



Potential Antioxidant Constituent from Leaf of *Rhizophora apiculata* an Typical Mangrove at Lempasing, South Lampung Coast

Potensi Kandungan Antioksidan dari Daun *Rhizophora apiculata* Mangrove Khas Lempasing, Pesisir Lampung Selatan

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ABSTRACT

The mangrove woodland is a distinct bush or brine habitat characterized by a coastal sedimentation environment in which fine sediment (often rich in organic matter) accumulates in areas protected from high energy waves. Mangrove forests thrive on the coasts of tropical and subtropical regions, including Indonesia. One of them is the Oil Mangrove (*R. apiculata*) which grows well on the coast of Lampung. Mangrove plants are tolerant to high salt levels, this special trait is due to the presence of secondary metabolites produced in response to various environmental stresses. Secondary metabolite compounds from mangroves have bioactivity such as antidiabetic, antimicrobial, antioxidant and anticancer. Exploration of secondary metabolites from mangroves, especially oil mangroves locally named as Bakau Minyak, which are widely grown in the Lempasing area, coastal Lampung for the development of medicinal compounds, has not been intensely reported. This investigation aims to study the potential phytochemistry profile of *R. apiculata*'s leaf methanol and ethyl acetate extract as an antioxidant. The results for phytochemistry showed that both extracts containing alkaloids, flavonoids, and steroids. The methanol extract contains tannin, whereas the ethyl acetate extract doesn't exist. The results of testing the antioxidant showed moderate IC₅₀. Based on the phytochemistry and antioxidant profile showed that the methanol extract had stronger antioxidant activity than the ethyl acetate extract.

Keywords: Oil mangrove leaf, mangrove, phytochemistry profile, Lampung

INTRODUCTION

Rhizophora (Rhizophoraceae) is a family that occupies both Atlantic East Pacific (AEP) and Indo West Pacific (IWP) biogeographic

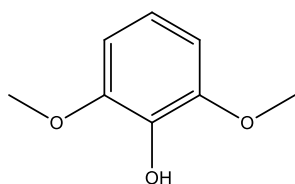
areas. In addition to its wide geographical distribution, *Rhizophora* is one of the most specific mangrove families (Hadi, 2016; Duke, 2017). This type of mangrove grows and develops in its habitat which has an extreme

environment and can change due to environmental influences in the form of high temperatures, tides, silt deposition, and the abundance of microorganisms. So this plant has good potential to be studied regarding the secondary metabolic compounds it contains (Mori et al, 2021; Eriani & Usman, 2017).

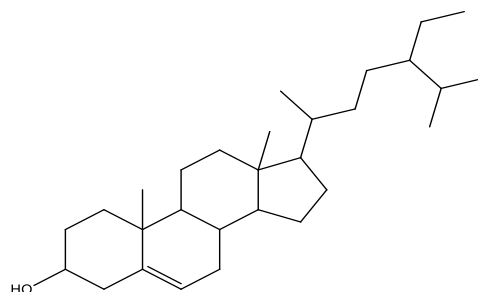
Along with qualitative and quantitative phytochemical analysis, leaf extract of *Rhizophora apiculata* showed the presence of 24 different compounds including silane, 2-cyclohexene, hexadecanoic acid, linoleic acid, linolenic acid, phytol and stearic acid. Pyridine is present as the main constituent with the highest percentage of area 12.47% (Eriani & Usman, 2017). Almost all parts of the plant *Rhizophora* sp. contains alkaloids, saponins, flavonoids and tannins (Hadi, 2016).

Mangroves have been known to produce steroids, alkaloids, and terpenoids that have biological benefits such as antidiabetic, antibacterial, anticancer, and antioxidant. The nature of the bioactivity in mangroves has impressed the pharmaceutical industry. This plant has been used traditionally to treat diarrhea and asthma, as well as the treatment of scurvy and rheumatism. *Rhizophora apiculata* or known as the Oil Mangrove in Lampung usually grows in the coastal areas of Lampung. This plant is hardy, rich in tannins, and has a high density, mainly used for construction and charcoal making (Kurniawan et al, 2021).

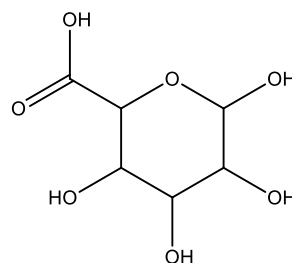
It can be seen that several structural compounds have been obtained in previous research on *Rhizophora*, including β -sitosterol from the root bark of *Rhizophora apiculata* as an antimicrobial (Kurniawan et al, 2021), syringol obtained from pyrolignic acid extract or wood liquid as an antioxidant (Loo et al, 2008), (+)-catechin from the tannin group obtained from the wood bark as an antioxidant (Rahim et al, 2008), and there is also a structure of uronic acid obtained from the leaves as an antiviral (Premanathan et al, 1999). These compounds can be seen in Figure 1.



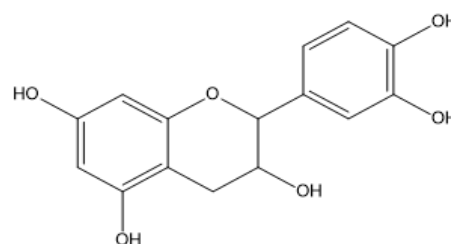
a. 2,6-Dimethoxyphenol - wood



b. β -sitosterol - root bark



c. Uronic Acid - leaf



d. (+)-catechin - wood bark

Figure 1. Secondary metabolite structure of *Rhizophora apiculata*

METHODS

Materials

The materials used in this study were the leaves of *Rhizophora apiculata*, methanol, iron (III) chloride solution, $MgCl_2$ powder, ethyl acetate, aquadest, Lieberman-Burchard reagents, Mayer's reagents and Dragendorff's reagents, 2,2-diphenyl-1-picrylhydrazyl (DPPH) solution.

Erlenmeyer and beaker glass, stirring rod, analytical scales, glassware, aluminum foil, measuring cup, spatula, test tubes, tube racks, micropipettes, filter paper, chamber, rotary evaporator, cuvette, UV-Vis spectrophotometer.

Sampling and Determination

Rhizophora apiculata leaves that is used for research was taken from Balai Besar Perikanan Budidaya Laut Lampung. The specimen is send to Herbarium Bogoriences-LIPI, Bogor to

determine the species as *R. apiculata*, under this specimen code HL124AB765. The determination of the *Rhizophora apiculata* plant aims to ensure and ensure that the ingredients used are truly *Rhizophora apiculata*.

Drying Method

The leaves of *R. apiculata* is washed and dried in a room so that the active substances contained are not damaged by direct sunlight. Furthermore, the dried leaf of the plant is blended until it becomes a coarse powder.

Maceration Process

In this research, sample was extracted using methanol or ethyl acetate solvent. The extract was made using maceration method, which is 100 g of coarse powder of sample put into a erlenmeyer glass and then added methanol as much as 350 ml, covered and allowed to soak for 3 days protected from light (every day stirring for at least 30 minutes). The extract is then filtered using filter paper so that it is obtained filtrate. This process was repeated 3 times.

Phytochemical Assay

1 g extract dissolved with 10 ml of methanol. And then, sample was taken as much as 1 ml (20 drops) using a pipette and then added into 7 test tubes for each sample extract (methanol and ethyl acetate extract). The first tube functions as a blank. The next six tubes is added with reagents for examination.

- **Alkaloid Examination**

The second tube is added Dragendorff reagent as much as 1 ml (20 drops), if orange, yellow to brown precipitate is formed indicating the presence of alkaloids. The third tube was added by Mayer reagent as much as 1 ml, if a white to yellowish sediment was formed indicating the presence of alkaloids (Saragih & Arsita, 2019).

- **Flavonoid Examination**

1 ml of the sample was added with 1 ml of $MgCl_2$ reagent which had been made by dissolving the $MgCl_2$ powder with 25 ml of aquadest. The presence of flavonoids is indicated by the presence of a red, yellow or orange color on the amyl alcohol layer (Rumagit, 2015).

- **Steroid and Terpenoid Examination**

1 ml extract was added with 1 ml of Lieberman-Burchard reagent. The formation of purple color indicates triterpenoid group content, whereas if green-blue color is formed, it indicates the presence of steroid group compounds (Setyawaty et al, 2020).

- **Saponin Examination**

1 ml extract was dissolved with 1 ml of distilled water in a test tube. After that, the test tube is shaken vigorously for about 5 minutes. A persistent foam will be formed which does not disappear on the addition of 1 drop of methanol indicating the content of saponins (Setyawaty et al, 2020).

- **Tannin Examination**

1 ml extract solution was reacted with iron (III) chloride solution. The results showed the presence of tannin compounds if a dark blue, black blue or greenish black solution was formed (Saragih & Arsita, 2019).

Antioxidant Assay

DPPH possesses a purple color, with a maximum absorption at 519 nm in ethanol; hence, scavenging the DPPH radical by coffee antioxidants will result in a decrease in absorption readings over time; the extent of decrease in DPPH absorption being proportional to the concentration of radicals that are being scavenged, according to the principle of Blois. Measurements are made using a UV-visible spectrophotometer at room temperature, and the scavenging capacity is represented as the percentage of DPPH radical inhibition. The DPPH assay is based on both electron transfer (SET) and hydrogen atom transfer (HAT) reactions. An advantage of the DPPH assay is that it is an easy, economic and rapid method to evaluate the radical scavenging activity of non-enzymatic antioxidants. Since DPPH is a stable radical, this assay considers not only the concentration of the tested sample, but also the reaction time and the temperature; both of which when controlled carefully enable this assay to be highly reproducible (Liang & Kitts, 2014).

1 g extract dissolved with 10 ml of methanol. And then, sample was taken as much as 0.2 ml using a micropipette added into the first tube. The next six tubes added with 0.1 ml fresh methanol using a micropipette. Then, do the mixing sequentially starting from the first

tube to the sixth tube, the last tube used for blank.

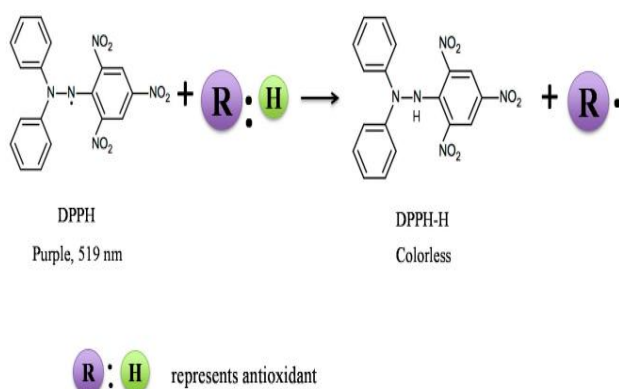


Figure 2. Antioxidant Reaction of DPPH (Liang & Kitts, 2014)

RESULT AND DISCUSSIONS

Result of Alkaloid

The results of the phytochemical analysis of the leaves extract of *R. apiculata* are presented in Table 1. The results of the phytochemical analysis showed that the leaves extract of *R. apiculata* contains alkaloid compounds. In this study, the leaves extract of *R. apiculata* was positive for alkaloid compounds due to the color change from green to yellowish-green in the methanol extract of the leaves of *R. apiculata* and from the ethyl acetate extract of the leaves of *R. apiculata* accompanied by the formation of a precipitate in the Mayer reagent test. While the positive results in the Dragendorff reagent test showed a color change to orange-green in the methanol extract and the ethyl acetate extract of the leaves of *R. apiculata*, the color changed to orange.

In identification of alkaloids with Mayer's reagent, a positive result was indicated by the formation of a precipitate. This precipitate is the result of the reaction of potassium ions (K^+) from potassium tetraiodomercurate(II) with nitrogen in the alkaloids to produce a precipitating potassium-alkaloid complex (Hadi, 2016).

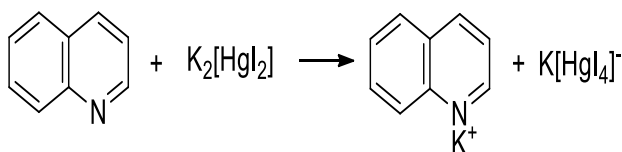


Figure 3. Alkaloid-Mayer Reaction

Result of Steroid and Terpenoid

Based on the test, the leaves extract of *R. apiculata* contains steroid and triterpenoid compounds. Test using Liebermann-Burchard reagent (acetic anhydride- H_2SO_4 Concentrated) which produces a blue-green color on most triterpenes and sterols (Harborne, 1984). The methanol extract of *R. apiculata* leaves showed a change from green to slightly reddish color which indicated the presence of terpenoid compounds. Ethyl acetate extract of *R. apiculata* leaves produces a slightly bluish color.

This color change is caused by an esterification reaction, namely the formation of ester compounds by steroid or triterpenoid compounds with acetic anhydride, resulting in a color change. The reaction between the carbocation formed from the reaction of acetic anhydride with acid reacts with the O atom in the OH group contained in steroid or triterpenoid compounds (Setyawaty et al, 2020).

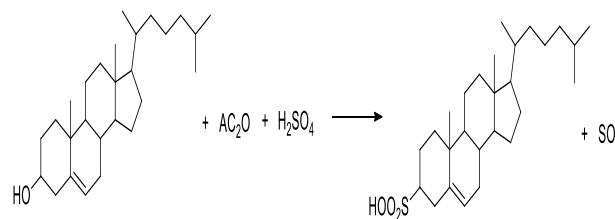


Figure 4. Reaction of Steroid and Terpenoid with Lieberman-Burchard

Result of Tannin

Positive results containing tannin compounds were only shown by the test sample of the *R. apiculata* leaves methanol extract with a color change to dark-green. Tannins are polar compounds and dissolve well in polar organic compounds, the polarity of tannin compounds is due to the presence of a hydroxyl group (-OH) attached to the benzene ring (C6) (Noer et al, 2018). Tannin test generally uses $FeCl_3$ reagent with different reactions depending on the group of reacting tannin compounds. Hydrolyzed tannin compounds produce a blue-black color and condensed tannins will produce a green-black color (Sangi et al, 2008). The formation of color changes in the extract after the addition of $FeCl_3$ was caused by the reaction of one of the hydroxyl groups of tannin compounds with $FeCl_3$ which formed a complex with Fe^{3+} ions.

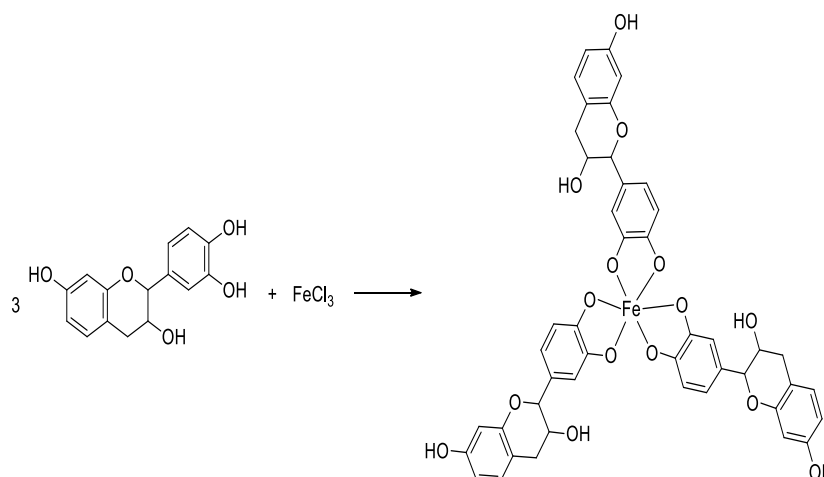


Figure 5. Reaction of Tanin with FeCl_3

Result of Saponin

The results of phytochemical tests showed that the extracts of *R. apiculata* leaves contain secondary metabolites: saponins, indicated by the presence of foam, in plants, saponins are spread evenly in parts such as roots, stems, tubers, leaves, seeds and fruit. Saponins are complex glycoside compounds that are compounds that condense a sugar with an organic hydroxyl compound that when hydrolyzed will produce sugar (glycon) and non-sugar (aglicon).

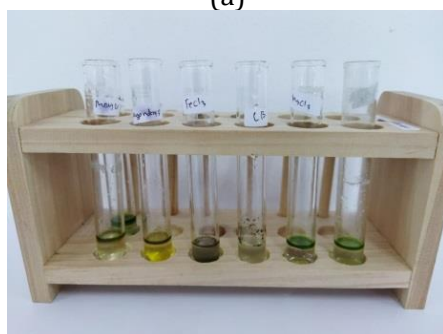
namely the properties of compounds with polar and nonpolar groups. When the extract is shaken with water, saponins can form micelles with non-polar groups facing in and polar groups facing out, this state looks like foam (Sangi et al, 2008). From the results of the saponin test of the methanol extract and ethyl acetate of the leaves of *R. apiculata*, both extracts showed negative results because there was no foam formation.

Result of Antioxidant Test

The antioxidant test was carried out using the DPPH method (2,2-diphenyl-1-picrylhydrazyl) with absorption measurements using a UV-Vis spectrophotometer at a wavelength of 517 nm. DPPH is a stable free radical compound with unpaired electrons that are delocalized throughout the molecule (Liang & Kitts, 2014). The principle of this method is the existence of bonds between the hydrogen atoms of antioxidant compounds with free electrons in radical compounds. The bond formed causes a change from free radicals (diphenylpicrylhydrazyl) to non-radical compounds (diphenylpicrylhydrazyl). Reduction of free radicals resulted in a color change of DPPH from purple to yellow DPPH-H (Setiawan et al, 2018).



(a)



(b)

Figure 6. Phytochemical assay of a) methanol extract and b) ethyl acetate extract from leaves of *Rhizophora apiculata*

The formation of foam is due to saponin compounds having surface active properties,



Figure 7. The results of Antioxidant assay

IC₅₀ was used as a parameter for determining antioxidant activity in samples using the DPPH method. IC₅₀ is the antioxidant concentration needed to reduce the initial DPPH concentration by 50%, so the lower the IC₅₀ value, the higher the antioxidant activity of the test sample (Rivero-Cruz et al 2020). Antioxidant testing used samples of crude methanol and ethyl acetate extracts of *R. apiculata* leaves with sample concentrations of 6.25%, 12.5%, 25%, 50%, and 100% for each extract. From measurements using a UV-vis spectrophotometer, the absorbance decreased

with increasing the concentration of the test sample. The ability of the test sample to inhibit oxidation reactions by radicals is expressed in %Radical Scavenging Activity (%RSA) or %inhibition (Huliselan, 2015). The test results show that the inhibition of DPPH by the extract is directly proportional to the concentration of the extract which can be seen in the Figure 8 (a), for the antioxidant test results for the methanol extract and the Figure 8 (b), shows the test results for the *R. apiculata* leaves ethyl acetate extract.

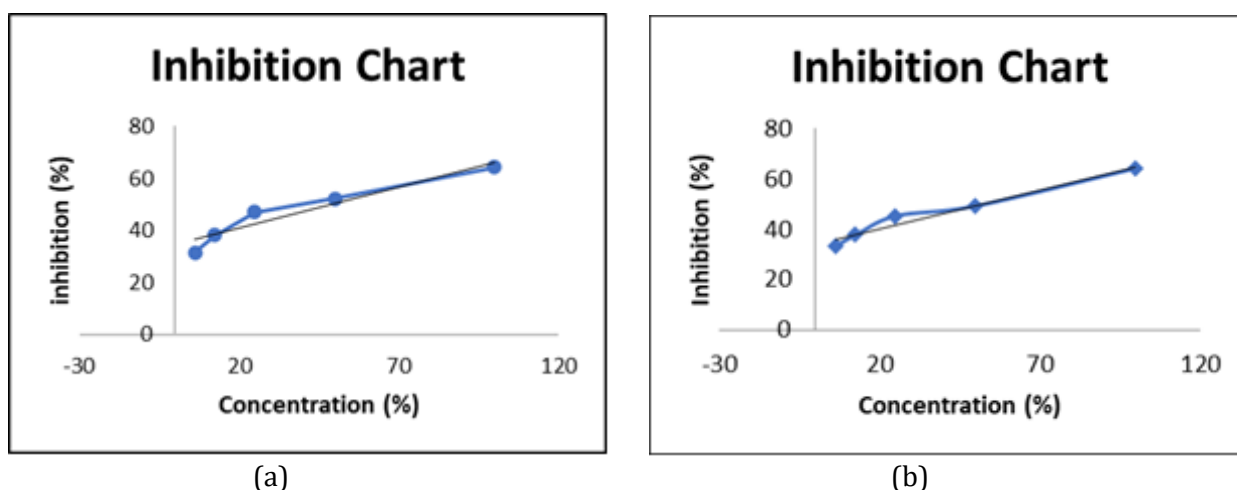


Figure 8. Inhibition chart of methanolic (a) and ethyl acetate (b) extract *R. apiculata*

Table 1. Phytochemistry and Antioxidant Profile

| Extract samples | Phytochemistry Profile | | | | | Antioxidant Activity |
|-----------------|------------------------|-----------|---------|---------|-------|----------------------|
| | Alkaloid | Flavonoid | Steroid | Saponin | Tanin | IC ₅₀ |
| MeOH | + | + | + | - | + | 49.202 |
| EtOAc | + | + | + | - | - | 51.960 |

Correlation between phytochemistry profile and antioxidant strength showed that the methanol extract, which has more secondary metabolite content, had stronger antioxidant activity than the ethyl acetate extract.

CONCLUSION

In this research, the antioxidant profile shown methanol as potential extract for further research. Although the IC₅₀ is moderate. The results for phytochemistry showed that both extracts containing alkaloids, flavonoids, and steroids. However, both extracts showed no saponin compound. For tannin, this content is exist in methanol extract and doesn't exist in ethyl acetate extract. Based on the research

results above, it can be concluded that mangrove leaf extract (*R. apiculata*) contained bioactive compounds that strongly in methanol extract.

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