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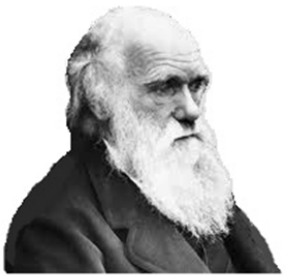

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# Total phenolic, flavonoid content and antioxidant capacity of stem bark, root, and leaves methanolic extract of *Rhizophora mucronata* Lam.

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**ABSTRACT**

The study was carried out to evaluate the phytochemical constituents, antioxidant activity and the content of total phenolic and flavonoid in the methanolic extract from stem bark, root, and leaves of *Rhizophora mucronata* from Karangsong, Indramayu Regency, West Java, Indonesia. The antioxidant activity was evaluated using 2,2-diphenyl-1-picrilhydrazyl (DPPH) assay. Total phenolic and flavonoid contents were determined according respectively to Folin-Ciocalteau method, and aluminium trichloride method. The methanol extract from stem bark of *R. mucronata* contained secondary metabolite such as phenolic, flavonoid, tannin and saponin. The stem bark of *R. mucronata* showed the highest phenolic and flavonoid content and possessed a higher antioxidant activity ($IC_{50}$ 84.80 µg·m/L) than that of root ($IC_{50}$ 166.95 µg·m/L) and leaves ($IC_{50}$ 90.51 µg·$mL^{-1}$). Total phenolic and flavonoid content in the stem bark of *R. mucronata* were 131.91 ±1.7 mg gallic acid equivalents/g and 902 ±0.7 mg quercetin equivalents/g, respectively.

***Keywords*:** Antioxidant, Phenolic, Flavonoid, Rhizophora mucronata, Secondary metabolites, *Rhizophora mucronata*, Indramayu Regency

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## 1. INTRODUCTION

A free radical is any atom or molecule that contains one or more unpaired electrons. The unpaired electrons alter the chemical reactivity of an atom or molecule, usually making it more reactive than the corresponding non-radical [3]. Free radicals can cause tissue damage by reacting with polyunsaturated fatty acids in cellular membranes, nucleotides in DNA, and critical sulfhydryl bonds in proteins. Free radicals can originate endogenously from normal metabolic reactions or exogenously as components of tobacco smoke and air pollutants and indirectly through the metabolism of certain solvents, drugs, and pesticides as well as through exposure to radiation. There is some evidence that free radical damage contributes to the etiology of many chronic health problems such as emphysema, cardiovascular and inflammatory diseases, cataracts, and cancer [5].

Halliwell (2007) reported that an antioxidant is any substance that delays, prevents or removes oxidative damage to a target molecule. Antioxidants are an inhibitor of the process of oxidation, even at relatively small concentration and thus have diverse physiological role in the body. Antioxidant constituents of the plant material act as radical scavengers, and helps in converting the radicals to less reactive species. A variety of free radical scavenging antioxidants is found in dietary sources like fruits, vegetables, and tea, etc. [5].

Medicinal plants contain phenolic compounds and these possessing powerful antioxidant activity. Especially flavonoids form a major class of phenolic compound present in many medicinal plants and are found to have a potential role in the prevention of various diseases via antioxidant activity [5]. Natural antioxidants are scattered in some parts of the plant, such as wood, root bark, leaves, fruit, flowers, seeds, and pollen. Natural antioxidants are antioxidants that are generally isolated from natural sources mostly from plants and fruits. Mangrove plants have been screened for their activities, such as antiviral, antibacterial, and anti-inflammatory [1]. *Rhizopora mucronata* is one type of mangrove which has the potential as a source of natural antioxidants (Figures 1 & 2) [12-30].

They possess active metabolites with some novel chemical structures which belong to diverse chemical classes such as alkaloids, phenol, steroids, terpenoids, tannins, etc. Flavonoids, phenols and tannins are secondary metabolite compounds that are believed to be responsible for antioxidant activity [8].

## 2. MATERIALS ANDMETHODS

### 2. 1. Chemicals and reagents

Methanol, 1,1-diphenyl-2-picrylhydrazyl (DPPH), ascorbic acid, chloroform, ammonium hydroxide, Wagner reagent, Meyer reagent, Mg powder, chloride acid, amyl alcohol, NaOH, ethanol, $FeCl_3$, acetic acid anhydride, concentrated sulfuric acid were used for the studies.

### 2. 2. Plant collection and extraction

The stem bark, root, and leaves of *R. mucronata* (Figures 1 and 2) were collected from Karangsong, Indramayu Regency, West Java. Collected plant materials were shade dried, powdered and used for extraction. The dried powder material of the stem bark, root, and leaves was extracted with methanol. The solvent was removed under pressure to obtain total extracts.

**(a)**

**(b)**

**Figure 1(a,b).** *Rhizopora mucronata* Lam.
Source: Personal Documentation, 2018

**(a)** **(b)** **(c)**

**Figure 2. (a)** Dried *R. mucronata's* stem bark, **(b)** Root of *R. mucronata*, **(c)** Leaves of *R. mucronata*
(Source: Personal Documentation, 2018)

*Rhizophora mucronata* Lam. is found in the Indo-Pacific region on the banks of rivers and on the edge of the sea. It is the only mangrove species to be found in East Africa. *R. mucronata* is native to Africa (in southeastern Egypt; eastern Ethiopia; eastern Kenya; Madagascar; Mauritius; Mozambique; the Seychelles; Somalia; eastern side of South Africa down to Nahoon, the most southern mangrove forest in Africa; southeastern Sudan; and eastern Tanzania); Asia (in Burma; Cambodia; India; Pakistan; Iran; Indonesia; the Ryukyu Islands of Japan; Malaysia; Papua New Guinea; the Philippines; Sri Lanka; Taiwan; Thailand; and Vietnam) the South Pacific (in the Solomon Islands; and Vanuatu), and Australia (in Northern Territory; and northern Queensland).

### 2. 3. Phytochemical screening

*Alkaloid*: The 0.5-gram sample was added a few drops of chloroform and stirred, then added a few drops of homogenized $NH_4OH$. The solution was filtered and the filtrate was then dissolved in 10 drops of 2N sulphuric acid. From formed 2 layers, top layer (chloroform layer) was taken. Then it was tested with alkaloid reagents, *i.e*. Meyer reagents and Wagner reagents [5].

Wagner reagent, made by pipetting 1.25 mL of distilled water added 0.31 g of iodine and 0.25 g of potassium iodide. Then it was diluted with distilled water into 25 mL in a pumpkin. This reagent is brown. Positive results are characterized by the formation of brown deposits.

Meyer reagent, made by 0.136 g $HgCl_2$ added 0.05 g potassium iodide. Then diluted with distilled water to 10 mL in a pumpkin. This reagent is colorless. Positive results are characterized by the formation of yellowish white sediment.

*Flavonoid*: The 1-gram sample was dissolved in 10 mL of methanol, boiled for 5 to 10 minutes. Divided into 3 tubes in a test tube. The "1" tube is added 0.1 g of Mg powder, 2 drops of concentrated HCl and a few drops of amyl alcohol. The "2" tube is added 2 drops of 2N $H_2SO_4$.

The "3" tube is given 2 drops of 10% NaOH. Positive results are marked by the change of color to red or orange, yellow, and brown.

*Triterpenoid*: The sample extract of 0.05 g was added 0.2 ml glacial acetic acid, then added 3 drops of concentrated sulfuric acid. Positive results are marked by the color setting to red and turn blue**.**

*Steroid***:** The sample extract of 0.05 g was dissolved in 0.5 mL of chloroform in a dry reaction tube. Then into the solution was added 0.125 mL glacial acetic acid and 0.038 mL of concentrated sulfuric acid. Positive results are marked by the change of color to red and turn into blue and green.

*Tannin*: The sample extract of 1 g was added 5 mL of water and boiled for 5 min, then taken 2 ml from the solution and added 2 drops of $FeCl_3$ 5% drop. Positive results are marked by the change of color to dark blue or dark green.

*Saponin*: Saponins can be detected by foam test in hot water. The foam is stable for 30 minutes and is not lost in the addition of 1 drop of 2N HCl, indicating the presence of saponins.

### 2. 4. DPPH radical scavenging activity

One mL of a solution of DPPH in methanol (0.1 mmol·$L^{-1}$) was mixed with 3.0 mL of extract in various concentrations during 30 min at room temperature in the dark, and the absorbance was recorded at 517 nm. The scavenging activity of the extracts was estimated based on the percentage of DPPH radical scavenged (I %) using equation

$$I = \frac{A\ \text{control} - A\ \text{sample}}{A\ \text{control}} \times 100\% \quad (1)$$

where: *A* sample is the absorbance of a sample solution, and *A* control is the absorbance of the control solution (containing all of the reagents, except the test sample).

### 2. 4. 1. Determination of total phenolic content

The amount of total phenolics in the extracts of *M. spinosa* leaves was measured using the Folin-Ciocalteu reagent method [10]. The ethanol solution of each extract (0.5 mL, l.0 mg·$mL^{-1}$) was added into test tubes containing 2.5 mL of 10% (*V*/*V*) Folin-Ciocalteau reagent and 2.0 mL of sodium carbonate (2%, *W*/*V*), and the tubes were shaken thoroughly. The mixture was incubated at 45 °C for 15 min with intermittent shaking. Absorbance was measured at 765 nmusing UV-Vis spectrophotometer. Gallic acid was used as a standard to obtain the calibration curve (ranging from 0 to 0.8 mg/mL), and the results were expressed as gallic acid equivalents in milligrams per gram (mg GAE/g) of dried extract.

### 2. 4. 2. Determination of total flavonoid content

A preliminary test for flavonoids was performed using the lead acetate, ferric chloride, ammonium hydroxide reagent, and the results were positive. Therefore an attempt was made to determine the total flavonoid content quantitatively. An aliquot of 0.5 mL of sample solution (1 mg·$mL^{-1}$) was mixed with 0.1 mL of 10% aluminum nitrate, 0.1 mL - 1 mol·$L^{-1}$ potassium acetate, and 4.3 mL of 80% ethanol. The reagents were thoroughly mixed and allowed to stand

for 40 min at room temperature; the absorbance in the supernatant was measured at 415 nm [22]. A yellow coloration of the mixture indicated the presence of flavonoids. Quercetin was used to plot a standard calibration curve, and the results were expressed as quercetin equivalents in milligrams per gram (mg QE/g) of dried extract.

### 2. 5. Phytochemical screening

The above results show that the extract of methanol from stem bark, root and leaves of *R. mucronata* contain secondary alkaloids, flavonoids, phenolic, terpenoid, tannins and saponins. Flavonoids, phenols and tannins are secondary metabolite compounds that are believed to be responsible for antioxidant activity. The results of the phytochemical analysis of the *R. mucronata* are presented in Table 1.

**Table 1.** Phytochemical content of *R. mucronata.*

| Phytocemical Content | Result of Methanol Extract | | |
|---|---|---|---|
| | Stem bark | Root | Leaves |
| Alkaloid | + | ++ | + |
| Flavonoid | +++ | +++ | +++ |
| Phenolic | +++ | +++ | +++ |
| Triterpenoid | – | – | – |
| Steroid | – | – | – |
| Taninn | +++ | +++ | +++ |
| Saponin | ++ | – | + |

(–) = Negative; (+) = Weak positive; (++) = Positive; (+++) = strong positive

### 2. 6. DPPH radical scavenging activity of *R. mucronata*

The antioxidant activity of *R. mucronata* was evaluated using DPPH method. This assay determines the scavenging of stable radical species of DPPH by antioxidants. The antioxidant activity of phenolic compounds is due to their redox properties, which play an important role in absorption and neutralization of free radicals [9].

DPPH radical is scavenged by antioxidants through the donation of proton forming the reduced DPPH. DPPH is one of the compounds that possesses a proton free radical and showed a maximum absorption at 517 nm. When DPPH encounter proton radical scavengers, its purple color fades rapidly. In DPPH radical scavenging assay, *R. mucronata* extracts showed the $IC_{50}$ value of stem bark extract was 84.8 $\mu g \cdot mL^{-1}$, $IC_{50}$ of root extract was 90.51 $\mu g \cdot mL^{-1}$, and $IC_{50}$ of leaves extract was 166.95 $\mu g \cdot mL^{-1}$. The IC50 of stem bark, root, and leaves extract from *R. mucronata* are presented in Table 2.

**Table 2.** $IC_{50}$ Values Methanol Extract of Stem Bark, Root, and Leaves *R. mucronata*

| Sample | $IC_{50}$ (μg·mL$^{-1}$) |
|---|---|
| Stem bark | 84.8 |
| Root | 90.51 |
| Leaves | 166.95 |

*R. mucronata* methanol extract has the potential for strong antioxidant activity which is supported by the flavonoids, phenols, and tannins in phytochemical screening.

According to Meskin *et al*. (2002), the antioxidant activity of phenol compounds is related to the structure of phenol compounds. The presence of hydroxyl or methoxy groups in the ortho or para position of benzoic acid, penilpropanoid or flavonoid (isoflavone) derivatives is known to increase the antioxidant activity of phenol compounds. While the presence of two hydroxyl groups in ortho or para positions can produce a stable quinoid structure, and the methoxy group in the *ortho* or *para* position is a donor electron that is effective in stabilizing the free radicals formed, thereby increasing the activity of phenol compounds.

### 2. 7. Total phenolic and total flavonoid content

Total phenolic content and total flavonoid content were determined from the calibration curves of gallic acid ($Y = 0.0066\ x + 0.0693$, $R^2 = 0.989$), and quercetin ($Y = 0.002x + 0.0496$, $R^2 = 0.9937$), respectively. The total phenolic and total flavonoid contents among the different part of *R. mucronata* are presented in Table 3. The results showed that stem bark extract possessed the highest phenolic [150.65 ±0.6 mg GAE/g of dry material] and flavonoid components [902 ±0.7 mg QE/g of dry material], followed by the root extract, while the leaves extract contained very limited polyphenolic compounds.

**Table 3.** Total phenolic and flavonoid contents of different parts of *R. mucronata.*

| Sample | Total phenolic content (mg GAE/g) | Total flavonoid content (mg QE/g) |
|---|---|---|
| Stem bark | 150.65 ± 0.6 | 902 ± 0.7 |
| Root | 131.91 ± 1.7 | 792 ± 0.28 |
| Leaves | 121.46 ± 3.1 | 562 ± 0.60 |

The contents of flavonoids and phenolic compounds were greater in stem bark extract than in root and leaves. There is compatibility with $IC_{50}$ that has been obtained. It is known that the highest $IC_{50}$ was in the stem bark extract. Different antioxidant abilities due to the content

of secondary metabolites contained in mangroves differ mainly in the content of phenolic compounds, flavonoids and tannins which are responsible for antioxidants.

## 3. CONCLUSION

This work revealed that extracts of the stem bark *R. mucronata* contain high levels of phenolics and flavonoids, and possess significant antioxidant activities. The methanol extract of stem bark *R. mucronata* exhibited remarkable free radical scavenging and antioxidant activity, which may be due to the presence of a high polyphenolic content.

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