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Antibacterial Activity of Mangrove Leaves Extract (Rhizopora Apiculata) Against *Salmonella typhi* Growth

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Abstract

Mangrove leaves (*Rhizopora apiculata*) contain antibacterial chemical compounds with antibacterial properties against various pathogens. *Salmonella typhi*, the causative agent of thyphoid fever, triggers a systemic infectious disease that can lead to complications and deaths if not treated properly. This study aimed to screen the phytochemical content of *R. apiculata* leaf extract, evaluate its antibacterial activity against *S. typhi*, and determine the optimal inhibitory concentration. Leaves were collected from the Sicanang mangrove forest in Belawan, North Sumatra, Indonesia, and extracted using the maceration method with 96% ethanol. Antibacterial effectiveness was assessed using disc diffusion method by measuring the zone of inhibition after exposure to mangrove leaf extract at concentrations of 40%, 60%, 80%, and 100%, with chloramphenicol as a positive control, to determine the most effective concentration for inhibiting the growth of *Salmonella typhi*. The results of the study showed that there were differences in inhibition zones in each group. Mangrove leaves extract at a concentration of 100% is the most effective in inhibiting the growth of *Salmonella typhi* compared to 80%, 60%, and 40% concentrations.

Keywords: Mangrove leaves, *rhizopora apiculata, salmonella typhi*

Introduction

Mangroves (*Rhizophora apiculata*) of the Rhizophoraceae family are widely distributed along the Indonesian coastline and have long been used in traditional medicine. Previous studies have highlighted their pharmacological potential, including anticancer, antitumor, anti-inflammatory, antifungal, antibacterial, antiviral, and antidiabetic properties.[1] Phytochemical analysis revealed that the mangrove leaves contain flavonoids, alkaloids, sterols, tannins, saponins, and phenols.[2,3] The environment and a plant's physiological adaptability affect an organism's capacity to create secondary metabolites.[4]

Several investigations have demonstrated the antibacterial activity of mangrove-derived extracts against diverse pathogens.[5,6] Given their promising antimicrobial potential in Indonesia and other regions, this study explores the antibacterial activity of *R. apiculata* leaf extract collected from the Sicanang mangrove forest, Belawan, North Sumatra, Indonesia.

Salmonella typhi is a bacterium that causes typhoid fever. Typhoid fever is a systemic infectious disease that, if not treated properly, can cause complications and death. It is usually spread through contaminated food or water. Despite considerable progress in water and sanitation facilities in most areas, typhoid fever is often found worldwide.[7,8] An estimated nine million people get typhoid fever, and 110,000 people die from it worldwide every year. The prevalence of typhoid in Indonesia is 1.60 %, and the age group 5–14 is the highest.[9] Moreover, according to the place of residence, typhoid fever cases are higher in rural areas than urban areas, with low levels of education and low economic conditions. The prevalence of typhoid varies from one region to another. The difference in the incidence of this disease between rural and urban areas is caused by the provision of

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drinking water, sanitation, and waste disposal.[10]

Typhoid fever treatment usually uses antimicrobial monotherapy, but the choice of drug and duration of therapy is optimal depending on the pattern of antimicrobial resistance.[11] The World Health Organization (WHO) recommends treating typhoid and paratyphoid (enteric fever) with azithromycin, ciprofloxacin, or ceftriaxone because of widespread resistance to first-line antimicrobials, such as chloramphenicol.[12] The prevalence of bacterial antibiotic resistance is increasing and becoming a significant problem in treating infectious diseases.[13] Therefore, the discovery of new antibacterial compounds is an important priority.[14] The discovery of bioactive compounds as an antibacterial in mangrove leaves is expected to lower incident drug resistance and increase efficacy for treating typhoid fever.

This study aimed to screen the phytochemical constituents of R. apiculata leaf extract, evaluate its antibacterial activity against S. typhi, and determine the optimal inhibitory concentration.

Methods

This experimental study was approved by the Health Research Ethics Committee of the Faculty of Medicine, Universitas Muhammadiyah Sumatera Utara (No. 1063/KEPK/FKUMSU/2023). This study involves six groups of experimental objects. Four groups receive mangrove leaf extracts with concentrations of 40%, 60%, 80%, and 100%, while two control groups receive chloramphenicol as a positive control and aquadest as a negative control. The measurement values taken in the treatment groups are compared with the control group. The sample in this research is *Salmonella typhi* ATCC 14028 bacteria. The number of research samples is calculated using the Federer formula: (n-1) (t-1)≥15; where is n: sample size, t: number of groups. There are four samples for each group, and the experiments are repeated four times, so the total sample used in this study is twenty-four. Data are collected by measuring the clear zone of *Salmonella typhi* growth using a vernier caliper.

Mangrove leaf extract uses the maceration method with 96% ethanol solvent. One kilogram of the mangrove leaves from Sicanang wisata hutan mangrove Belawan, Sumatera Utara, Indonesia is washed clean and dried without direct sunlight exposure. Then, the mangrove leaves are blended and sifted into powder. The mangrove leaf powder is soaked in 3 liters of 96% ethanol solvent for the first 6 hours, stirred occasionally, then left for 18 hours. The mass is separated by filtration, then evaporated using a rotary evaporator until a thick extract is obtained, and a phytochemical test is carried out. The extract obtained is diluted using DMSO (Dimethyl Sulfoxide) solvent to make extracts with concentrations of 40%, 60%, 80%, and 100%.[12]

Moreover, this study uses phytochemical test of mangrove leaf extract. The phenol test is carried out by adding 1% FeCl3 to 2 ml of mangrove leaf extract, then observing whether there was a color change. It is positive when the color turns blackish blue. The Alkaloid test is carried out by mixing 2 ml of mangrove leaf extract with 2 ml of HCl and Mayer's reagent, then observing whether there is a color change. It is positive when a white precipitate forms. The flavonoid test is carried out by mixing several ml of mangrove leaf extract with 5 ml of ethanol, then adding several drops of concentrated HCl and 1.5 grams of magnesium. It is positive when it turns red. The tannin test is carried out by mixing 2 ml of mangrove leaf extract with $FeCl_3$ and 2-3 drops of H_2SO_4 solution, then observing whether there is a color change. Positive if it turns brownish yellow. The Saponin test was carried out by mixing 2 ml of mangrove leaf extract with 5 ml of distilled water, shaking until stable foam formed, then adding another 1 drop of HCl 2N. It is positive when foam forms remain stable. The Steroid test is carried out by adding one drop of anhydrous acetic acid and one drop of concentrated sulfuric acid (Liebermann Burchard reagent) to the mangrove leaf extract. It is positive if it turns blue or green.[10]

The diffusion method inhibition test is conducted by making a suspension of Salmonella typhi colonies in 0.9% NaCl with a McFarland turbidity standard of 0.5 (comparable to the density of a bacterial suspension with a 1.5 x 10^8 CFU/mL). Blank discs are first sterilized by autoclaving. Mangrove leaf extract testing is carried out by immersing a sterilized blank disk in each extract concentration with a volume of 1 ml for 15 minutes so the solution could be properly absorbed into the blank disk. Next, the suspension of *Salmonella typhi* bacterial colonies is planted using a sterile tube evenly over the entire surface of the Muller Hinton Agar. Then, the blank disk containing the test material is placed in the middle of the agar surface using sterile tweezers and pressed slightly to adhere well. After that, it is incubated at 37ºC for 18-24 hours. Then, the resistance of the clear zone

around the additional blank disk is measured using a vernier caliper.[13]

Statistical tests are used to determine the differences in the clear zone between the four treatment groups and the control groups. The normality and homogeneity tests found that the data in this study were not normally distributed and homogeneous. As a result, the Kruskal-Wallis test is carried out and followed by the Mann-Whitney test. All tests are considered significant if the p-value is >0.05.

Table 1 Phytochemical Screening Result of Mangrove Leaf Extract

Phytochemical Test	Result
Flavonoid	Positive
Alkaloid	Positive
Sterol	Positive
Tannin	Positive
Saponin	Positive
Phenol	Positive

Results

Phytochemical screening of *Rhizophora apiculata* leaf extract confirmed the presence of flavonoids, alkaloids, sterols, tannins, saponins, and phenols (Table 1).

Table 2 shows that the average diameter of growth inhibition in the negative control group was 0 mm, in the positive control group was 20.50 mm, in the 40% concentration group was 11.75 mm, in the 60% concentration was 12.50 mm, in the 80% concentration was 13.50 mm, and in the 100% concentration was 15 mm.

The average diameter of growth inhibition in the negative control group was 0 mm, in the positive control group was 20.50 mm, in the 40% concentration group was 11.75 mm, in the 60% concentration was 12.50 mm, in the 80% concentration was 13.50 mm, and in the 100% concentration was 15 mm. In the Krusskal Wallis test, a p-value was obtained at 0.002 (p-value<0.05), and there were differences in the diameter of the inhibition zone between treatment groups. Then, the Mann-Whitney test was conducted to determine which groups had different inhibition zones.

Table 3 shows that the inhibition zone of

Table 2 Inhibitory Zone of Mangrove Leaf Extract Against *Salmonella typhi*

Repetition	Diameter of Inhibition of the Growth of *Salmonella typhi* (mm)						p-value
	Concentration of Mangrove Leaf Extract (*Rhizopora apiculata*)				Control		
	40%	60%	80%	100%	Positive	Negative	
1	10	11	11	13	21	0	
2	11	11	13	14	20	0	
3	13	14	14	16	20	0	0.002*
4	13	14	16	17	21	0	
Mean	11.75	12.50	13.50	15.00	20.50	0	

Krusskal-Wallis test, *significant difference (p<0.05)

Table 3 Significant Differences Among All Groups

Group	Control -	Control +	40%	60%	80%	100%
Control -						
Control +	0.013*					
40%	0.013*	0.019*				
60%	0.013*	0.018*	0.369			
80%	0.014*	0.019*	0.180	0.544		
100%	0.014*	0.019*	0.038*	0.137	0.304	

The mean values of the groups were significantly different; *significant different (p<0.005)

the negative control group had a significant difference from the inhibition zone of the positive group, the 40%, 60%, 80%, and 100% groups. The inhibitory zone of the 40% group had no significant difference with 60% and 80% but had significant differences with 100%. The inhibitory zone of the 60% group had no significant difference between 80% and 100%, and the inhibitory zone of the 80% group had no significant difference with 100%.

Discussion

The mangrove leaves in this study were extracted using the maceration method by soaking the samples using an organic solvent at room temperature. This method is known to be very profitable in isolating bioactive compounds from natural materials. Immersing the sample makes the cell walls and cell membranes break down due to the presence of the difference in pressure inside and outside the cell. Therefore, secondary metabolites in the cytoplasm are dissolved in the organic solvent used, and compound extraction will be perfect according to the length of sample immersion.[14] The extract used to test the antibacterial activity of mangrove leaves at concentrations of 40%, 60%, 80%, and 100% was dissolved using Dimethyl Sulfoxide (DMSO) solvent. DMSO is a solvent that can dissolve almost all polar and non-polar compounds. In addition, DMSO does not inhibit bacterial growth, so it will not interfere with the results of observations of antibacterial activity tests.[15]

Phytochemical tests are qualitative tests that aim to determine the bioactive components contained in a material. The phytochemical test in this study showed positive results in the flavonoids, alkaloids, sterols, tannins, saponins, and phenols. The results of several previous studies on phytochemical screening of mangrove leaf extracts were in line with this study, stating that positive mangrove leaf extracts contain flavonoids, alkaloids, sterols, tannins, saponins, and phenols.[10,16] Such compounds are known to interfere with bacterial systems, providing a bacteriostatic effect). Prior studies have also confirmed the antibacterial potential of mangrove extracts against *Staphylococcus aureus* and *Escherichia coli*, supporting the broad-spectrum activity of mangrove-derived compounds..[7,17,18] Previous research concluded the potential of mangrove leaf extract as an antibacterial for gram-positive and gram-negative bacteria.

Chloramphenicol was used as a positive control. This antibiotic acts as a bacteriostatic agent by inhibiting protein synthesis through interference with peptidyl transferase on the 50S ribosomal subunit.[19] In this study, chloramphenicol produced a mean inhibition zone of 20.50 mm against S. typhi, which according to the National Committee for Clinical and Laboratory Standards (NCCLS), falls within the sensitive category for Enterobacteriaceae (>18 mm).[20]

In this study, the antibacterial activity of mangrove leaf extract was determined against the growth of *Salmonella typhi* based on the diameter of the clear zone produced. The antibacterial activity of mangrove leaf extract was found in all test groups, and the greatest inhibitory power was found in the concentration of 100%. The data analysis shows a difference in inhibitory power between the chloramphenicol group and the mangrove leaf extract group with concentrations of 40%, 60%, 80%, and 100%. This shows that although mangrove leaf extract can inhibit the growth of *Salmonella typhi* bacteria, its effectiveness is not as good as chloramphenicol. This could be due to the antibacterial effect of mangrove leaf extract being lower than the antibiotic chloramphenicol. Still, it could also be due to the lack of absorption of the mangrove leaf extract soak into the test blank disk or the lack of antibacterial compounds extracted from the mangrove leaf sample, which can cause a decrease in the antibacterial effect.
In conclusion, *R. apiculata* leaf extract exhibits antibacterial activity against *S. typhi*, with maximal inhibition at 100% concentration. Although its activity was less than chloramphenicol, the extract demonstrates potential as a natural antibacterial agent and may serve as a complementary or alternative therapy, particularly in the context of rising antimicrobial resistance.

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