Journal of Pharmacology Experiment and Pharmaceutical Technology
DOI: 10.64015xxxx

Identification of Secondary Metabolite Compounds in Black Mangrove Leaves *(Rhizophora mucronata* Lamk.*)*

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ABSTRACT

Indonesia has a very large mangrove forest area, reaching nearly 3.5 million hectares or about 25% of the world's total mangrove forests, with one of the dominant species being *Rhizophora apiculata* which grows in muddy tropical coastal areas. Plant extracts are a potential source of natural antimicrobial compounds because they contain various active substances with bacteriostatic properties that can be utilized as alternative therapies. This study aimed to identify the phytochemical metabolite compounds contained in mangrove leaf extract. The research design used in this study was a laboratory experimental design with a qualitative approach including the identification of flavonoids, tannins, saponins, and alkaloids. The results of the study showed that the phytochemical screening carried out on the mangrove leaf extract indicated the presence of several secondary metabolites, namely flavonoids, tannins, and saponins, which showed positive results in each test. These results conclude that mangrove leaf extract contains phytochemical metabolites including flavonoids, tannins, and saponins.

Keywords: mangrove leaf extract, phytochemical metabolites, flavonoids, tannins, saponins

INTRODUCTION

Extracts from natural sources, such as plants, have become objects of research in efforts to discover antimicrobial compounds that can be used as alternative therapies. Natural or herbal compounds, whether in pure form or as extracts, have potential as sources of medicine because they contain various chemical compounds. The active substances in these plants possess bacteriostatic properties, which can be utilized as alternative treatments in antibacterial therapy (Muslim et al., 2020).

Indonesia has a very extensive mangrove forest area. The Ministry of Environment and Forestry reported in 2021 that the total area of mangrove forests in Indonesia reaches nearly 3.5 million hectares, or about 25% of the total mangrove forests in the world. One of the most common mangrove species found in Indonesia is *Rhizophora apiculata*. This species commonly grows in brackish and muddy areas within tropical regions. *Rhizophora apiculata* has characteristics such as hard wood, stilt roots, opposite leaves with the lower leaf surface having a yellowish-green color, and a fast growth rate, reaching heights of approximately 15–30 meters (Azhari et al, 2022)

Mangrove plants are widely utilized as raw materials for medicines and cosmetics due to the presence of active compounds contained in them. Research conducted by ,(Akasia et al., 2021) reported that mangrove plants of the species *Rhizophora apiculata* contain many secondary metabolites such as phenols, alkaloids, flavonoids, tannins, saponins, and steroids which can be utilized as antiviral, antifungal, and anticancer agents. These secondary metabolites were identified through phytochemical tests on methanol extracts of mangrove leaves.

In the study by (Ramli et al., 2020), it was reported that *Rhizophora apiculata* can be used as a treatment for diarrhea, nausea, antiviral therapy, and as a hypoglycemic agent. In addition, the bark of the mangrove plant *Rhizophora apiculata* contains tannins that function as natural antioxidants. Black mangrove leaves have the potential to be developed as medicinal raw materials; therefore, their safety and benefits need to be improved through further research and development (Aji et al., 2023). Mangrove leaves contain secondary metabolites such as tannins, carotenoids, phenols, chlorophyll, and alkaloids (Ridlo et al., 2017). Research conducted by (Kasitowati et al., 2017) showed that mangrove leaf extract of *Rhizophora mucronata* contains flavonoid, alkaloid, and tannin compounds which have potential as natural antioxidants.The presence of these compounds indicates that mangrove leaves have considerable potential to be developed as a source of natural active ingredients.

This study aims to identify the phytochemical metabolite compounds contained in mangrove leaf extract *(Rhizophora mucronata Lamk.)* through qualitative phytochemical tests, particularly for flavonoids, tannins, saponins, and alkaloids. This identification was conducted to determine the bioactive potential contained in mangrove leaves as a source of natural ingredients with medicinal properties, as well as to provide a scientific basis for their utilization in the fields of pharmacy, health, and the development of traditional medicines. In addition, the results of this study are expected to enrich scientific information regarding the phytochemical composition of mangrove plants that have the potential to be further developed.

METHODS

The research design used in this study was a laboratory experimental design with a qualitative approach. This research was conducted through a series of controlled stages in the laboratory, including the extraction process of mangrove leaves *(Rhizophora mucronata Lamk.)* using 96% ethanol as a solvent. The equipment used in this study included glassware (beaker glass, measuring cylinder, dish, iwaki pyrex), blender (Ossel), stirring rod, chamber, Buchner funnel (Pyrex), Erlenmeyer flask (Iwaki), hot plate, knife, dropper pipette, Rotary Evaporator (Buchi), spatula, analytical balance (Ohaus), and water bath. The materials used in this study were ethanolic extract of mangrove leaves (Rhizophora mucronata Lam.), 96% ethanol, distilled water (Quadrant), $FeCl_3$, HCl, and Mayer, Wagner, and Dragendorff reagents.

Black Mangrove Leaf Simplisia *(Rhizophora mucronata Lamk.)*

The simplisia of black mangrove leaves *(Rhizophora mucronata Lamk.)* was obtained from the coastal area of Muara Mundu, Mundu Pesisir Village, Cirebon Regency. A total of 6.7 kg of leaves were collected. After collection, the leaves were washed and dried under sunlight for 7 days. Once dried, dry sorting was carried out to remove any remaining impurities attached to the dried black mangrove leaf simplisia. Subsequently, the dried leaves were ground and sieved to obtain black mangrove leaf powder. The resulting powder was stored in a container protected from light (Septiani, 2020).

Extraction of Black Mangrove Leaves (*Rhizophora Mucronata Lamk)*

To obtain mangrove leaf extract, a maceration process was carried out with a ratio of 1:10. A total of 250 grams of mangrove leaves were soaked in 2,500 mL of 96% ethanol and then left to stand for 5 × 24 hours. After that, the filtrate was separated using a 100-mesh sieve. To obtain a concentrated extract, the solution was evaporated. The 96% ethanol extract of mangrove leaves was then ready to be used for antibacterial activity testing (Sulastri et al., 2020).

After maceration for 5 days, filtration was carried out using a Buchner funnel to separate the filtrate from the residue. The resulting filtrate was then concentrated by evaporation using a rotary evaporator and water bath to obtain a thick extract of black mangrove leaves (*Rhizophora mucronata Lamk*.).

$$\text{Yield (\%)} = \frac{\text{Weight of the obtained extract (g)}}{\text{Initial simplicia weight (g)}} \times 100\%$$

Flavonoid Identification

A total of 2 mL of mangrove leaf extract sample was added with 5 drops of concentrated HCl in a test tube and then heated on a hot plate for 5 minutes. The formation of a brick-red color indicates a positive result for the presence of flavonoids (Putra et al., 2023).

DOI: 10.64015xxxx

Tanin Identification

A total of 2 mL of the sample was placed into a test tube and 5 drops of $FeCl_3$ solution were added. The color change was then observed. A color change to blue or dark green indicates that the mangrove leaves are positive for tannins (Humairah et al., 2022).

Saponin Identification

A total of 0.5 grams of mangrove leaf extract was placed into a test tube, then 10 mL of distilled water was added and shaken vertically for 10 seconds. The formation of stable foam with a height of 1–10 cm indicates a positive result for the presence of (Putri & Lubis, 2022)

Alkaloid Identification

A total of 0.5 grams of mangrove leaf extract was placed into a beaker glass, then 1 mL of 2N HCl and 9 mL of distilled water were added. The mixture was heated for 2 minutes, then cooled, filtered, and the filtrate was used for the alkaloid test. The solution was divided into three test tubes. Tube 1 was added with 2–3 drops of Dragendorff reagent, tube 2 with 2–3 drops of Mayer reagent, and tube 3 with 2–3 drops of Wagner reagent. A positive result is indicated by the formation of an orange precipitate in the sample (Nurwahidah et al., 2024).(Nurwahidah et al., 2024)

RESULTS AND DISCUSSION

Based on the extraction process of mangrove leaf ethanol extract, the amount of active compounds obtained from an extraction process will affect the percentage yield. Several factors influence the success of extraction, including the type of solvent, solvent-to-sample ratio, particle size, temperature, extraction time, and extraction method. A higher yield percentage indicates a greater amount of active compounds extracted from the sample (Pawarti et al., 2023). A thick extract weighing 64 grams with a greenish-brown color was obtained, with a yield value of 25.6%. This yield indicates the effectiveness of the extraction process, as determined by Kementerian Kesehatan Republik Indonesia (Kemenkes RI), which states that a good extraction yield is > 10% (Puspitasari & Wulandari, 2017). The yield obtained in this study can be compared with the results of previous studies (Muldianah et al., 2023) which reported a lower yield of gendola leaf extract, approximately 10%, indicating that the method used in this study was likely more effective or that the raw material contained a higher level of active compounds. In addition, a study by (Wijaya et al., 2022) also showed variability in extraction yield depending on the extraction method, with yields varying between 15% and 25% depending on the material and techniques used. The yield results can be seen in **table 1**

Table 1. Percentage Yield (% Yield) of Mangrove Leaf *(Rhizophora Mucronata Lamk)*

Powder Weight	Thick Extract Weight	% Yield
250 Grams	64 Grams	25,6%

Based on the results of the phytochemical screening that has been conducted, the mangrove leaf extract showed positive results in the flavonoid, tannin, and saponin tests. Meanwhile, the alkaloid test showed negative results because no precipitate was formed after the addition of Mayer, Wagner, or Dragendorff reagents. The results of the phytochemical screening can be seen in **table 2.**

Table 2. Phytochemical Screening Results

Phytochemical Test	Reagent	Observation	Description
Flavonoids	HCl	Formation of a brick-red color	(+)
Tannins	FeCl3	Color change to dark green	(+)
Saponins	Aquadest	Formation of stable foam with a height of 1 cm	(+)
Alkaloids	Mayer Dragendroff Wagner	No orange precipitate formed, no yellowish-white precipitate, and no brown to black precipitate formed	(-)

Description: (+) Contains the tested compound

(−) Does not contain the tested

Phytochemical compounds are various types of compounds that occur naturally in plants, and some of them can produce activity in biological systems (Huang et al., 2016). Secondary metabolites are chemical compounds produced by plants in forms that differ

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from one species to another. These secondary metabolites are produced as a form of defense against disturbances from other organisms and environmental factors (Li et al., 2020). There are approximately 200,000 types of secondary metabolite products; therefore, to identify the types of secondary metabolites, classification is necessary based on nostructural characteristics, biosynthetic pathways, and other origins (Kusbiantoro & Purwaningrum, 2018).

Flavonoids are one of the secondary metabolites found in plants. In general, the basic structure of flavonoids is a polar compound because it contains –OH groups that form hydrogen bonds (Satria et al., 2022). Flavonoids are secondary metabolites that can be used as antimicrobial agents, treatments for wound infections, antifungal, antiviral, anticancer, and antitumor agents. In addition, flavonoids can also function as antibacterial, anti-allergic, cytotoxic, and antihypertensive agents. In plants, flavonoids are found in several parts such as roots, leaves, flowers, and fruits. Flavonoids are present in all green plants and are secondary metabolites that exhibit various pharmacological activities (Goa et al, 2018).

Flavonoid identification in this study used the Bate-Smith test. The Bate-Smith test was carried out by adding concentrated HCl to hydrolyze flavonoids by breaking the O-glycosyl bond to produce its aglycone form. The glycosyl group, which acts as an electrophile, will be displaced by H^+ from the acid. The heating process aims to accelerate the hydrolysis reaction (Widyasari & Yuspita Sari, 2021). In this study, the mangrove leaf extract *(Rhizophora mucronata Lamk.)* showed a positive result for flavonoids indicated by the formation of a brick-red color. The brick-red color can be seen in Figure 1. This result is consistent with the study by (Suedi et al, 2025), which reported that a color change to red in both samples indicates a positive result for anthocyanidin-type flavonoids. The results of flavonoid identification can be seen in **Figure 1.**

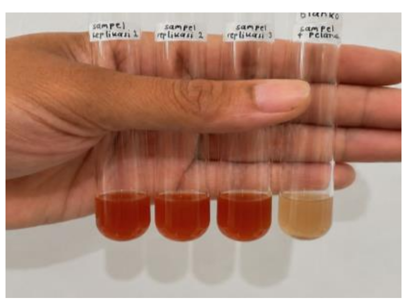

Figure 1. Results of Flavonoids Identification

Tannins are found in plants and are synthesized within their tissues (Hidayah, 2016). Tannins are polymeric phenolic compounds with large molecular weights. They are polyphenolic compounds with complex hydroxyl groups and have diverse structures with high molecular weights ranging from approximately 500 to 20,000 Da (Elgailani & Ishak, 2016). The potential of tannins in the health field includes their role as antioxidants (Sulasiyah et al., 2018).

In this study, the mangrove leaf extract *(Rhizophora mucronata Lamk.)* tested positive for tannins, indicated by the formation of a dark green color.This result is consistent with the study by (Kasih et al, 2022) which reported that mangrove plants contain tannin compounds, indicated by the formation of an ink-blue or dark green color in the sample. The formation of a dark green color in the extract after the addition of $FeCl_3$ occurs because tannins form complex compounds with $FeCl_3$ (Halimu et al., 2017). The results of tannin identification can be seen in **Figure 2**.

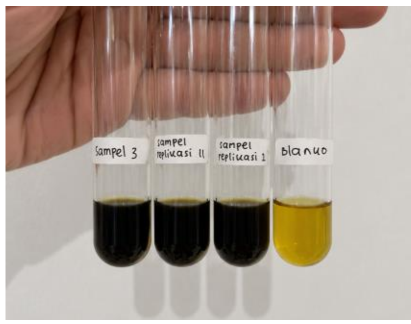

Figure 2. Results of Tannins Identification

Saponins are natural surfactants and will form foam when subjected to strong shaking (Minarno, 2016). Saponins can be found in the roots, bark, leaves, seeds, and fruits of plants and function as a defense system (Hidayah, 2016). Saponins have specific physical, chemical, and biological properties, making them potentially useful in the health field as medicinal compounds (Sulasiyah et al., 2018).

The results of the saponin test showed that the mangrove leaf extract tested positive for saponins, indicated by the formation of stable foam with a height of 1 cm. The foam formed because the hydrophilic groups in saponins bind to water, while the hydrophobic groups interact with air, resulting in foam formation (Sulistyarini et al, 2020). The formation of this foam is the result of a hydrolysis reaction, in which saponin compounds undergo hydrolysis into glycone and aglycone forms (Bhernama, 2020). Another study by (Kasih et al, 2022) also reported that the saponin test of mangrove leaves showed the formation of foam. The results of saponin identification can be seen in **Figure 3.**

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Figure 3. Results of Saponins Identification

Alkaloids are one of the groups of secondary metabolites that possess important physiological activities and are widely found in nature. In general, alkaloids are organic salts that are solid and crystalline, although their physicochemical properties (such as color) may vary depending on their structure. Alkaloids have various biological effects, including the ability to affect the nervous system (as stimulants, hypotensive agents, or sedatives), and they also exhibit analgesic and antibacterial properties, as well as applications in the treatment of heart disease (Salwa et al., 2025)

The identification of alkaloids showed that mangrove leaves *(Rhizophora mucronata Lamk.)* do not contain alkaloid compounds. The test results can be seen in **Figure 4**. A negative result is indicated by the absence of an orange precipitate with Dragendorff reagent, a brown precipitate with Wagner reagent, and a white precipitate with Mayer reagent. A study by Hasibuan et al. (2020) stated that the analysis of alkaloids in the ethanol extract of *Avicennia marina* leaves using two different methods also showed negative results. The negative result was indicated by the absence of a white precipitate with the reagent. Research conducted by Darkwah et al., (2018) stated that the alkaloid content in plants tends to decrease and its concentration weakens as the plant ages. In addition, Lantah et al. (2017) reported that alkaloids are not heat-resistant, indicating that alkaloids are sensitive to high temperatures. These phytochemical compounds can easily be damaged when exposed to high temperatures, either during the heating process or during the drying of simplicia. Research by Jairaman et al. (2019) also reported that *A. marina* leaves from Muthukadu Lake, India showed positive results for phytochemical compounds such as alkaloids, flavonoids, and terpenoids, while saponins, tannins, and steroids were negative. This indicates that the same plant species may have different phytochemical contents due to various factors. The extraction of phytochemical compounds can be influenced by factors originating from the sample as well as from the extraction process. Sample-related factors include plant parts, plant origin, particle size, drying method, and moisture content. Extraction factors include the type of solvent, extraction method, solvent ratio, temperature, and extraction time (Shaikh & Patil, 2020). The results of alkaloid identification can be seen in **Figure 4.**

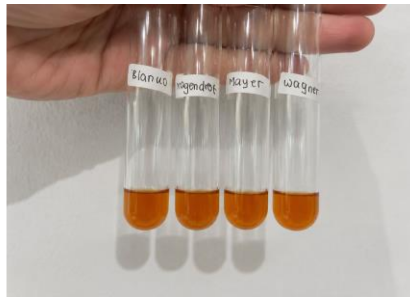

Figure 4. Results of alkaloid Identification

CONCLUSION

Based on the stages of phytochemical screening tests that have been carried out, it can be determined that the extract of mangrove leaves *(Rhizophora mucronata Lamk.)* in this study contains several secondary metabolite compounds, namely flavonoids, tannins, and saponins, which showed positive results in each test. Meanwhile, the alkaloid test did not show a positive result, indicating that alkaloid compounds were not detected in the mangrove leaves in this study.

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