrove *Rhizophora apiculata*. Sampel yang digunakan merupakan daun mangrove tua yang diekstrak menggunakan metanol 70% dengan metode maserasi dan diuji aktivitas antioksidan menggunakan metode DPPH. Hasil penelitian menunjukkan bahwa persen inhibisi 50-1000 ppm ekstrak daun mangrove berkisar antara 56-91% dengan IC<sub>50</sub> 30,86 µg/mL. Ekstrak daun mangrove tua memiliki kemampuan antioksidan sangat kuat (IC<sub>50</sub><50 µg/mL).

**Kata Kunci:** komponen bioaktif, DPPH, mangrove



## INTRODUCTION

Mangroves play an important role in maintaining biodiversity by providing habitats for various aquatic and terrestrial organisms. They also contribute to ecosystem services such as carbon sequestration, coastaline stabilization, and water filtration (Arifanti et al., 2022). From an ethnobotanical perspective, the wood and bark of mangrove trees are utilized for firewood, charcoal production, and tannin extraction for natural dyes (C. A'in & B. Sulardiono, 2017; Rumengan et al., 2021; Rusila Noor et al., 2006).

*Rhizophora apiculata*, commonly known as mangrove oil. Most parts of the mangrove oil are used as medicine by coastal communities in Indonesia due to their beneficial active ingredients (Akasia et al., 2021). Mangrove leaves have been widely utilized by communities in the food, pharmaceutical, nutraceutical, and cosmeceutical industries. As a country with high mangrove vegetation diversity, Indonesia holds various untapped mangrove potentials, particularly as a raw material for medicine (Mahmiah et al., 2021). Mangroves have several species believed by communities to have medicinal properties. All parts of the mangrove tree have unique advantages that can be used as antioxidants, antidiabetics, and antibacterials.

Research by Akasia et al., (2021), based on phytochemical testing of mangroves from Tuban (Bali), extracted using methanol solvent, found that *R. apiculata* positively contained phenols, alkaloids, flavonoids, tannins, saponins, steroids, and negatively contained terpenoid compounds. The analysis of *R. apiculata* ethanol leaves extract by Maulana and Sasmito (2021) stated that this extract has the highest antioxidant activity with an IC<sub>50</sub> of 49.45 ppm. The maturity level of the leaves have an effect on sensory characteristics, bioactive compound content, and nutritional value. The age of the leaves can be identified based on its position on the stem: the third leaf from the tip (indicating physiologically young leaves), the sixth leaf from the tip (indicating semi-mature leaves), and the eighth leaf from the tip (indicating physiologically mature leaves) (Maleke et al., 2024).

This research aims to identify the antioxidant activity of methanol extracts from the leaves of mature *R. apiculata* mangrove, which can be used as natural

antioxidant preparations for the pharmaceutical, nutraceutical, and cosmeceutical industries.

## RESEARCH METHOD

### Preparation and Extraction

Samples of mature leaves from *R. apiculata* mangrove located at the base of the trunk were obtained from the BAPPL Serang mangrove conservation area in Banten, which is a conservation area directly adjacent to community cultivation areas (shrimp farms). The collected mangrove leaves were placed in plastic bags and immediately transported to the Laboratory of Raw Material Characteristics, Department of Aquatic Product Technology, Bogor Agricultural Institute. The mature mangrove leaves are washed clean and then dried at room temperature for up to 14 days. The extraction method refers Arifin et al., (2023), the dried mangrove leaves were ground using a grinder and then sieved until a powder is obtained. The resulting powder was then dissolved using 70% methanol (1:5), the solution was macerated for 2x24 hours using an orbital shaker, then evaporated using a rotary vacuum evaporator at 40°C until a paste-like extract (crude extract) was obtained. The resulting extract was tested for antioxidant activity.

### Antioxidant Activity

Antioxidant activity testing refers to the SNI 8623:2018 method. The analysis using 1,1-diphenyl-2-picrylhydrazyl (DPPH). Stock solution of the sample was prepared beforehand, dissolving 2 mg of the sample in 5 mL of ethanol solvent (Merck). Then, extract solutions are prepared at concentrations of 50, 100, 150, 200, and 250 ppm. The DPPH solution used is 0.76 mM (3 mg DPPH dissolved in 10 mL ethanol solvent). A total of 4 mL of extract solution from each concentration is mixed with 1 mL of DPPH (4:1), incubated for 30 minutes in the dark, and then 180–200 µL of each was pipetted into a microplate. Absorbance was measured using a spectrophotometer at a wavelength of 517 nm. The positive controls used ascorbic acid at concentrations of 1, 2, 3, 4, and 5 ppm. The blanks used a mixture of solvent and DPPH. The percentage of inhibition can be calculated using the formula:

$$\%DPPH = \frac{\text{blank absorbance} - \text{sample absorbance}}{\text{blank absorbance}} \times 100\%$$

## RESULTS AND DISCUSSION

Mangroves are known to be rich in secondary metabolites such as flavonoids, phenolics, and tannins, which have high antioxidant activity (Fadilah et al., 2023; Mile et al., 2021). These compounds can neutralize free radicals, prevent oxidative damage to cells, and have wide applications in health, pharmacy, and the food industry (Cadamuro et al., 2021; Mohd Mokhtar et al., 2022). The secondary metabolites produced by the mangrove *R. apiculata* are also expected to influence antioxidant activity against free radicals (Mutik et al., 2022). Several studies have shown that extract of mangrove leaves contain significant phenolic compounds, which play a crucial role in antioxidant activity. Every part of the mangrove plant contains active components that can be identified through initial phytochemical analysis screening.

The content found in the aqueous extracts of *Rhizophora apiculata* and *Avicennia marina* includes tannins, saponins, and steroids (Ramli et al., 2020), saponins, flavonoids, tannins, and triterpenoids in the ethanol extract of *R. apiculata* mangrove leaves (Maulana & Sasmito, 2021), saponins, phenols, flavonoids, anthraquinones, and alkaloids in the methanol extract of the bark of *R. apiculata* (Vittaya et al., 2022), as well as steroids and phenols in the methanol extract of the stem of *R. apiculata* (Egra et al., 2023). Various studies have shown the diversity of phytochemical content in different parts of mangrove plants, which contributes to their antioxidant activity. To strengthen these findings, the results of antioxidant activity tests of *R. apiculata* mangrove leaves extracts obtained in this study are presented in Table 1.

Table 1. Antioxidant Activity of Mangrove Leaf Extract

Concentration (µg/mL)	Absorbance	Sample	% Inhibition	IC <sub>50</sub> (µg/mL)
50	0,359	0,324	56,157	
100	0,315	0,280	62,111	
250	0,183	0,148	79,973	30,86
500	0,142	0,107	85,521	
750	0,112	0,077	89,581	

Information: IC<sub>50</sub><50 µg/mL (very strong); IC<sub>50</sub> 50-100 µg/mL (strong); IC<sub>50</sub> 101-150 µg/mL (moderate); IC<sub>50</sub> 150-200 µg/mL (weak); (Sari et al., 2024).

Source: Data Processing

The antioxidant activity results in Table 1 show that the IC<sub>50</sub> of the methanol extract of mangrove leaves is 30.86 µg/mL and is classified as very strong, different from the results of Mutik et al., (2022), where the methanol extract of mangrove leaves *R. apiculata* from the waters of Teluk Awur, Jepara, has antioxidant activity with an IC<sub>50</sub> value of 85.999 ppm. The antioxidant activity of *R. apiculata* mangrove leaf extract from the Tomini Bay coast has an IC<sub>50</sub> of 96.68±0.68 µg/mL (Dewanto et al., 2021). The antioxidant activity of *R. mucronata* mangrove leaf extract from the Tugurejo mangrove area in Semarang was highest in the methanol extract with an IC<sub>50</sub> value of 113.41 ppm and was classified as a moderate antioxidant (Ridlo et al., 2017). Differences in compound content and activity are likely due to the influence of habitat and mangrove age. The growing environment plays a role in determining the levels of flavonoids, phenols, and antioxidant activity in leaves in the

research (Utomo et al., 2020). Increased environmental temperature tends to increase the production of flavonoids, phenolic compounds, and antioxidant activity. However, these environmental conditions did not show a significant effect on chlorophyll and carotenoid content. According to Bachtiar et al. (2023), one important factor contributing to the composition and effectiveness of phenolic compounds is the habitat in which the plants grow. The antioxidant activity of plant extracts depends on the species, extraction method, season, and sampling location (Budhiyanti et al., 2012). During the growth phase, plants produce secondary metabolites and bioactive compounds in varying amounts, which are influenced by morphological changes and increased leaf age (Farhoosh et al., 2007).

## CONCLUSION

The antioxidant activity of methanol extract from mature mangrove leaves, with an IC<sub>50</sub> of 30.86 µg/mL, is

categorized as a very strong antioxidant that can be recommended as a natural antioxidant alternative that can be utilized by the pharmaceutical, nutraceutical, and cosmeceutical industries.

## RECOMMENDATIONS

Additional phytochemical analysis and antioxidant activity testing using alternative methods are needed to validate the obtained data. Furthermore, samples should be collected based on tree age and from additional locations to assess variations in phenolic compound content.

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