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Phytochemical Composition and Antioxidant Activity of Leaf Extracts from Three *Rhizophora* Species from Bontang Waters, Indonesia

Dewi E. Bulan[1,2]*, Nurfadilah Nurfadilah[1], Muhammad R. Syahrir[1], Andi Mismawati[3], Arifuddin K. Torambung[4], Maulida Rachmawati[5]

[1]*Department of Aquatic Resource Management, Faculty of Fisheries and Marine Science, Mulawarman University, Samarinda, East Kalimantan, Indonesia*
[2]*Research Center for Drugs and Cosmetics from Tropical Rainforest Resources, Mulawarman University, Samarinda, Indonesia*
[3]*Department of Aquaculture, Faculty of Fisheries and Marine Science, Mulawarman University, Samarinda, East Kalimantan, Indonesia*
[4]*Department of Forestry, Faculty of Forestry, Mulawarman University, Samarinda, East Kalimantan, Indonesia*
[5]*Department of Agricultural Product Technology, Faculty of Agricultural, Mulawarman University, Samarinda, East Kalimantan, Indonesia*

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ABSTRACT

The *Rhizophora* genus is a prominent mangrove distributed in tropical Bontang waters. However, exploration of antioxidant potential has received very little attention. The present study was, therefore, conducted to investigate the phytochemical properties and antioxidant activity of three *Rhizophora* species from Bontang waters in Indonesia. Leaves of *Rhizophora mucronata*, *Rhizophora apiculata*, and *Rhizophora stylosa* were extracted with methanol (polar target molecules) and ethyl acetate (semi-polar target molecule) using the successive extraction method. The extractive yields of the two solvents were determined, and the three *Rhizophora* crude extracts were subjected to standard phytochemical screening. The DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging assay was used to assess the antioxidant activity of the three *Rhizophora* extracts. The results showed that ethanol extractive yields were higher in all samples than ethyl acetate extractive yields. The phytochemical screening results indicated that the ethyl acetate extract contains a greater variety of metabolites than the methanol extract. Both ethyl acetate and methanol extracts demonstrated significant anti-DPPH radical activity. The *R. mucronata* extract in methanol had the highest antioxidant activity, with an IC_{50} value of 0.04±0.00 g/ml, followed by the *R. stylosa* extract at 0.37±0.09 g/mL, and the *R. apiculata* extract at 5.06 ± 0.41 g/mL. Meanwhile, the IC_{50} values of the *R. apiculata*, *R. stylosa*, and *R. mucronata* extracts in ethyl acetate were 0.72±0.07, 1.16±0.04, and 2.34±0.12 g/mL, respectively. The findings of the study suggest that the ethyl acetate and methanol extracts of *Rhizophora mucronata*, *Rhizophora stylosa*, and *Rhizophora apiculata* have the potential to be developed as natural antioxidants.

Keywords: Antioxidant, Bontang waters, Phytochemical properties, *Rhizophora*.

Introduction

The tropical rainforest of East Kalimantan is well-known for its diverse flora and fauna, which includes mangroves. *Avicennia*, *Bruguiera*, *Rhizophora*, *Ceriops*, and *Sonneratia* are some of the mangrove genera found in the intertidal zone at Bontang, one of East Kalimantan's coastal areas in Indonesia.[1] Mangroves play an important role in ecosystems, serving as a buffer zone against erosion, a means of stabilizing sedimentation, a means of cleaning contaminated coastal water, and a source of various products like wood, fuel, food, medicine, and bioactive substances.[2-7] Previous research on mangroves has identified secondary metabolites with phytochemical properties that can be used as a source for functional foods, medications, dyes, and the treatment of hayseed as folk medicine. It has also provided information about their biological activities.[6-9]

*Corresponding author. E mail: dewi.embong@fpik.unmul.ac.id
Tel: +6281350834836

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Additionally, *Rhizophora mucronata* and *R. apiculata* have been used to treat various diseases, such as dysentery, fever, diarrhea, nausea, hematuria, diabetes, hemorrhages, and blood in the urine.[2,6,12,15] Some research on *Rhizophora* species from different countries has focused on examining their cytotoxicity, antibacterial, and antioxidant properties.[10-13] In India, *R. mucronata* and *R. apiculata* leaves were processed to make tea with antioxidant and antibacterial properties.[14-15] Meanwhile, in Indonesia, *R. stylosa* fruit is used to make tea and coffee with recognized physiological benefits like rejuvenation and fitness improvement.[1]

Mangroves have also been utilized as traditional medicines by coastal communities to treat conditions like fever, vaginal discharge, ulcers, sores, and hypertension, suggesting that this species has certain compounds that can treat various diseases.[16] However, no study has examined the phytochemical constituents and antioxidant activity of a leaf extract from the genus *Rhizophora*, specifically from the Bontang variety. Moreover, the different climatic conditions and edaphic factors between Bontang and other countries may have an impact on the chemical composition and biological activities of mangroves. The three species of *Rhizophora*, which are widespread and abundant genera in Bontang waters, must thus be studied for their phytochemical composition and antioxidant potential.

Thus, the study was aimed at determining the phytochemical properties and antioxidant activity of *R. mucronata*, *R. stylosa*, and *R. apiculata* from Bontang waters in Indonesia.

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Materials and Methods

Source of plant materials

Fresh leaves of the mangrove species *R. mucronata*, *R. stylosa*, and *R. apiculata* were collected from Panjang Island, Bontang waters, East Kalimantan, Indonesia between April and August 2019 (Figure 1). The mangrove samples were identified at the Laboratory of Plant Anatomy and Systematics, Faculty of Mathematics and Science, University of Mulawarman under the identification number 95/UN17.8.5.7.16/HA/VI/2019. The samples were cleaned with tap water, rinsed with distilled water, and then allowed to air dry. Using an electronic blender, the dry samples were pulverized to produce tiny particles for extraction.

Preparation of various Rhizophora extracts

Throughout the maceration process, ethyl acetate was used for the semi-polar target molecule and methanol was used for the polar target compound. The extraction process was maintained using successive extraction methods by increasing the polarity of the solvent. One hundred grams (100 g) of plant materials were dissolved in 300 mL of solvent to create the extract. The ethyl acetate was the first solvent suspended in the powder and filtered every 48 hours at room temperature, followed by methanol. The procedure was repeated until the extracted solution was crystal clear using the same powder. Under a partial vacuum and at 40°C, the resultant extracts were combined and concentrated with a rotary evaporator (Buchi, Switzerland). The ethyl acetate and methanol extracts were continuously prepared for the phytochemical analysis and DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging test.

The percentage of yield was calculated using the formula:

$$\%\,Yield = \frac{WCE}{WSP} \times 100$$

Where WCE is the weight of crude extract, and WSP is the weight of the dried sample.

Phytochemical screening of Rhizophora extracts

Standard procedures were employed to conduct the phytochemical screening of the three *Rhizophora* extracts. To test for alkaloids, 1 mL of plant extract was combined with either 0.5 mL of Dragendorff reagent or 0.5 mL of Mayer reagent. A positive alkaloid test resulted in the formation of orange turbidity or yellow precipitate, respectively.[17-19] The presence of pale pink colour in a mixture of 1 mL sample and 5 mL diluted HCl indicated that the test for anthocyanin was positive.[17,18] The cardiac glycoside positive test was characterized by the formation of a brown ring between layers after appropriately combining a sample volume of 5 mL with 2 mL of glacial acetic acid and 1 mL of sulphuric acid.[20] Saponin's positive test was indicated by the presence of foam After mixing 1 mL of the sample with 2 mL of dH_2O and carefully shaking for 1 minute.[21,22] Phenol content was determined with lead acetate, which involved the addition of 1 mL of sample solution to 2 mL of 10% lead acetate. A brown precipitate was indicative of the presence of phenol.[2] In testing for flavonoids, 1 mL of the sample was combined with a few drops of 20% sodium hydroxide. The presence of flavonoids was indicated by the formation of yellow deposits after the addition of a 1% hydrochloric acid solution.[20] Tannins were determined using a ferric chloride test, which involved mixing 1 mL of the sample with 2 mL of 5% ferric chloride. A change in colour to brownish green or deep blue indicated the presence of tannins.[17,18] The steroid and terpenoid test was achieved when 1 mL of the sample solution was mixed with 10 mL of chloroform. Then, 10 mL of sulphuric acid was cautiously and gradually added until there were two layers. A positive terpenoid was indicated by a reddish-brown colour, while a positive steroid was shown by a reddish-brown colour that was formed on the ring after slow stirring.[22]

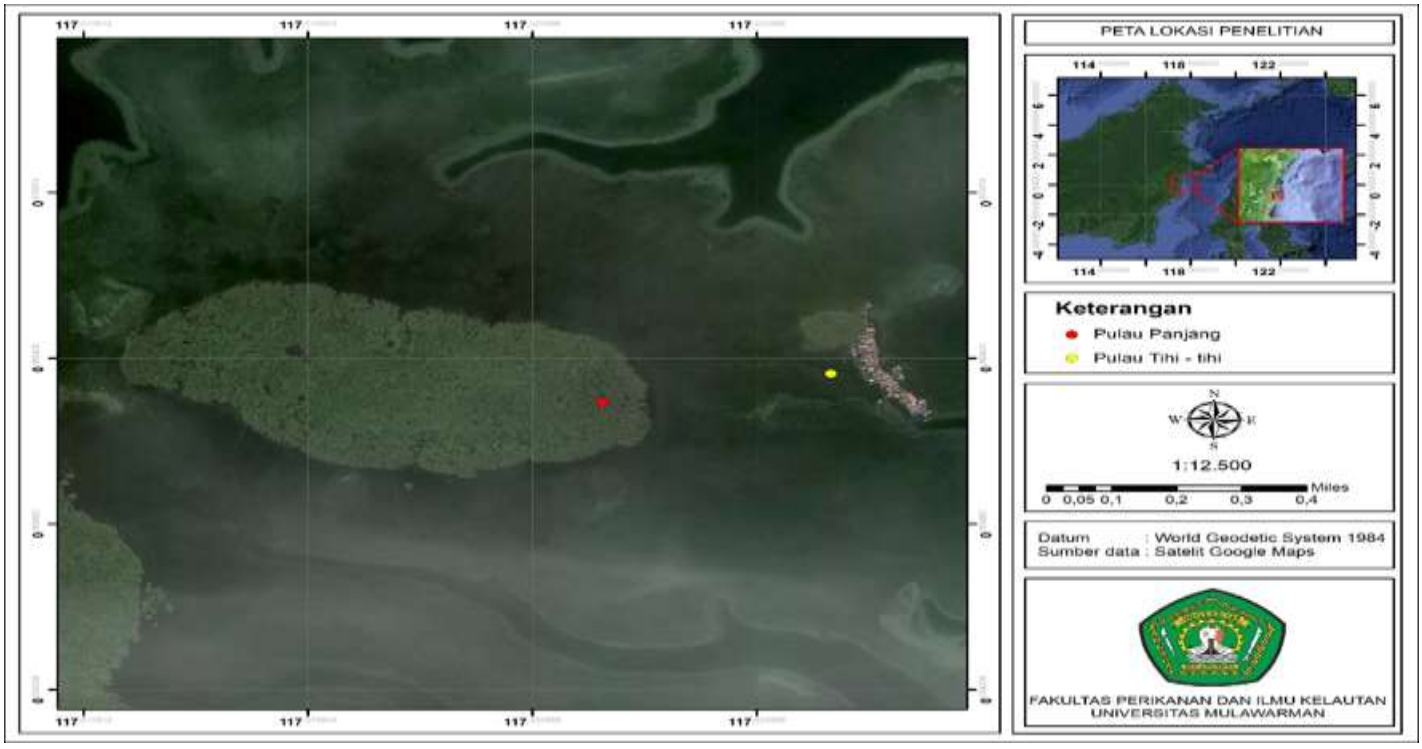

Figure 1: The sampling study site at Panjang Island, Bontang, East Kalimantan, Indonesia.

DPPH radical scavenging activity of Rhizophora extracts

The DPPH radical scavenging assay was conducted using the method of Wang *et al*.,[23] and Mismawati *et al*.,[24] with slight modifications to evaluate the antioxidant activity of the methanol and ethyl acetate extracts. The initial solution, which serves as both a positive control and the solvent for extract extraction, was made at concentrations of 10, 25, 75, and 100 g/mL. Each concentration of extract solutions was combined in a 1:1 ratio with DPPH (40 g/mL). Ascorbic acid was

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employed as the positive control and methanol as the negative control. After 30 minutes of incubation at room temperature in the dark, the absorbance was measured at 517 nm wavelength using a UV-Vis spectrophotometer. The percentage of DPPH scavenging activity was calculated using the formula:

$$\%\ \text{scavenging activity} = \frac{A0-A1}{A0} \times 100\ \%$$

Where A_1 is the absorbance of the sample, and A_0 is the absorbance of the control without the sample.

Statistical analysis
All data are presented as mean ± standard deviation (SD), and they were analyzed using a one-way analysis of variance (ANOVA). The statistical analysis was performed with Microsoft Excel (2010 edition), and $p < 0.05$ was considered to be statistically significant.

Results and Discussion

Extractive yield of the extracts from the leaves of Rhizophora species
Progressively more research has been conducted on the extraction and isolation of bioactive compounds from plant materials for application in food or medicine. The extraction of the polar and semi-polar bioactive compounds was carried out using the selected solvents that affect the efficiency of the bioactive compounds. Polar target molecules were extracted with methanol and semi-polar target compounds with ethyl acetate solvent. The results indicated that raising the polarity of the solvent for each sample increased the yield percentages. All samples had higher methanol extractive yields than ethyl acetate extractive yields as depicted in Table 1. Different extraction techniques, extraction conditions (solvent/sample ratio, type of solvent, extraction time, and temperature), and sample preparation (degradation and size reduction) may all have an impact on the final extraction yield. [25,26] The increasing percentage yield of methanol extract indicated that the sample contains more polar than the semi-polar components (lower percentage yield of ethyl acetate extract).

Phytochemical composition of the extracts from Rhizophora species
Qualitative evaluation of the primary phytochemical properties of all samples was carried out against alkaloids, anthocyanin, cardiac glycoside, saponins, phenols, flavonoids, tannins, steroids, and terpenoids. Alkaloids, cardiac glycoside, saponins, phenols, tannins, steroids, and terpenoids were found in the methanol extract of *R. mucronata*. However, anthocyanins and flavonoids were absent. *Rhizophora stylosa* methanol extract contains no anthocyanins, cardiac glycosides, or saponins. Meanwhile, alkaloids, saponins, and phenols were detectable in the methanol extract of *R. apiculata*. Anthocyanins and cardiac glycosides were absent from the ethyl acetate extract of *R. mucronata*. However, practically all phytochemicals, except anthocyanins, were present in the ethyl acetate extract of *R. stylosa* and *R. apiculata*. The result of primary phytochemical screening demonstrated a greater variety of chemical components from ethyl acetate extract than methanol extract, particularly for species of *R. stylosa* and *R. apiculate* (Tables 2 and 3). The phytochemical properties of each species in the two extractive solvents varied.
The effectiveness of the extraction process to produce secondary metabolites is influenced by several variables, including extraction method, temperature, extraction time, phytochemical content, and extractive solvents.[25-29] According to a study by Throung,[26] the polarity of a solvent significantly affects the number of chemical components present, the success of the extraction yield, and biological activity. Alkaloids, phenolics, saponins, cardiac glycosides, flavonoids, tannins, steroids, and terpenoids are only a few of the phytochemical components that have been linked to a variety of bioactivities. [26,28,31,32]

DPPH radical scavenging activity of the extracts from Rhizophora species
The DPPH radical scavenging assay is widely used to evaluate the potency of a plant extract to neutralize free radicals, especially organic radicals.

Table 1: The ethyl acetate and methanol extractive yield (%) of *Rhizophora mucronata, R. stylosa,* and *R. apiculate*

Sample	Extractive yield (%)	
	Ethyl acetate	Methanol
R. mucronata	3.97	6.30
R. stylosa	3.94	4.34
R. apiculata	2.86	4.79

Table 2: Phytochemical properties of methanol extract of *Rhizophora* species.

Phytochemical test	*R. mucronata*	*R. stylosa*	*R. apiculata*
Alkaloids	+	+	+
Anthocyanins	-	-	-
Cardiac Glikosida	+	-	-
Saponins	+	-	+
Phenols	+	+	+
Flavonoids	-	+	-
Tannins	+	+	-
Steroids	+	+	-
Terpenoids	+	+	-

+: Present; -: Absent

Table 3: Phytochemical screening of ethyl acetate extract of *Rhizophora* species

Phytochemical test	*R. mucronata*	*R. stylosa*	*R. apiculata*
Alkaloids	+	+	+
Anthocyanins	-	-	-
Cardiac Glikosida	-	+	+
Saponins	+	+	+
Phenols	+	+	+
Flavonoids	+	+	+
Tannins	+	+	+
Steroids	+	+	+
Terpenoids	+	+	+

+: Present; -: Absent

The ability of an extract or vitamin C (as a positive control) to donate a hydrogen electron and bind with the nitrogen radical in DPPH causes the purple colour solution of the DPPH radical to change to light yellow. The scavenging activity of each species varied in methanol and ethyl acetate extracts, which increased by increasing the concentrations. The DPPH radical scavenging activity method was used to measure significant antioxidant activity. As shown in Figure 1, both methanol and ethyl acetate extracts showed high antioxidant activity. The IC_{50} of the *R. apiculata, R. stylosa*, and *R. mucronata* extracts in ethyl acetate were respectively 0.72±0.07, 1.16±0.04, and 2.34±0.12 µg/mL. Meanwhile, 0.04±0.00 µg/mL of *R. mucronata* extract, 0.37±0.09 µg/mL of *R. stylosa* extract, and 5.06±0.41 µg/mL of *R. apiculata* extract were found using methanol as a solvent.
Ascorbic acid, used as a positive control, demonstrated increased action against the DPPH radical with an IC_{50} of 0.0029± 0.00 µg/mL.
The potential biological activity of methanol and ethyl acetate extracts is implicated by the presence of chemical components that are active chemicals, as revealed by the results of the phytochemical screening of each species (Tables 2 and 3). *R. mucronata* exhibited the greatest inhibition in the methanol extract, followed by *R. stylosa* and *R. apiculata*. Meanwhile, in the ethyl acetate extract, the highest

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inhibition was observed in the *R. apiculata*, followed by *R. stylosa*, and the lowest was *R. mucronata*. Oxidative inhibition of methanol extracts of *R. mucronata* and *R. stylosa* is greater than ethyl acetate extracts, although inversely proportional to *R. apiculata*. The high antioxidant capacity of the *Rhizophora* genus is likely associated with the high phenolic content.[33] Previous research indicated that high phenolic compounds, alkaloids, and terpenoids are associated with powerful antioxidant activity.[30-32] In addition, phytochemical constituents such as alkaloids, tannins, saponins, and terpenoids are also reported to act as scavengers and antioxidant chain breakers.[1,34-36] Phenol derivatives such as flavonoids and tannins have antioxidant activity that may be influenced by the position and number of hydroxyl groups. The instability of phenol radical derivatives is caused by the radical neutralization process that involves transferring an electron from H atoms. By transferring electrons from the hydroxyl group to the hydrogen atom, the number and position of the hydroxyl group can normalize the electron shortage.[37-38]

A previous investigation found that *R. mucronata* extracts in methanol and ethyl acetate had high activity, with IC_{50} values of 36.17 and 28.53 mg/mL, respectively. Interestingly, the antioxidant activity of the methanol and ethyl acetate extracts of the *Rhizophora* species from Bontang waters was higher than in other studies,[1,13,39] which may be due to variations in the natural environment and edaphic characteristics of their habitat. Extensive research is required to determine the environmental conditions that affect the occurrence of active chemicals in the mangroves. The results of this study are the first to show the significant antioxidant potential of natural mangroves from Bontang waters. This offers a great opportunity to discover more active compounds in mangroves from Bontang on a large scale.

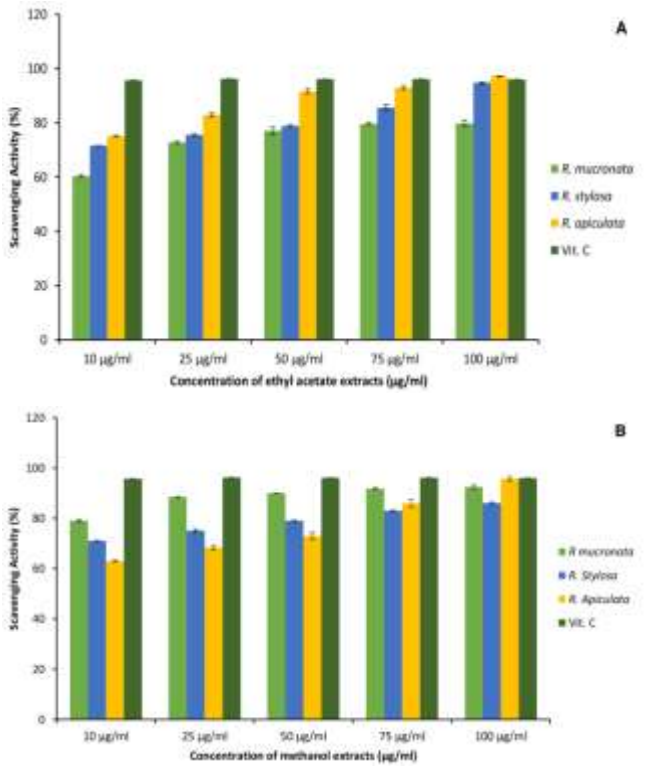

Figure 1: The DPPH (1,1-diphenyl-2-picrylhydrazyl) scavenging activity of ethyl acetate extract (A) and methanol extract (B) of *Rhizophora* species.

Conclusion

The findings of this study reveal that *Rhizophora mucronata*, *Rhizophora stylosa*, and *Rhizophora apiculata* exhibit strong DPPH radical activity in ethyl acetate and methanol extracts. This activity is consistent with the presence of phytochemicals in these plants. As a result, these phytochemical properties may be explored for the development of natural antioxidants. The possibility of using the three *Rhizophora* species as natural antioxidants is very high.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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