Pharmaciana
Vol.12, No.3, Nov 2022, Page. 364-371
ISSN: 2088 4559; e-ISSN: 2477 0256
DOI: 10.12928/pharmaciana.v12i3.22475

Antibacterial activity of methanol extract *Rhizophora mucronata* leaves toward *Salmonella typhi*: leading the typhoid fever

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Submitted: 15-12-2021 ***Reviewed: 25-05-2022*** ***Accepted: 21-10-2022***

ABSTRACT

The community has utilized mangrove extensively, particularly as a component of traditional medicine. *Rhizophora mucronata* is one species that possess antibacterial, antiviral, antifungal, and insecticidal properties. According to reports, *R. mucronata* has antibacterial properties against the *Salmonella typhi* bacteria that cause typhoid fever. This research aims to obtain a methanol extract of *R. mucronata* leaves and assess its antibacterial potential as natural new medicine, particularly for treating typhoid fever. The extraction method is maceration with a 70 percent methanol solvent. Well, diffusion is utilized to determine antibacterial activity. Alkaloid, flavonoid, saponin, steroid, tannin, and triterpenoid are the chemical compounds identified in the methanol extract of *R. mucronata* leaves (MERmL). Ten percent concentration of MERmL exhibited moderate antibacterial activity (7.97±0.25 mm), whereas 30 percent concentration (11.380.29 mm) and 50 percent concentration (16.07±0.40 mm) exhibited intense antibacterial activity. Based on these findings, *R. mucronata* leaf methanol extracts with higher concentrations have more potent antibacterial activity against *S. typhi* in typhoid fever treatments.

Keywords: antibacterial, methanol extract, *Rhizophora mucronata*, *Salmonella typhi*, typhoid

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INTRODUCTION

Mangrove is one of the estuary ecosystems widely utilized by coastal communities. Today, one of the advantages of mangroves is their ability to act as a restorative material. Historically, certain mangrove species have been used as insecticides and natural pesticides (Noor, 2006). The majority of mangrove plants have medicinal properties (Purnobasuki, 2004). Mangroves have also been reported to possess antimicrobial properties due to their high concentration of alkaloid compounds, flavonoids, phenols, terpenoids, steroids, and saponins (Kordi, 2012). *Rhizophora mucronata* is one type of mangrove that exhibits antibacterial, antiviral, and antifungal activity. Mangrove leaf extract is reported to possess antibacterial and antifungal properties against *Escherichia coli* and *Penicillium digitatum* (Amirkaveei & Behbahani, 2011; Pimpliskar, 2011). Additionally, other studies have demonstrated that stem extracts of *Rhizophora mucronata* possess antimicrobial activity against *Escherichia coli*, *Salmonella typhi*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* (Feliatra, 2002; Joel & Bhimba, 2010; Puspitasar et al., 2012). The previous research tested *Rhizophora mucronata* leaf extract as an antifouling and antibacterial for *Staphylococcus aureus*, which causes skin infections.

Salmonella typhi is one of the bacteria that can cause typhoid fever in humans. The disease is present in almost every country but is particularly prevalent in developing countries such as Indonesia. Typhoid fever is prevalent in Asia at a rate of 900/10,000 per year (Widoyono, 2008). Typhoid fever is 1.5 percent in Indonesia, which translates to 1,500/100,000 Indonesia (Herawati & Ghani, 2009). Typhoid fever is treated medically using the trilogy principle of rest, diet, and antimicrobials. The antibiotic chloramphenicol is the first-line treatment for typhoid fever. However, *Salmonella typhi* is currently widely reported to be resistant to chloramphenicol (Sidabutar & Satari, 2016). Typhoid fever must be treated with natural plant-based ingredients. According to (Dewoto, 2007), natural ingredients from plants containing active substances such as alkaloids, flavonoids, tannins, saponins, and terpenes have therapeutic properties. They can be used to treat various conditions. Thus, a pharmacological study of *R. mucronata* is necessary to determine its potential as a typhoid fever drug.

MATERIALS AND METHOD

Materials

Rhizophora mucronata leaves collected in Kajhu Village, Baitussalam, Aceh Besar District, D.I Aceh Province, Indonesia. The solvent used for the extraction process is a 70% methanol solution. Materials used for chemical analysis include Dragendorff reagent solution, Mayer's reagent solution, HCl, Mg powder, CH_3COOH, iron (III) chloride solution 10%. Ingredients used for the antibacterial test include aqua-dest, NaCl 0.9%, Mueller Hinton Agar (MHA) media, McFarland standard 0.5, and chloramphenicol, Bacterial test used is *Salmonella typhi.* All materials used are from STIKes Assyifa Aceh Chemistry Laboratory.

The instrument used in this study were analytical scales (CAMRY), blender (Miyako), autoclave (YX-24H), ovens (DESPATCH), incubator, rotary evaporator, vortex (Thermolyne), micropipettes (Acuma), glassware (pyrex), spreaders drigalski glass, cork borer, Whatman filter paper.

Methods

Sample preparation

The sample of *R. mucronata* leaves is washed with running water to remove impurities. However, dry in the sun and cover with a cloth. The dried leaves (simplicia) are mashed in a blender to reduce the particle size and increase the surface area of the leaf powder. The powder is then stored in a dry container that is well covered and away from moisture.

Extraction

Simplicia leaves (50 g) of *R. mucronata* were weighed and soaked in 750 mL of 70% methanol solvent in a glass jar for 48 hours (Cseke et al., 2006). After 48 hours, the solution is filtered using

Whatman paper and evaluated using a rotary evaporator set to 50°C to obtain paste extracts. The paste is then weighed to determine the yield percentage obtained.

Bacterial strains

The bacterial suspension was made by taking several *Salmonella typhi* using ose and then inserted into a test tube that contained 10 ml of NaCl 0.9%. The concentration of bacteria is equated to the standard 0.5 Mc Farland (Cockerill et al., 2012).

Antibacterial test

Antibacterial activity is determined using the suitable diffusion method. Take up to 1 mL of bacterial suspension and drip it onto the Mueller Hinton Agar (MHA) media surface. Flatten with a glass spreader and silence for 5 minutes. Then a well was made using a cork borer with a diameter of 5 mm. Each Petri dish is constructed with five wells. Each well is filled to a maximum volume of 50 L, depending on the treatment group. The concentrations of *R. mucronata* methanol leaf extract used in this study are 10%, 30%, and 50%. The positive control is chloramphenicol, while the negative control is the extraction solvent, methanol 70%. The bacteria were incubated at 37°C for 48 hours, and the diameter of the bland zone was measured.

Phytochemical screening

Identification of chemical compounds in methanol extract of *R. mucronata* leaves is carried out by phytochemical screening, including examining alkaloid compounds, flavonoids, saponins, steroids, tannins, and triterpenoids (Cseke et al., 2006).

Alkaloid test

On a porcelain cup, 2 mL of extract is evaporated. After that, the residue is dissolved in 5 mL HCl 2 M. The obtained solution was divided into three test tubes. As a blank, three drops of HCl 2 M are added to the first tube. Three drops of Dragendorff reagent are added to the second tube, and three drops of Mayer reagents are added to the third tube; the Dragendorff reagent will form orange deposits. On the other hand, Mayer's reagents will form yellow deposits indicating alkaloids' presence.

Flavonoids test

Add up to 2 mL of extract to hot water to taste, then simmer for 5 minutes before filtering. Filter up to 5 mL, add 0.05 Mg powder and 1 mL concentrated HCl, and vigorously shake. Positive tests produce the colors red, yellow, or orange.

Saponin test

A total of 2-3 ml of extract is added to the test tube, followed by 10 ml of hot water and cooling. The test tube is then vigorously shaken for 10 seconds, and one drop of HCl 2 N is added. A positive test forms a stable froth that reaches a height of 1-10 cm and remains stable for at least 10 minutes.

Steroid and triterpenoid test

A total of 2 mL extract was added along with two drops of glacial CH_3COOH. The solution is gently shaken and set aside for a few minutes. Blue or green indicates steroids, while purple or red indicates triterpenoids.

Tannin test

1 mL of extract is added, along with a few drops of 10% iron (III) chloride solution. The presence of tannins is indicated by a dark blue or greenish-black color.

Data Analysis

The antibacterial activity of methanol extract leaves *R. mucronata* was analyzed using Analysis of Variants and Duncan's Multiple Range Test at a 5% significance level. Conduct data analysis using

the SPSS 22.0 program. The data on identifying the bioactive compound found in *R. mucronata* leaves are analyzed descriptively.

RESULT AND DISCUSSION

Extraction of bioactive compounds Rhizophora mucronata ***leaves***

Using methanol as a solvent, the leaf extract of *Rhizophora mucronata* has a paste-like consistency and a blackish-green hue. In addition to attracting active components, methanol also attracts chlorophyll, so the extracted material is typically green. The extraction of methanol against the leaves of *R. mucronata* yields a crude extract. The yield of the extract is determined by comparing the weight of the produced extract to the initial extracted sample. The yield of *R. mucronata* extracted with methanol is 22.58 percent. These findings suggest that the active compounds on the leaves of *R. mucronata* dissolve in methanol. Methanol is an alcohol-based solvent. Alcohol, according to (Harborne, 1987), is an excellent, versatile solvent for extracting depleted bioactive compounds. Alkaloid compounds, phenolic components, carotenoids, tannins, sugars, amino acids, and glycosides are extracted by methanol solvents.

Identification of bioactive compounds Rhizophora mucronata leaves

Table 1 displays the phytochemical test outcomes for the methanol leaf extract of *R. mucronata*. The production of secondary metabolites compensates for biotic and abiotic environment interactions. Compounds of secondary metabolites prevent pathogenic bacterial infections (Kelman et al., 2000). Active components detected in *R. mucronata* are tannins, alkaloids, flavonoids, terpenoids, and saponins (Joel & Bhimba, 2010; Puspitasar et al., 2012). (Nurdiani et al., 2012) and (Tarman et al., 2014) also reported that methanol extract leaves of *R. mucronata* contain alkaloids, tannins, saponins, flavonoids, and triterpenoids as active constituents.

Table 1. Results of identification of bioactive compounds of *R. mucronata* leaves

Phytochemical Test	Results	Evidence
Alkaloid		
a. Dragendorff	An orange precipitate is formed	+
b. Mayer	A yellow precipitate is formed	+
Flavonoid	Yellow color formed	+
Saponnin	Stable foam formation	+
Steroid	No color change	-
Tannin	Greenish black color	+
Triterpenoid	Formation of red	+

* (+) = positif test (exist); (-) = negatift test (not exist)

Antibacterial activity *R. mucronata*

The well's method for determining antibacterial activity is characterized by forming clear zones around the wells. This method determines the diameter of the transparent area surrounding the well that exhibits antibacterial activity. The diameter of the tasteless zone provides information regarding the susceptibility of bacteria to antibiotics or other antibacterial test materials, as measured by the clear area. The diameter of the bland zone is calculated in millimeters (mm) by subtracting 5 mm from the overall diameter. The diameter of the inhibition zone is then classified as an antibacterial activity using David & Stout's classification system (Davis & Stout, 1971).

The concentrations of *R. mucronata* leaves used in this study were 10%, 30%, and 50%. Two percent of Chloramphenicol was used as a positive control, and 70% of methanol was used as a negative control. Table 2 contains the test results for the bland taste of the methanol extract of *R. mucronata* leaves against *Salmonella typhi*.

Table 2. After 48 hours of incubation, the methanol extract of *R. mucronata* leaves was tested for its ability to inhibit *Salmonella typhi*

Treatment*	Diameter of inhibition zone (mm)**
Control -	0.00±0.00 a
Control +	18.83±1.04 e
MERmL 10%	7.97±0.25 b
MERmL 30%	11.83±0.29 c
MERmL 50%	16.07±0.40 d

*MERmL= Methanol Extract of *Rhizophora mucronata* Leaves; **The numbers preceding the same letter are identical, ±SD

Chloramphenicol was used as a positive control in this study. It is well known that chloramphenicol has a broad spectrum, inhibiting both Gram-positive and Gram-negative bacteria. (Brahmachary et al., 2004) states that chloramphenicol inhibits protein synthesis by preventing the amino terminus of acyl t-RNA from joining peptidyl transferase. While the negative control was 70% methanol to determine if there was a solvent effect on the growth of *Salmonella typhi*, it was clear that the test substance, not the solvent, had antibacterial activity. Contrary to expectations, the negative control treatment exhibited no antibacterial activity, while the positive control (Chloramphenicol) demonstrated a significant antibacterial activity (18.83±1.04 mm) against *Salmonella thypi* bacteria. Antibacterial strength is categorized as extreme (> 20 mm), strong (10-20 mm), medium (5-10 mm), and weak (5 mm) (Davis & Stout, 1971).

The formation of an inhibition zone against *Salmonella typhi* was due to the presence of a secondary antibacterial metabolite produced by *R. mucronata*. The inhibition does not originate from the maceration-derived solvent. The diameter of the inhibition zone against *Salmonella typhi* was 0.00±0.00 mm, according to the results of the antibacterial test performed on the negative control, as shown in Table 1. Maceration utilizes methanol as an organic solvent to attract secondary metabolites in plant cells. The bioactivity of secondary metabolites against pathogenic bacterial species is unaffected by organic solvents (Khalil, 2012).

At a 5% significance level, Duncan's Multiple Range Test (DMRT) revealed a significant difference in the diameter of the *Salmonella typhi* bacterial growth zone (Table 1). Higher concentrations of methanol extract of *R. mucronata* leaf (MERmL) demonstrate potent antibacterial activity. A sterile growing zone indicates resistance to *Salmonella typhi*. MERmL at a 10% concentration has moderate antibacterial activity (7.97±0.25 mm), whereas MERmL at a 30% or 50% concentration has high antibacterial activity (16.07±0.40 mm). The antibacterial activity of MERmL 50 percent against *Salmonella typhi* was greater than that of MERmL 30 percent and 10 percent. It is indicated by the letter d in the 50 percent MERmL treatment, the 30 percent MERmL treatment is indicated by the letter c, and the 10 percent MERmL treatment is indicated by the letter b. (Kusuma et al., 2011) demonstrated that the *R. mucronata* leaf Methanol extract has antibacterial activity in the 14-16 mm range. (Nurdiani et al., 2012) demonstrated that a methanol extract of *R. mucronata* leaves inhibits the growth of bacteria with a bland zone diameter between 6.1 and 6.4 mm. (Tarman et al., 2014) report that a methanol extract of *R. mucronata* leaves possesses antibacterial activity with a 3-12 mm zone of inactivity. In addition, (Rante et al., 2016) reported that a methanol extract of *R. mucronata* mangrove leaves possesses significant antibacterial activity with a blank zone diameter ranging from 10.7 to 12.03 mm.

Each secondary metabolite compound possesses a distinct antibacterial action mechanism. Secondary metabolite compounds inhibit bacterial growth by first harming the cell wall. Instability in the cell wall disrupts the selective permeability, active transport function, and control of the protein composition of bacterial cells, resulting in shape loss and cell lysis.

According to phytochemical tests, the leaves of *R. mucronata* contain alkaloids, flavonoids, saponins, tannins, and triterpenoids. Alkaloids are the most prevalent nitrogen-containing secondary metabolites in plant and animal tissues. The majority of alkaloid compounds are derived from plants. Numerous plant parts contain alkaloids, including flowers, seeds, leaves, twigs, roots, and bark (Fattorusso & Scafati, 2008). Alkaloid compounds function by inhibiting cell wall synthesis (González- Lamothe et al., 2009).

Flavonoid compounds can permeate peptidoglycan because flavonoids are also polar. Flavonoids inhibit bacterial growth by causing damage to the permeability of bacterial cell walls, microsomes, and lysosomes. As a result of the interaction between flavonoids and bacterial DNA, flavonoids can also release transduction energy to the bacterial cytoplasmic membrane. Additionally, flavonoids can inhibit the motility of bacteria. The hydroxyl group present in the structure of flavonoid compounds leads to alterations in organic components and nutrient transport, which ultimately have toxic effects on bacteria (Redha, 2010).

Tannins have antibacterial activity due to their ability to inhibit bacterial cell adhesion via enzymes and interfere with protein transport in the inner layer of bacterial cells, according to (Masduki, 1996). Tannins also target bacterial cell wall polypeptides, resulting in the imperfect formation of bacterial cell walls. Tannins are antibacterial because they inhibit the enzymes reverse transcriptase and DNA topoisomerase, preventing the formation of bacterial cells, as described by (Kordi, 2012).

Additionally, the *R. mucronata* plant also contains triterpenoids to tannins, flavonoids, and alkaloids. Triterpenoids are antibacterial because they form strong polymer bonds with porins (transmembrane proteins) on the outer membrane of the bacterial cell wall, thereby destroying the porin. Damage to the porin, which regulates nutrient entry and exit, will decrease the permeability of the bacterial cell wall due to the inhibitory compounds. The permeability of the bacterial cell wall will impede the entry and exit of nutrients and other compounds, inhibiting bacterial growth or killing bacterial cells (Robinson, 1995).

CONCLUSION

According to the research findings, higher concentrations of Methanol extract of *R. mucronata* leaves (MERmL) demonstrated potent antibacterial properties. It is indicated by the presence of *Salmonella thypi* bacteria-resistant growth zones. Giving MERmL at a concentration of 10% has a moderate antibacterial effect (7.97±0.25 mm), whereas giving MERmL at a concentration of 30% (11.830.29 mm) or 50% (16.07±0.40 mm) has a strong antibacterial effect.

ACKNOWLEDGEMENT

The authors would like to thanks the LPPM STIKes Assyifa Aceh, LPPM Universitas Pasifik Morotai, and LPPM Universitas Sembilan belas November who have given permission to collaborate to conduct this research.

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