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The Potential Bioactive Compound of *Rhizophora Apiculata* Mangrove Fruit Extract in Inhibiting the Growth of *Salmonella typhi* Bacteria

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Abstract: Infections caused by *Salmonella typhi* remain a serious global health problem, particularly due to the increasing bacterial resistance to conventional antibiotics. Therefore, the search for effective natural antibacterial sources is urgently needed. *Rhizophora apiculata*, a mangrove plant known to contain various bioactive compounds, has the potential to be an alternative antibacterial source. This study aims to identify the phytochemical content in *R. apiculata* fruit extract and evaluate its antibacterial activity against *S. typhi* using the disc diffusion method and bacterial cell morphology analysis via SEM. The phytochemical screening results revealed that the extract contains phenolics, tannins, alkaloids, and flavonoids. However, saponins and steroids were not detected. The antibacterial activity test showed that the extract at a concentration of 250 mg/mL significantly ($p < 0.05$) inhibited the growth of *S. typhi*, with the largest inhibition zone (11.6 mm). SEM observations supported this finding by showing a decrease in the number of bacterial cells at extract concentrations of 125 mg/mL and 250 mg/mL, where the highest concentration caused the most bacterial cell death. This study demonstrates the potential of *R. apiculata* fruit extract as an effective natural antibacterial source for the development of alternative treatments for *S. typhi* infections.

Keywords: Antibacterial; Bacterial Cells; Fruit; Mangrove; Phytochemicals

Introduction

Typhoid fever is a significant public health problem, particularly in developing countries like Indonesia. While there is no single, up-to-date national data, various reports and studies indicate that the incidence of typhoid fever is estimated to range from 350 to 810 cases per 100,000 population annually (Oktaria et al., 2025). Data from 2018 showed that this disease was the third most common cause of death, with 41,081 cases. These figures vary because data is often collected from small-scale studies or in specific regions (Muthmainnah et al., 2024). Furthermore, typhoid fever is also a leading cause of death, ranking 15th among causes of death in those aged 14-45, with a mortality rate of around 0.6-5% of total cases. Therefore, typhoid fever continues to require serious attention in prevention and management efforts (Dhawy et al., 2024).

Typhoid fever is a significant public health problem, especially in developing countries. The primary cause is the bacterium *Salmonella typhi*, which is spread through contaminated food or water (Kumar et al., 2025). Typhoid treatment generally uses antibiotics, but excessive and inappropriate use of antibiotics has led to the emergence of bacterial resistance, reducing treatment effectiveness. Therefore, alternative therapies derived from natural ingredients with potential as antibacterial agents are needed(Buzilă et al., 2025).

One plant with potential for development as a natural antibacterial agent is *Rhizophora apiculata*, a type of mangrove commonly found in coastal areas of Indonesia (Mulia et al., 2023). This plant is known to

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contain various bioactive compounds such as tannins, flavonoids, saponins, and alkaloids, which have been reported to have antibacterial activity. However, research on the effectiveness of *R. apiculata* fruit extract against *S. typhi* has not yet been conducted (Ramya et al., 2023).

This study aims to evaluate the potential of *Rhizophora apiculata* fruit extract in inhibiting the growth of *Salmonella typhi* through antibacterial activity testing and observing bacterial cell structure using Scanning Electron Microscopy (SEM) (Sianipar et al., 2025).

This research is urgent in the field of health and natural resource utilization, which has the potential to discover new bioactive compounds from *R. apiculata* fruit that could be an alternative treatment for typhoid, offering a safer and more effective solution (Setyawan et al., 2022). Environmentally and economically, Indonesia is rich in mangrove ecosystems, but the utilization of its fruit is still minimal (Sarkar et al., 2024). This research can pave the way for the sustainable use of local natural resources, potentially creating affordable pharmaceutical products, while also supporting the economy of coastal communities (Sunkur et al., 2024; Lovelock et al., 2024). The results of this study are expected to contribute to the development of natural ingredients as an alternative treatment for bacterial infections that is safer and more environmentally friendly. It is hoped that the results of this study will contribute to the development of natural ingredients as safer and more environmentally friendly alternative treatments for bacterial infections.

Method

Preparation of herbal medicines

The sorted fruit is washed with clean water to remove dirt, soil, and microorganisms attached to the surface. To speed up the drying process and increase the evaporation surface area, the fruit can be cut into small pieces. Drying can be done in an oven at 50°C. Once dry, the material is dried further if necessary, then ground using a blender or flour mill to form a powder.

The sorted fruit is washed with clean water to remove dirt, soil, and microorganisms attached to the surface. Next, the fruit is cut into small pieces to speed up the drying process and increase the evaporation surface area. The drying process is carried out in an oven at 50°C until the material is completely dry. Afterward, the dried material is ground using a blender to form a herbal medicine powder ready for use in the extraction process (Suryani et al., 2025).

Extraction Process

Rhizophora apiculata fruit extract is prepared using the maceration method. The resulting powdered medicinal plants are weighed according to requirements and then soaked in 96% ethanol at a 1:10 ratio in a closed container. The maceration process is carried out for 24 hours at room temperature, with occasional stirring to maximize the dissolution of the active compounds. After the soaking process is complete, the mixture is filtered using filter paper to separate the pulp from the filtrate. The resulting filtrate is then evaporated using a rotary evaporator or heated at a low temperature of 60°C to remove the solvent, until a thick extract is obtained. This thick extract is then stored in a closed container and kept in a cool place until ready for use in phytochemical and antibacterial activity tests (Sianipar et al., 2025).

Qualitative phytochemical tests

The flavonoid test is performed by adding a concentrated magnesium and HCl solution to the extract; the formation of a yellow color indicates the presence of flavonoids. The phenolic test is performed by adding a few drops of 1% $FeCl_3$ solution to the extract; a dark brown or blackish-green color indicates the presence of phenolic compounds. For the tannin test, a drop of 1% $FeCl_3$ solution is added to the extract; a color change to blackish-brown indicates the presence of tannins. The steroid test is performed by adding a mixture of chloroform and concentrated H_2SO_4; the formation of a reddish-brown ring at the boundary between the two layers indicates the presence of steroids. The saponin test is performed by vigorously shaking the extract in a test tube containing water; the formation of a stable foam that persists for 10 minutes indicates the presence of saponins. For the alkaloid test, Dragendorff's reagent is added to the extract; the formation of a brown precipitate indicates the presence of alkaloid compounds (Kenitasari et al., 2023; Sianipar et al., 2024).

Antibacterial Testing

The antibacterial activity of *R. apiculata* fruit extract against *Salmonella typhi* was tested using the disc diffusion method. Activated *S. typhi* bacterial cultures were grown on nutrient agar media using a spread plate technique using sterile cotton swabs. Sterile paper discs (6 mm in diameter) were soaked in extracts at different concentrations: 0 mg/mL (negative control), 125 mg/mL, and 250 mg/mL, then placed on the surface of the bacterial-infused media. Each treatment was replicated three times. The plates were then incubated at 37°C for 24 hours. After incubation, the zone of inhibition formed around the discs was measured using a ruler or caliper in millimeters. The area of the zone of inhibition reflects the antibacterial effectiveness of the extract in inhibiting the growth of *S. typhi* (Shaikh et al., 2023).

Scanning Electron Microscopy (SEM) Analysis

To evaluate the effect of *R. apiculata* fruit extract on *S. typhi* cells microscopically, SEM analysis was performed. Bacteria incubated with the extract at specific concentrations were centrifuged and then washed with phosphate buffer solution to remove residual medium. Next, the bacterial slides were fixed using a 2.5% glutaraldehyde solution for several hours to maintain cell structure. After fixation, the slides were dried through a stepwise dehydration process using a series of ethanol solvents of increasing concentrations. The dried slides were then coated with a thin layer of gold using a sputter coater to clearly observe the cell surfaces in the SEM (Sianipar et al., 2025).

Data Analysis

The experiment was conducted with three replications based on a factorial Completely Randomized Design (CRD) with one treatment, namely different concentrations (0, 125, and 250 mg/mL of sample extract). The data were subjected to a one-way analysis of variance (ANOVA) to analyze the effect of the treatment on the observed parameters. A p-value ≤ 0.05 indicates a significant effect of the treatment on the test parameters (Sianipar et al., 2025).

Result and Discussion

Phytochemical test

Phytochemical testing of the ethanol extract of *R. apiculata* fruit was conducted using a qualitative phytochemical test method using a dye test reagent (Figure 1). This phytochemical test is used to determine the bioactive compounds present in the sample extract, resulting in positive and negative results for the test compounds (Kumaradewi et al., 2021). Based on the positive color change test for phytochemical content, the fruit with the highest phytochemical content, with three positive results (Fernandes et al., 2025), consisted of phenolics (blackish brown), tannins (brownish green), alkaloids (brown), and flavonoids (dark yellow), while other phytochemical components, such as saponins and steroids, were absent (Chowdhury et al., 2024).

The high content of phenolics, tannins, and alkaloids in the fruit indicates that *R. apiculata* has the highest bioactive potential due to its most complete phytochemical content (Safrida et al., 2023). Testing for tannins and phenolics with the addition of $FeCl_3$ thickens to a brown color. This color change occurs when $FeCl_3$ reacts with one of the hydroxyl groups present in the tannin and phenolic compounds (Artati et al., 2025). Phenolic compounds play a role in forming stable phenoxy radicals in oxidation reactions, and tannins function as binding agents that can stabilize lipid fractions, thus preventing oxidation that can damage fats (Chihomvu et al., 2024). High flavonoid content acts as an antibacterial. As an antibacterial, flavonoids' mechanism of action involves damaging bacterial cell structures, such as cell walls and membranes, thus causing bacterial death (Sarkar et al., 2024).

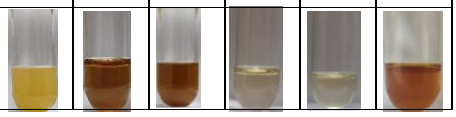
Phytochemical Screening	Flavonoid	Phenolic	Tannin	Steroids	Saponin	Alkaloids
Qualitative Test	++	+++	+++	-	-	+++
Observation						

Figure 1. Phytochemical screening

Antibacterial activity

The antibacterial activity of *R. apiculata* fruit was tested in vitro. This test was conducted using the disc diffusion method against a type of pathogenic bacteria, namely *S. typhi* (Rao et al., 2023). The test results were in the form of an inhibition zone, indicating the strength of antibacterial activity, as shown in Table 1. Based on research by Wijayanti et al. (2021) , a concentration of 250 mg/mL of the antibacterial test extract was able to inhibit *S. typhimurium* and *L. monocytogenes* bacteria (Chelliah et al., 2023).

The *S. typhi* bacteria test showed that *R. apiculata* fruit extract with a concentration of 250 mg/mL was able to inhibit *S. typhi* bacteria with a larger inhibition zone. This happened because the number of active compounds in the extract, such as natural antibacterial compounds, such as flavonoids, tannins, and alkaloids, also increased with increasing concentration (Popescu, et al., 2025). These compounds work by disrupting the structure and function of bacterial cells, such as damaging cell membranes, inhibiting protein synthesis, or disrupting bacterial metabolism (Anbessa et al., 2024). With higher concentrations, the number of active compounds that interact with bacteria becomes greater, so that the extract's ability to inhibit bacterial growth is greater and forms a wider inhibition zone (Neagu et al., 2024). Therefore, a high extract concentration is directly proportional to its effectiveness in inhibiting the growth of *S. typhi* (Parboteeah et al., 2023).

Table 1. Inhibitory Zone

Bacteria	Inhibitory Zone (mm)		
	0 mg/mL	125 mg/mL	250 mg/mL
S.typhi	0	6.0^{b}	11.6^{a}

Note: Different letters indicate significant differences in concentration (P<0.05).

The SEM test results support the antibacterial activity test results, indicating a higher bacterial cell count at a concentration of 0. However, after the

addition of *R. apiculata* fruit extract at concentrations of 125 and 250 mg/mL, the bacterial cell count decreased. The 250 mg/mL concentration showed the lowest bacterial cell count, due to the extract's effect on bacterial cell death (Hisada et al., 2023).

These results indicate that *Rhizophora apiculata* fruit extract has strong antibacterial activity against *Salmonella typhi* and acts concentration-dependently. This means that the higher the concentration of the extract used, the greater its ability to inhibit and kill bacterial cells. The decrease in bacterial cell count observed through SEM (Figure 2) at concentrations of 125 mg/mL and especially at 250 mg/mL indicates that the active compounds in the extract are capable of damaging bacterial cell structure or disrupting their vital functions, leading to death. Therefore, these findings support the antibacterial activity test results and indicate that *R. apiculata* fruit extract has the potential to be used as a natural antibacterial agent to control infections caused by *S. typhi* (Sianipar et al., 2025).

Most previous research tends to focus on more common plant parts, such as leaves or stems, which have already been extensively explored (Tandi et al., 2023). By examining the fruit, there is great potential to discover previously unidentified bioactive compounds, which may have different mechanisms of action or stronger activity against resistant *Salmonella typhi* bacteria (Fadilah et al., 2023). Furthermore, utilizing the fruit supports the concept of sustainability and optimal use of natural resources. However, this research also faces challenges. The availability of raw materials from the fruit is seasonal, which can hinder large-scale production. Furthermore, because the fruit is rarely studied, data on its safety and toxicity are still very limited, requiring a series of additional, time-consuming tests. Overall, despite these limitations, this research offers a new and promising approach to finding alternative solutions to the problem of antibiotic resistance.

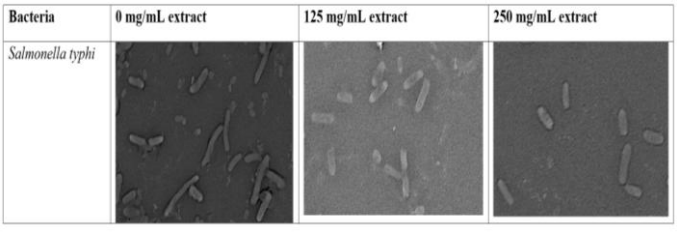


Figure 2. *S. typhi* bacterial cells using SEM

Conclusion

Rhizophora apiculata fruit extract contains phytochemical compounds such as phenolics, tannins, alkaloids, and flavonoids that contribute to its antibacterial activity. The extract with a concentration of 250 mg/mL proved to be the most effective in inhibiting the growth of *Salmonella typhi*, indicated by the largest inhibition zone and a significant decrease in the number of bacterial cells in microscopic observations using SEM. These findings indicate that *R. apiculata* has potential as a natural antibacterial source that can be further developed as an alternative treatment to overcome *S. typhi* bacterial infections.

In the future, these findings could form the basis for the development of phytopharmaceutical products, namely standardized plant-based medicines. This process requires a series of further studies, such as the isolation and identification of specific active compounds, clinical testing on animals, and even human clinical trials to prove their safety and effectiveness. Furthermore, this research also encourages the sustainable conservation of mangrove ecosystems, as their utilization can raise awareness of their economic value, not only as abrasion prevention but also as a valuable medical resource.

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Conseptualization, H.F.S.; methodology, M.P.S. and W.O.B.B.; validation, H.F.S.; resources, H.F.S.; writing original draft preparation, H.F.S., and M.P.S. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest
The authors declared that there are no conflicts of interest.

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