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Article

Potency of Mangrove Leaves (*Rhizophora mucronata*) Containing Bioactive Compounds as Source of Antioxidant: A Review

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Abstract

Bioactive compounds derived from plants are gaining attention worldwide because they have beneficial effects on humans. *Rhizophora mucronata* is a species of mangrove plant that has slightly dark leaves and glossy green on the top of the leaves and pale green on the bottom and acts as a natural antioxidant which has traditionally been used as a food ingredient and traditional medicine. Therefore, this article was written to review the bioactive compounds in *Rhizophora mucronata* leaves, antioxidant levels and the benefits of antioxidants in food and health products. This article summarizes relevant literature regarding *R. mucronata* which was tested using Phytochemical screening, FTIR and DPPH (1,1-diphenyl-2-picrylhydrazyl) methods. Phytochemical analysis revealed the presence of alkaloids, flavonoids, steroids, phenolics, tannins and saponins. Based on the FTIR test, *R. mucronata* leaf extract showed the presence of –OH, C-H (aromatic), C=C (aromatic), and C-O groups. The antioxidant capacity expressed in IC_{50} (mg/mL) shows that *R. mucronata* has the potential to be used as a source of natural antioxidants. Antioxidants can play a role in improving the sensory quality of a food product and as a natural bactericidal so that it can be used to maintain aquatic biota and food preservatives. In addition, antioxidants contained in mangroves can prevent aging, wrinkles, cancer, obesity, and diabetes mellitus.

Keywords: bioactive compounds, leaves, mangrove, *Rhizophora mucronata.*

1. Introduction

Indonesia is an archipelagic country rich in natural resources. One of the natural wealth that can be utilized its natural potential is mangrove plants or commonly known as mangrove plants [1]. Mangroves are widespread in tropical and subtropical regions, mostly found along saltwater tidal zones in the protected coastlines of the Indo-Pacific (India to Australia) [2]. Mangroves are referred to as coastal plants, tidal plants and brackish plants because they grow well on coral beaches or thin sandy coral reef land, or on beaches that have alluvial soil types [3]. Mangroves are included in supporting plants of various types of coastal ecosystems, estuaries and deltas in tropical and sub-tropical regions [4]. One of the characteristics of mangrove plants is that they have roots that come to the surface. The appearance of mangroves is like a stretch of shrubs that separates land from sea so that mangroves can be said to be transitional ecosystems between land and sea [5].

Around 202 types of mangrove species in Indonesia have been identified and thrive because the tropical climate is an ideal place for mangrove

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plant growth [6]. One type of mangrove that is abundant in Indonesia's tropical rainforests is *Rhizophora mucronata* [7]. R. *mucronata* is one of the mangrove plant species belonging to the *Rhizophoraceae* family and has a large distribution in the Indo-Pacific region [8]. *Rhizophora mucronata* is a type of true mangrove plant that has a root system with a rhizophore type or supporting roots that grow down from the stem to the ground and plunge into the ground. Morphologically, *R. mucronata* can grow up to a height of 3-4 meters with thick, elliptical, green leaves. *Rhizophora mucronata* has creamy white flowers and produces cigar-shaped fruits (propagul) [9].

Traditionally *Rhizophora mucronata* has been used as food and beverage ingredients, vegetables, tanning agents, dyes, slimming, antidiarrheal and anti-vomiting [10]. In addition, it can be used as traditional medicine, such as antifungal, anti-viral, anti-cancer and anti-tumor drugs [11]. The presence of a class of secondary metabolite compounds in plants provides various biological activities. Chemotaxonomy, *R. mucronata* contains secondary metabolites such as alkaloids, tannins, terpenoids, saponins, phenolics, flavonoids and glycosides [12]. Methanol extract of *R. mucronata* contains bioactive compounds identified as Quinizarin so that *R. mucronata* has antibacterial, antioxidant and cytotoxic properties [13]. Crude extract of R. mucronate fruit acts as an anticancer because of the presence of terpenoid compounds [14]) and acts as an antidiabetic because it can reduce glucosal levels [15].

Rhizophora mucronata mangrove leaves contain 2-(2ethoxy ethanol, kau-16-ene and benzophenon, phenolic compounds flavonoid group, phenolic acids, phenolic acids, dihydroflavonol tannins, caffeic acid, vanillic acid, p-hydroxy benzoate acid, tannins, alkaloids, coumarins, flavonoids, phenols and polyphenols, quinones, resins, saponins, phytosterols, xanthoprotin, pigments (chlorophyll, carotenoids) and sugars [16]. The leaves of *R. mucronata* are rich in phenolic and triterpenoid compounds. Phenolic compounds in *R. mucronata* methanol extract make *R. mucronata* potential as a skin protector against ROS so that it can prevent aging and wrinkles [9]. Based on the potential possessed by *Rhizophora mucronata,* the writing of this article aims to examine the characteristics of phenolic compounds based on *Rhizophora mucronata* mangrove leaf extract which have the potential as antioxidants.

2. Discussion

2.1. Bioactive compounds as source of antioxidant

Antioxidants are compounds that have the ability to inhibit, delay, prevent or slow down oxidation reactions, even at low concentrations. Mangrove plants (*Rhizophora mucronata*) are plants that have potential as natural antioxidants [17]. Antioxidants contained in natural ingredients can generally be obtained through the extraction process. The extraction process of mangrove leaf samples (*Rhizophora mucronata*) was carried out by maceration method [7]. Maceration is a simplisia extraction process carried out at room temperature using solvents and shaken several times [18]. The yield is affected by the size of the sample powder, the length of maceration time, and the solvent used. The smaller size of mangrove leaf powder (*Rhizophora mucronata*) will produce a higher yield because the contact area between the material and the solvent is wider so that the solvent more easily breaks down the cell wall [19]. In addition, the longer the extraction time will cause the solubility of the bioactive components in the solution to increase and the extract will also increase until the solution reaches its saturation point [18].

Maceration extraction is carried out by soaking mangrove leaf powder using certain solvents with a ratio of leaves to solvents used 1: 3 within a certain time frame [19]. Each solvent has a different character in the uptake of bioactive compounds based on their polarity [18]. There is a difference in the percentage of yield produced in samples extracted stratified with n-hexane, ethyl acetate and methanol solvents. The highest yield was produced in extraction with methanol (3.27%), followed by ethyl acetate extract, and n-hexane. This shows that the leaves of *R. mucronata* mangroves contain many compounds that are polar [16]. This is in line with the statement which states that the extraction of mangroves (*R. mucronata* Lamk.) is best when extracted under polar conditions with methanol [6]. Ethanol extract of *R. mucronata* Lamk. fruit yields a yield of about 9.76% to 10.95%. The extraction results using leaf methanol solvent (*Rhizophora mucronata*)

are green in color and yellow on the upper wall of the boundary [7]. Visually, maceration extraction will produce a brownish-green color if using methanol solvent, brownish-green if using ethyl acetate solvent, and yellowish-brown with n-hexane solvent (Table 1). The green color is thought to come from chlorophyll while the brown color is thought to come from carotenoids and other pigments [16].

Maserat obtained from maceration extraction evaporated using a rotavapor tool will produce a viscous extract that can be used to determine the bioactivity of secondary metabolite compounds in mangrove plants. The method that can be used in identifying the content of secondary metabolite compounds contained in samples is phytochemical screening [20]. Secondary metabolite compounds as potential antioxidants contained in mangrove leaves (*Rhizophora mucronata*) macerated with methanol solvents, namely alkaloids, flavonoids, steroids, phenolics, and saponins [7]. The results that have been obtained are also supported by research that has been conducted on dichloromethane and ethyl acetate extracts of *Rhizophora mucronata* mangrove leaves which are proven to contain both alkaloid compounds, flavonoids, tannins and phenolics. Unidentified compounds in dichloromethane and ethyl acetate extracts of *Rhizophora mucronata* mangrove leaves are saponins and terpenoid compounds [15].

Table 1. Extraction yield of *R. mucronata* leaves

Solvent	Yield (%)	Color	Reference
Methanol	3.27	Brownish green	[16]
Ethyl acetate	2.32	Brownish green	
n-Hexane	0.18	Yellowish-brown	

Compounds contained in mangrove leaves have a function as antioxidants. Flavonoids are a class of phenolic compounds that contain many hydroxyl groups so that they can transfer electrons to free radical compounds. Similarly, alkaloid compounds that can increase the production of hydroxy groups (OH^-) so that they have the ability as antioxidants [21]. Tannin compounds are phenolics in the form of phenol polymerics that function as antioxidants [6] and have polar properties that can dissolve in methanol [20]. Phenolic components have aromatic structures that bind to one or more hydroxyl groups [6]. Saponins are bioactive compounds that belong to the group of glycoside compounds, terpenoids are forms of compounds with a large structure and derived from isoperne units (C5). Steroids exist in the form of glycosides or compounds consisting of sugar and aglicon [20].

The Fourier Transform Infra-Red (FTIR) test was performed to determine the functional group profile on *R. mucronata* leaves. FTIR spectra show the presence of typical peaks of flavonoids, namely 3319, 2973, 1607, 1444, 1042, and 878 cm-1. The wavenumber 3319 indicates the absorption of the –OH group. The presence of C-H alkenes is determined at wavenumbers 2973 cm-1 while wavenumbers 1607 and 878 cm-1 indicate the presence of C=C of aromatic rings and C-O [22]. These results are similar to the research which states that the infrared spectrum results of methanol extract of *R. mucronate* leaves have peaks in waves 3424, 2927, 1631, and 1449 which indicate the presence of –OH, C-H (aromatic), C = C (aromatic), and C-O groups, indicating that isolates are compounds of phenolic groups such as flavonoids and tannins [23].

2.2. Evaluation of Antioxidant Activity using DPPH

Antioxidant test with DPPH method (1,1-diphenyl-2-picrylhydrazyl) is a quantitative test method widely used to evaluate the potential of plant extracts to neutralize free radicals [24]. This test can be observed visually, namely the color change from dark purple to pale yellow [15]. Color change is caused by a reaction between antioxidant compounds and free radicals through the mechanism of hydrogen atom donation. The conjugated double bond in DPPH is reduced due to the capture of one electron by an antioxidant compound which causes no chance for that electron to resonate where this change can be measured and recorded with a spectrophotometer [25]. This is in accordance with the statement that

the testing of the antioxidant activity of the DPPH method on *R. mucronata* leaf extract was carried out using a UV-Vis spectrophotometer with a wavelength of 517 nm [15].

The antioxidant value of an extract is determined based on the regression value equation between concentration and percent inhibition so that the IC_{50} value is known [24]. The IC_{50} value is the concentration of the extract solution which causes a reduction in DPPH activity by 50% [15]. The IC_{50} value of the tested extract was obtained from compounds contained in the extract and fraction, where both the extract and the fraction are a collection of several secondary metabolite compounds that have not been separated [24]. *Rhizophora mucronata* (Lamk.) known as black mangrove belongs to the Rhizophoraceae family [21]. This mangrove plant grows in the tropics and its fruits are commonly used as food and traditional medicine by the community around the coast. The abundance of active compounds detected through phytochemical tests determines the high antioxidant activity in crude extracts. Based on Table 2, the maturity of *R. mucronata* fruit has an effect on the IC_{50} value produced in the DPPH test. Old *R. mucronata* fruits produce lower IC_{50} values compared to young *R. mucronata* which signifies higher antioxidant activity [6].

Based on Table 2, dichloromethane and ethyl acetate extracts of *Rhizophora mucronata* mangrove leaves have potential as strong antioxidants because they have IC_{50} values in the range of 50-100 ppm [15]. Methanol extract has the strongest antioxidant activity compared to ethyl acetate and n-hexane extracts because it has the smallest IC_{50} value [16]. The use of methanol solvents in extracts is able to dissolve almost all components both nonpolar, semipolar, and polar. The antioxidant content in the bark of *R. mucronata* is included in the strong category [26]. Bark samples on *Rhizophora mucronata* mangrove trees from different waters produced different IC_{50} values. This is due to differences in age, geographical location and climate that will affect the content of bioactive compounds in mangrove plants. In addition, different antioxidant abilities are caused by the content of secondary metabolites that act as antioxidant supporting compounds such as phenolic compounds, flavonoids, and tannins contained in different mangroves. Polluted environment causes mangrove trees to be depressed so that they contain higher secondary metabolites [20].

There is a positive correlation between the high content of phenolic group compounds and antioxidant activity [24]. Antioxidant activity in a sample is influenced by the total phenolic content in the sample. The higher the amount of phenol compounds and flavonoid bioactives in a sample will provide higher antioxidant activity so that the IC_{50} value will be lower [27]. DPPH free radical reducing activity by Rhizophora mangrove extract proves that mangroves have the ability as antioxidants and illustrates the effectiveness, prevention ability and repair mechanism in overcoming damage to biological systems.

Table 2. Antioxidant activities of various parts of *R. mucronata* extract using DPPH methods

Sample	Solvent	IC_{50} (ppm)	Reference
Unriped fruits	Methanol	58.468	[6]
Unriped fruits	Methanol	10.257	
Leaves	Dichloromethane	70.38	[15]
Leaves	Ethyl acetate	59.89	
Leaves	n-Hexane	151.13	[16]
Leaves	Methanol	113.41	
Leaves	Ethyl acetate	184.78	
Trunk bark	Methanol	84.80	[26]
Trunk bark	Methanol	65.59	

2.3. Application of antioxidant in food production and health improvement

Secondary metabolites are known to exhibit significant and substantial antioxidant properties, not only by their ability to donate electrons but also by the chelate ability of metals [28]. *Rhizophora mucronata* mangrove leaves contain secondary metabolite compounds that act as antioxidants such as alkaloids, flavonoids, tannins and phenolics [15]. Antioxidants have an important role in maintaining quality and increasing the hedonic value of a food product or ingredient. According to Utari et al. (2018), the high antioxidants in *Avicennia marina* mangrove fruit can

be used as a natural preservative. The use of mangrove fruit fiber in tilapia can extend shelf life. This is because phytochemical compounds will damage the cytoplasmic membrane and kill epidermal cells so that they act as antibacterial or antimicrobial. There was a sensory increase in chocolate treated with the addition of Rhizophora apiculata extract. Antioxidant compounds in mangroves can inhibit fat oxidation in chocolate bars indicated by unchanged texture and the absence of fat bloom after storage for 14 days. Antioxidants make the color and appearance of brown which is preferred due to some antioxidant components of tannins and flavonoids whose pigments are dark in color [29]. Besides being used in food products, *R. mucronata* stem and leaf extracts contain phytochemicals that act as natural bactericides because they can inhibit the growth of pathogenic bacteria that attack freshwater biota, namely *Aeromonas hydrophila* [30].

Mangroves have the potential to be used as a source of antioxidants derived from nature that can be used as traditional medicine. *R. mucronata* fruit extract as a potential source of anticancer agents against leukemia cancer cells. Terpenoid compounds in crude extract of *R. mucronata* fruit act as anticancer with moderate toxicity at IC_{50} values of 398 µg mL^{-1} against P388 murine leukemia cells. Mangroves exhibit pronounced antioxidant activity and enzyme inhibition. The leaves of *R. mucronata* are rich in phenolic and triterpenoid compounds [14]. Compounds make *R. mucronata* potential as a skin protector against ROS so that it can prevent aging and wrinkles. *R. mucronata* fruit extract can suppress pancreatic lipase activity the most, which is 101.02mg OE/g followed by root extract 88.32mg OE/g which plays an important role in obesity management [9]. Antioxidants can also play a role in overcoming degenerative diseases such as diabetes mellitus. This is evidenced by the increase in antioxidants such as the enzyme catalase can ward off free radicals and lower blood glucose levels. Testing the antidiabetic activity of dichloromethane extract of *Rhizophora mucronata* mangrove leaves at a dose of 250 mg/kg BW can reduce mouse glucose levels by 68.78% [15].

3. Conclusions

The results of antioxidant tests on *Rhizophora mucronata* mangroves are proven to have the ability as natural antioxidants. *Rhizophora mucronata* has potential as an antioxidant, especially in the leaves, because it contains secondary metabolites and phenolic compounds. Phenolic compounds found in mangrove leaves (*Rhizophora mucronata*) include alkaloids, flavonoids, steroids, phenolics, tannins and saponins. Based on the FTIR test, R. mucronate leaf extract showed the presence of –OH, C-H (aromatic), C=C (aromatic), and C-O groups The potential of *R. mucronata* as an antioxidant is determined based on the IC_{50} value. The lower the IC_{50} value, the more secondary metabolite compounds so that the potential as an antioxidant will be stronger. Antioxidants can play a role in improving the sensory quality of a food product and as a natural bactericidal so that it can be used to maintain aquatic biota and food preservatives. In addition, antioxidants contained in mangroves can prevent aging, wrinkles, cancer, obesity, and diabetes mellitus.

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