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Phytochemical Test of Mangrove *Avicennia alba*, *Rhizophora apiculata and Sonneratia alba* from Musi River Estuary, South Sumatera

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Abstract

Mangrove is one of the plants that has the potential to be developed into medicinal plants. However, further research is needed to prove scientifically the content of secondary metabolites in it. This study aims to identify the secondary metabolites contained from leaves and roots in Avicennia alba, Rhizophora apiculata and Sonneratia alba. The leaves and roots of mangroves extracted by maceration using ethyl acetate. The secondary metabolites contained in mangrove samples obtained by doing phytochemical tests through color tests. Phytochemical test results showed that secondary metabolites contained in mangrove Avicennia alba are flavonoids, steroids/triterpenoids, saponins, and tannins/phenols in leaf samples, whereas in its root samples it contains flavonoids and steroids/triterpenoid compounds. Sonneratia alba contains flavonoids, steroids/triterpenoids, saponins and tannins/phenols in its leaf samples, while the root samples contain flavonoids, steroids/triterpenoids and tannins/phenols compounds. The leaf samples of mangrove Rhizophora apiculatacontains flavonoid and steroid/triterpenoid compounds whereas in its root samples contains flavonoids, steroids/triterpenoids, saponins as well as tannins/phenols.

Keywords : Color-test, ethyl acetate, identification, mangrove, secondary metabolites.

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1. Introduction

Mangroves are able to grow and thrive in the estuary area and have a typical adaptation pattern to face of environmental pressures such as high salinity, high temperature, direct sunlight exposure and the abundance of microorganisms and microorganisms [1]. Mangrove growth is strongly influenced by environmental factors, which the physical and chemical properties of its fluctuating habitats are influenced by tides, sludge deposition, as well as the composition of organic materials so that mangroves form certain zoning systems [2]. The ability of mangroves to survive with extreme environmental pressure shows that the chemical compounds were contained and it can protect mangroves from cell damage.

Plants that can live in extreme environmental conditions such as mangroves have bioactive compounds that protect them from cell and tissue damage. Secondary metabolites as bioactive compounds are related to the chemical content in plants, so that these plants can act as medicinal plants. Secondary metabolites found in the mangroves include alkaloid compounds, phenolics, steroids, and terpenoids[3]; [4].Based on several reference studies, the *Avicennia sp* generally contains alkaloid compounds, flavonoids, saponins, steroids and terpenoids [6]; [7]; [8]; [16]. *Rhizophora* sp generally contains alkaloid compounds, flavonoids, steroids, tannins/phenols [5]; [11]; [19]; [20]; [24]; [40]. *Sonneratia* sp generally contains flavonoid, steroid, triterpenoid, tannin/phenol compounds [14]; [28]; [30].

A.alba, R.apiculata and *S.alba*are some types of mangrove plants that have several function as antibacterial, anticancer, antimalarial, antitoxic, antinematode , antioxidant, anti-inflammatory, antifungal and other various pharmacological benefits [6]; [16]; [18]; [19]; [31]; [35]; [39]; [43]. Phytochemical tests were carried out to prove some of these pharmacological functions scientifically. Phytochemical test was conducted to determine whether there were bioactive components found in the test sample. Phytochemical

tests included alkaloid test, steroid/triterpenoid test, flavonoid, saponin, and tannin-phenolic. In this research, phytochemical test was done through color test which used modification procedure of [7]; [34] research.

2. Materials and Methods

This research was carried out by laboratory experimental method. Mangrove samples was conducted on March 31, 2018 in Musi River Estuary, South Sumatera and laboratory experiments were conducted from April to July 2018 in Marine Bioecology Laboratory, Marine Science Study Program, and Pharmaceutical Biology Laboratory, Pharmaceutical Study Program, Faculty of Mathematic and Natural Sciences Sriwijaya University.

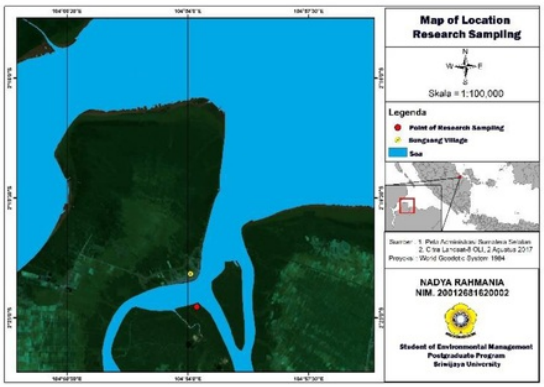

Figure 1. Map of location. Red point (research sampling) and yellow point (Sungsang village)

Tools and Objects

The tools used in the research includes; aluminum foil, vial bottle, reagent bottle, Bunsen, blender, funnel, grinder, rubber band, filter paper, 100 ml measuring flask, mortar, drop plate, drop pipette, spirits, spatula, test tube, digital scales, glass jar, vacum rotary evaporator.

The materials used in the study include; leaf and root samples (*A. alba, R. apiculata* and *S. alba*), Ethyl Acetate, $FeCl_3$, concentrated HCl, Metals Mg, NaOH, Dragendorff Reagents, Liberman-Burchard Reagents, Meyer Reagents, Wagner Reagents, and Aquades.

Research Procedure (Modification from Rastuti *et al* 2012; Jacoeb and Zahidah, 2013)

1.1 Sample Preparation

Leaves and roots of mangrove samples that have been washed from dirt are then dried by aerated at room temperature for ± 3 weeks, and then mashed with mortar and blender, while root samples are ground using a grinder and blender until each sample becomes powder.

1.2 Extraction

Each sample are weighed and put it into a glass jar to be extracted by maceration method using ethyl acetate until all the powder sample was submerged for 2x24 hours while stirring occasionally. Then filtered with filter paper and funnel. The maceration process were repeated 3 times until the extract became clear. Then each sample that has been done from maceration process was evaporated using vacuum rotary evaporator to separate the solvent and extract. Each extract that has been done evaporated was aerated for a while so that the remaining solvent can evaporate itself.

2.3 Phytochemical test using color test

a. Alkaloid Test

Each extract samples 0.5 gram (leaf and root) was taken and put into a test tube, then added 10 ml of ammonia solution in chloroform then shake it for 1 minute, then filtered into a test tube. The results of the filter (filtrate) are added 4-5 drops of H_2SO_4 and shake until well blended, leave it until it forms two layers. The top layer (water phase) are separated and tested with reagents Meyer, Wagner and Dragendorf and then observed the changes that formed.

b. Flavonoid Test

- Method 1: Using Mg metal + HCL

Each extract samples 0.5 gram was put into the test tube and then added with 5 ml of ethanol, then heated for about 5 minutes. Then the extract was filtered, then added a few drops of concentrated HCL into the filtrate. Next, added about 0.2 mg of magnesium powder (metal Mg). Positive contain flavonoids when the red color appears on the sample.

- Method 2: Using 10% NaOH

0.5 gram of each extract samples was put into the test tube and then added with 5 ml of methanol, then heated for about 5 minutes and then filtered to take the filtrate. Added 2-3 drops of 10% NaOH into the filtrate, and then observed the color changes that are formed, if the color changes to a reddish yellow-orange, it means the sample is positive containing flavonoids in it.

c. Steroid and Triterpenoid Test

Each extract samples 0.5 gram is put into a test tube and then dissolved it with chloroform, then drops the sample for about 3-4 drops on the drop plate and left to dry. After drying, add anhydrous acetic acid and stir evenly. Add 2-3 drops of concentrated sulfuric acid. Observe the results that occurs, if the color changes to blue-green, it means positive steroid, if the

color changes to purple, it means positive triterpenoid.

d. Saponin Test

Each extract samples 0.5 grams was inserted into the test tube and added enough distilled water, boiled it for about 2 minutes. Samples that have boiled will produce the foams, and then cooled the sample for a while and then shake it harder until its well distributed. If there are foams showed up, and the foams are stable for about 1 minutes, it means there are saponin compounds in the test sample.

e. Tannin-Phenolic Test

Each extract samples 0.5 gram was put into a test tube and 3-4 drops of distilled water were added, then heated it for a while and leave it for a while until its cooled. Then added $FeCl_3$, observe the color changes that occurs. If brownish-green color were formed, it means test samples were positive containing tannin. Meanwhile, phenol compounds are shown by the formation of dark blue or blackish green.

3. Results and Discussion

Samples that obtained from the field are first cleaned using running water to get rid of the dirt such as soil or mud. Each sample then dried (aerated) without exposing it to direct sunlight. This drying process were carried out for 2-3 weeks, then the samples were mashed using a blender and mortar, while the root sample, which is quite large, were mashed using a grinder. Root and leaf samples from each mangrove that has been mashed, were macerated for 2x24 hours, and repeated it three times (until the samples became clear) at room temperature using a ratio of 1:5 between ethyl acetate solvent and sample. The weight of the samples extracted by maceration method were presented in Table 1 below.

Table 1. The weight of each mangrove samples

Sample code	Sample weight (gram)
AAroot	100,04
AAleaf	108,36
AMroot	88,14
AMleaf	100,34
RAroot	55,14
RAleaf	105,41
SAroot	40,37
SAleaf	106,54

*description: AA : *Avicennia alba*
RA : *Rhizophora apiculata*
SA : *Sonneratia alba*

The maceration results that have been filtered using filter paper and funnel are then evaporated at 40-50 °C to obtain a dry extract in the form of a blackish brown paste, which is then weighed. The weight of extracts from evaporation is presented in Table 2 below.

Table 2.The weight of each extract samples after evaporation process

Sample code	Sample weight (gram)
AAroot	3,23
AAleaf	6,12
RAroot	1,39
RAleaf	5,91
SAroot	1,14
SAleaf	4,22

Ethyl acetate concentrated extract obtained from maceration which has been concentrated through the evaporation process then aerated, so that the remaining solvents can evaporate naturally. When compared to the weight of each concentrated extracts sample after evaporation and powder samples before the maceration is really different. The maceration results of the color of leaf samples is greenish yellow (after 3 times repetition), and the maceration results of root samples is dark-greenish yellow to clear (after 3 times repetition). This can be influenced by the use of solvents at the maceration stage, which in this study used ethyl acetate. According to [24], based on the polarity, ethyl acetate solvents are included in the type of semi-polar solvents, and the extract results, especially in leaf samples, show a green color from chlorophyll. Then the statement was supported by [21] which states that semi-polar compounds such as chlorophyll will dissolve in semi-polar solvents such as ethyl acetate. [9] also mentioned that extraction results that match quality standards, can also be influenced by several factors, such as the extraction method used, the particle size of the extract material, the conditions and also the storage time, the length of extraction time and the ratio between the amount of solvent to the sample.

The type of solvent will determine the results of the extract and binding of different secondary metabolites depending on the type of solvent that used, so that the results of samples after evaporation with ethyl solvents will produce thick samples like pasta, while using methanol solvents, after evaporation process, samples still contains water. The polarity of the active compounds of various samples were different, so that the active compounds contained will be extracted only by the solvents with the same polarity level as the polarity of these components which causes the percentage of the extract to be different as well [16]; [37]. Furthermore, each sample was carried out by phytochemical testing process through color test

The phytochemical test was conducted to determine the class of secondary metabolites contained in mangrove samples, such as alkaloids, flavonoids, steroids, triterpenoids, saponins and tannins. The class of secondary metabolites contained in the sample extract can be known through changes in color, precipitation or foam formation that occurs in accordance with the reagents used [43]. Indicator of the secondary metabolites presence in phytochemical tests through color change observation will be presented in table 3. Meanwhile phytochemical test results on mangrove samples in this research will be presented in table 4 below.

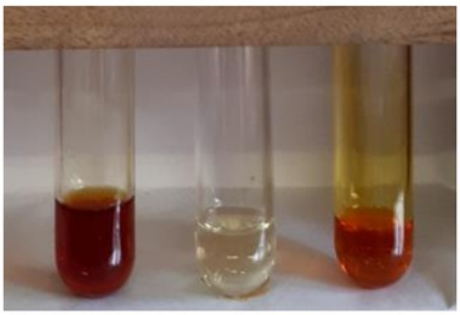

Figure 2. Alkaloid test (negative) a. Wagner treatment; b. Meyer treatment; c. Dragendorff treatment)

Table 4. Phytochemical test result from the sample

Species	Alkaloid			Flavonoid	Steroid/ Triterpenoid	Saponin	Tanin/Phenol
	Wagner	Meyer	Dragendorff				
AAleaf	-	-	-	+	+	+	+
AAroot	-	-	-	+	+	-	-
RAleaf	-	-	-	+	+	-	-
RAroot	-	-	-	+	+	+	+
SAleaf	-	-	-	+	+	+	+
SAroot	-	-	-	+	+	+	+

Based on the table above, all samples tested for phytochemical through color tests contained different metabolite compounds. The typical color shown in each sample based on the color change indicator makes it easy to identify the secondary metabolite class. Based on the test results shown in table4, it is known that all test samples contain flavonoid compounds in the leaves and roots (Figure 1).

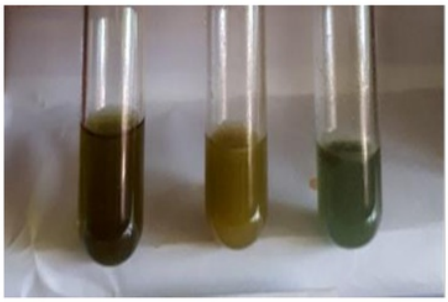

Figure 1. Flavonoid test (positive) a. samples before treatment; b. Samples after given NaOH 10% treatment; c. Samples after given HCl + Mg treatment)

The presence of flavonoid content in all test samples is indicated by the change in color in the sediment to red, yellow or green after being treated. The discovery of flavonoids in all test samples can be due to the fact that flavonoids are a group of natural secondary metabolite compounds that are often found in plants [32]; [38]. Flavonoids are divided into groups such as flavanols, glycosylflavones and flavones, and acts as antioxidants [39], meanwhile, according to the results of [16], although the extract produced by ethyl acetate solvent is relatively small, due to the semi-polar ethyl acetate properties, it causes ethyl acetate can extract various polar and non-polar antioxidant compounds. This is also supported by [42] which states that the extraction results using ethyl acetate solvents contain more isoflavone components (including flavonoids) both non polar and polar so the results of this study that found flavonoid content in all samples test can be verified.

Based on the color test, mangrove extract using ethyl acetate solvent contained negative alkaloids (Figure 2). This is indicated by the absence of red color in the treatment using Wagner reagent, nothing of white yellowish color appeared in the Meyer reagent treatment, nor the appearance of brown in the sample after Dragendorff reagent added. Basically, almost all of the plants contains an alkaloid because the alkaloid is a group of secondary metabolites that are widespread in plants [4], but in this study, alkaloid were not found in the test sample that can be caused by several factors such as the use of solvent ethyl acetate that are semi-polar makes alkaloid (which is polar) insoluble perfectly so that the amounts of alkaloids are dissolved in the

sample may be very little and causes no discoloration.

Based on the test results, steroid components were also found in all test samples (Figure 3). In the steroid test, Liebermann-Burchard reagent was used which consisted of concentrated sulfuric acid and acetic acid anhydride. The use of this reagent is to test the characteristics of unsaturated sterols (alcohol steroids) which are characterized by the formation of green rings derived from the reaction between sterols and acetic acid anhydride (CH_3COOH which is then added of concentrated sulfuric acid (H_2SO_4) [34]. Positive result shows the presence of steroid soluble in ethyl acetate solvents with the appearance of green color in each sample extract, while if using other solvents, positive result shows the appearance of blue or purple color [13], according to the type of solvent used during the extraction process.

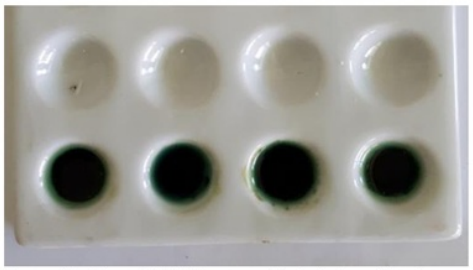

Figure 3. Steroid/Triterpenoid test (positive)

Positive result which contains tannins/phenolics compound are found in all test samples except for *Avicennia alba* root sample. The structure of the tannin compound consists of a benzene ring (C6) which binds to the group phenolic -OH (hydroxyl) [29] that cause tannin as polar and can be dissolved excellently in a polar organic solvent (methanol) but also can dissolve in semi-polar solvents such as ethyl acetate, but it may affect its binding compound, so the tannins amount that were dissolved may be too small (imperfect) causing no color changes that occurs as in *Avicennia alba* root sample. This is also supported by the statement of [23] which states that phenolic compounds such as tannins are obtained more in the leaves in high concentrations, especially in the form of tannin compounds, and also supported by [36] that ethyl acetate solvent is suitable for extracting phenolic compounds in the leaves.

Tannins a very complex component of organic substances, consisting of phenolic compounds that are difficult to separate and difficult to crystallize, precipitate proteins from their solutions and berate compounds with these proteins [10]. [3]also found secondary metabolite compounds contained in plants generally spread evenly to all parts of the plant, but in different amount, and can be extracted according to their character and solvent properties based on the theory of like dissolves like which means that secondary metabolites will dissolve easily in solvents that have similar polarity properties (polar compounds soluble in polar solvents) [1]; [2]; [13].

The sample which positively contains tannin and phenolic showing the color changes into brownish green or blackish green. Tannin belong to the derivative component of phenolic compounds which have a phenol group, together with flavonoids, phenols hydroquinone and phenyl propanol [13]; [38] which is soluble in polar solvents and semipolar, and has a major role as an antioxidant and protect plants from excessive light damage[15], as antibacterial, anticycotoxic, anti-diarrhea. [26] also mentioned that the more tannin contains in the plant, the greater its antioxidant activity, because tannins are composed of polyphenol compounds that have free radical capture activity. Meanwhile, tannin can also protecting plant from animal attacks (predators) during the growth period in certain parts such as in parts of immature fruit [4]; [30]. Avicenniaceae, Rhizophoraceae, and Sonneratiaceae are mangrove families that are rich in tannin sources [4].

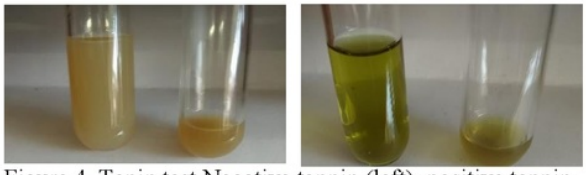

Figure 4. Tanin test Negative tannin (left), positive tannin (right)

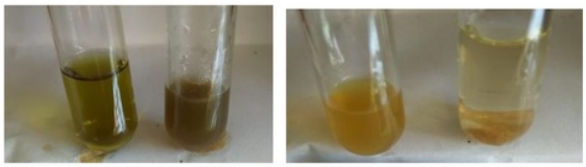

Figure 5. Phenolic test Positive phenol (left), negative phenol (right)

Saponin compound were positively detected in *Avicennia alba* leaf, *Sonneratia alba* leaf and *Rhizophora apiculata* root, which extracted using ethyl acetate solvent, shown by the formation of a stable foam after shook in the test tube as shown in Figure 6 below.

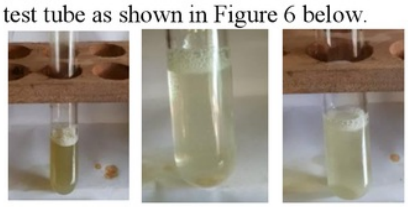

Figure 6. Positive saponin in the sample (left: *Avicennia alba* leaf; center: *Rhizophora apiculata* root; right: *Sonneratia alba* leaf)

Positive results obtained in S.alba, in accordance with the results of research conducted by [12] on the same

mangrove samples, which obtained positive results containing saponins even though the concentration was weak. If the foam that appears on the sample after being left for 10 minutes remains stable and does not disappear and a height of about 1-2 cm indicates positive saponins with foam shaped like a white foam bath. [13] explains, saponin is a type of glycoside, which is generally found in many plant and characterized as foam and easily soluble in polar solvents, also soluble in semi-polar solvents, but insoluble in non-polar solvents. The existence of stable foam formation is a reliable proof of the presence of saponins in the extract. In addition, the results of research by [22] obtained positive results of saponins when isolating saponin compounds from mangrove *Bruguiera gymnorhiza*. Furthermore, the results of research that has been done by [27] conducted flavonoid and saponin compound in the leaves of *Avicennia* sp. Meanwhile the research conducted by [33] also states that *Sonneratia alba* leaf extract contains more saponin than the saponin that was obtained from *Avicennia* sp and *Rhizophora* sp leaf extract. In this study, samples of *Avicennia alba* leaf forms a foam, but the foam is unstable and only last a few seconds and then disappear that indicating negative result, as in Figure 7 below.

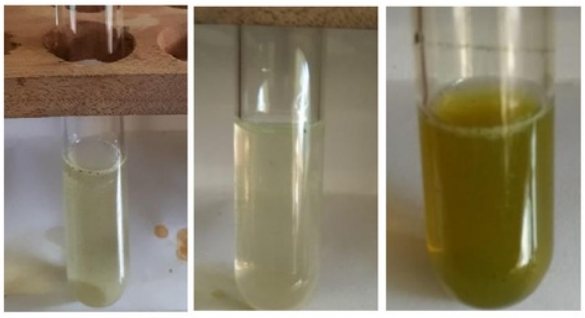

Figure 7. Negative saponin (left: *Avicennia alba* root; center: *Sonneratia alba* root; right: *Rhizophora apiculata* leaf)

4. Conclusion

Based on the results of the research that has been done, it can be concluded that:

1. Secondary metabolite compounds contained in the mangrove extract were flavonoids, steroids/triterpenoids, saponins, and tannins/phenols. *Avicennia alba* contains flavonoids, steroids/triterpenoids, saponins, and tannins/phenols in leaf samples, whereas in its root samples contains flavonoids and steroids/triterpenoid compounds. *Sonneratia alba* contains flavonoids, steroids/teriterpenoids, saponins and tannins/phenols in its leaf samples, while the root samples contain flavonoids, steroids/teriterpenoids and tannins/phenols compounds. The leaf samples of mangrove *Rhizophora apiculata* contains flavonoid and steroid/triterpenoid compounds whereas in its root samples contains flavonoids, steroids/triterpenoids, saponins as well as tannins/phenols. These compounds were found in several samples (leaves or roots only), except flavonoids which were found in all samples including leaves and roots. Meanwhile alkaloid compounds were not found in all test samples.
2. The use of semi-polar ethyl acetate solvents influences the amount of secondary metabolites which are extracted so that it affects the phytochemical color test results in the study because secondary metabolites can be extracted well if the polarity is the same as solvent polarity (principles "*like dissolves like*").

5. Acknowledgement

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