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Phytochemical Composition and Antibacterial Activity of *Barringtonia asiatica* (L.) Kurz Fruit Peel Extracts against *Staphylococcus aureus* and *Escherichia coli*

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Abstract: The present study investigated the phytochemical constituents and antibacterial potential of methanol, ethyl acetate, and *n*-hexane extracts obtained from the fruit peel of *Barringtonia asiatica* against the bacteria *Staphylococcus aureus* and *Escherichia coli*. The powdered peel material was initially extracted through a maceration process using methanol as the solvent, after which the extract was fractionated with ethyl acetate and *n*-hexane to obtain different polarity fractions. Qualitative phytochemical screening was conducted to determine the presence of secondary metabolites, whereas antibacterial activity was assessed using the agar diffusion technique at concentrations ranging from 100 to 500 mg/ml. The phytochemical evaluation revealed that the methanol extract contained several bioactive compounds, including flavonoids, tannins, and terpenoids. In the antibacterial test, the ethyl acetate fraction demonstrated the highest inhibitory effect on both test microorganisms at a concentration of 500 mg/ml, producing inhibition zones of 16.42 mm against *S. aureus* and 11.37 mm against *E. coli*. Meanwhile, the methanol extract produced inhibition zones of 13.30 mm and 10.63 mm, respectively. The *n*-hexane fraction exhibited comparatively weaker activity, with inhibition zones measuring 7.45 mm against *S. aureus* and 7.32 mm against *E. coli*. Overall, these findings suggest that extracts derived from the fruit peel of *B. asiatica* possess antibacterial properties, with the ethyl acetate fraction showing the most pronounced inhibitory activity among the tested extracts.

Keywords: *Barringtonia asiatica* L. Kurz, Fruit Peel Extract, Phytochemical, Antibacterial Activity, *S. aureus* and *E. coli*

Introduction

Infectious diseases triggered by pathogenic bacteria continue to represent a major global health concern, particularly infections associated with *Staphylococcus aureus* and *Escherichia coli* (Suhaera et al, 2025). These microorganisms are frequently implicated in a variety of clinical conditions, including skin infections, gastrointestinal disorders, and hospital-acquired infections (Suhaera et al, 2025). The growing prevalence of resistance to commonly used antibiotics among these bacteria has intensified the search for alternative antibacterial agents that are safer, effective, and sustainable, particularly those derived from natural sources (Khumaidi et al, 2022). Indonesia is recognized as one of the world's megabiodiversity countries, possessing abundant plant resources with considerable

potential for the discovery of pharmacologically active compounds (Khumaidi et al, 2022). Among these plants, *Barringtonia asiatica* (L.) Kurz, belonging to the Lecythidaceae family, has attracted attention due to its diverse biological properties. Traditionally, this coastal plant has been utilized by local communities as a fish poison as well as in various traditional medicinal practices (Mara et al, 2024) (Timog et al, 2025). Phytochemical investigations have revealed that the plant contains numerous secondary metabolites, including saponins, flavonoids, alkaloids, tannins, terpenoids, and phenolic compounds, which are known to exhibit several biological activities such as antibacterial effects (Maliangkay et al, 2021) (Mara et al, 2024). Previous investigations of *B. asiatica* have mainly focused on its seeds and leaves, whereas studies examining the fruit peel remain relatively scarce (Khumaidi et al, 2022) (Maliangkay et al, 2021) (Mara et al, 2024) (Suhaera et al, 2025). In fact, fruit peel is commonly discarded as agricultural waste, despite its potential to contain substantial levels of bioactive compounds (Khumaidi et al, 2022). Furthermore, the selection of extraction solvents with varying polarity—such as methanol (polar), ethyl acetate (semi-polar), and *n*-hexane (non-polar)—can significantly influence the types and quantities of phytochemical constituents obtained, which may subsequently affect the antibacterial activity of the resulting extracts (Khumaidi et al, 2022) (Maliangkay et al, 2021) (Mara et al, 2024). From a theoretical perspective, variations in phytochemical composition caused by differences in solvent polarity may contribute to distinct antibacterial mechanisms. These mechanisms include disruption of bacterial cell walls, alteration of membrane permeability, inhibition of protein synthesis, and inactivation of essential bacterial enzymes (Maliangkay et al, 2021) (Mara et al, 2024). Compounds such as saponins and flavonoids, which have been widely reported in *B. asiatica*, are known to compromise the structural integrity of bacterial cell membranes and suppress the growth of both Gram-positive and Gram-negative pathogenic bacteria (Mara et al, 2024). Based on these considerations, investigating the phytochemical profile and antibacterial properties of methanol, ethyl acetate, and *n*-hexane extracts derived from the fruit peel of *B. asiatica* against *S. aureus* and *E. coli* is of considerable importance. The findings of this study are expected to provide scientific evidence supporting the development of *B. asiatica* as a potential natural antibacterial source, while also promoting the utilization of fruit peel waste as a value-added raw material in pharmaceutical applications.

Methodology

Tools and Materials

The equipment used in this study included a rotary evaporator (Heidolph WB 2000); measuring cups, beakers, erlenmeyer flasks, base flasks, and test tubes (Pyrex); glass funnels and separating funnels; droppers and micropipettes (Eppendorf); spatulas; water baths; vial bottles; thin layer chromatography plate; stirring rods; aluminum foil; cotton, cotton buds, and test tube racks; blenders; refrigerators (Toshiba); osse needles; petri dishes; incubators (Fiber Scientific); disc paper; bunsen burners; vernier calipers; autoclaves (Yamata SN 20); tweezers; analytical balances (Mettler AE 200); and laminar air flow (LAF). The materials used in this study included *Barringtonia asiatica* fruit peel; methanol, ethyl acetate, and *n*-

hexane (Merck); distilled water; 5% $FeCl_3$ solution; 1% $CeSO_4$ solution in 10% H_2SO_4; Wagner, Mayer, Bouchardat, and Dragendorff reagents; dimethyl sulfoxide (DMSO); Nutrient Broth (NB), Nutrient Agar (NA), and Mueller Hinton Agar (MHA) media; McFarland standard; and *Staphylococcus aureus* and *Escherichia coli cultures*.

Sample Provision

The sample studied was *Barringtonia asiatica* fruit peel obtained from the Biotechnology Road of the Faculty of Mathematics and Natural Sciences, University of North Sumatra, Medan. The *Barringtonia asiatica* fruit peel was blended until 350 g of fruit skin powder was obtained.

Extraction of *Barringtonia asiatica* Fruit Peel

Barringtonia asiatica fruit peel powder was weighed at 350 g, then macerated with approximately 4 L of methanol until all samples were submerged and left for 24 hours. The macerate was filtered to obtain the methanol extract of *Barringtonia asiatica* fruit peel. The methanol extract of *Barringtonia asiatica* fruit peel was concentrated over a water bath to obtain a solid methanol extract. The obtained solid methanol extract was then dissolved with ethyl acetate to form a methanol precipitate and ethyl acetate extract. The methanol precipitate was separated for phytochemical screening, after which the solid methanol extract was evaporated with a water bath for subsequent testing of its antibacterial activity against *S. aureus* and *E. coli* bacteria. Ethyl acetate extract is concentrated using a water bath to obtain a solid ethyl acetate extract. The solid extract obtained is evaporated until all the ethyl acetate evaporates. The solid extract is dissolved in methanol and partitioned with *n*-hexane to form two layers. The lower layer is ethyl acetate and the upper layer is *n*-hexane. The partition is repeated repeatedly using *n*-hexane solvent until the *n*-hexane layer is clear. Ethyl acetate and *n*-hexane extracts were reconcentrated with a water bath to obtain solid ethyl acetate and solid *n*-hexane extracts. The solid extracts of ethyl acetate and *n*-hexane were then subjected to antibacterial activity testing against *S. aureus* and *E. coli* bacteria.

Phytochemical Screening of *Barringtonia asiatica* Fruit Peel

Tannin Test

Methanol extract of the *B. asiatica* fruit peel is placed in a test tube, then 5% $FeCl_3$ is added. If a black solution forms, then it is positive for tannin.

Terpenoid Test

Methanol extract of *B. asiatica* fruit peel is dripped onto a thin layer chromatography plate, 1% $CeSO_4$ is added, then heated. If a brownish red color forms, it is positive for terpenoids.

Alkaloid Test

Methanol extract of *B. asiatica* fruit peel is placed in three test tubes. Tube I is dripped with Bouchardat reagent; if a brown precipitate forms, it is positive for alkaloids. Tube II is treated with Meyer's reagent; if a white precipitate forms, it is positive for alkaloids. Tube III is treated with Dragendorff's reagent; if an orange precipitate forms, it is positive for alkaloids.

Saponin Test

A portion of the methanol extract obtained from the fruit peel of *B. asiatica* was transferred into a test tube, followed by the addition of 10 ml of distilled water. The mixture was then shaken vigorously to allow thorough mixing. The formation of stable foam indicates the presence of saponins in the sample.

Flavonoid Test

Methanol extract of *B. asiatica* fruit peel is placed in a test tube, then ethyl acetate and $FeCl_3$ reagent are added. If a black solution forms, the result is positive for flavonoids.

Antibacterial Activity Test Extracts of *Barringtonia asiatica* Fruit Peel

Preparation of Nutrient Agar (NA) Media

A quantity of 7 g of NA was transferred into an erlenmeyer flask and mixed with 250 ml of distilled water. The mixture was heated while stirring until the medium dissolved completely and reached the boiling point. Afterward, the prepared medium was sterilized in an autoclave at 121 °C for 15 minutes.

Preparation of Slant Agar Media and Bacterial Culture Stock

Sterile 3 ml of NA medium is placed in a sterile test tube and left at room temperature until it solidifies at an angle of 30-45°. A culture of *Staphylococcus aureus* bacteria from the main strain is taken with a sterile inoculating needle and then inoculated onto the surface of the NA slant by streaking, then incubated at 35 °C for 18-24 hours. The same procedure was performed for the *Escherichia coli* bacterial culture.

Preparation of Mueller Hinton Agar (MHA) Media

A total of 10.2 g of MHA medium was transferred into an erlenmeyer flask and mixed with 300 ml of distilled water. The mixture was heated until the medium dissolved completely and reached boiling. Subsequently, the medium was sterilized using an autoclave at 121 °C for 15 minutes.

Bacterial Inoculum Production

A total of 3.25 g of Nutrient Broth (NB) was weighed and dissolved in 250 ml of distilled water in an erlenmeyer flask. The mixture was heated until the medium was completely dissolved and reached boiling. Afterward, the solution was sterilized in an autoclave at 121 °C for 15 minutes and allowed to cool. Bacterial colonies of *Staphylococcus aureus* were then aseptically collected from the culture stock using a sterile inoculating needle and transferred into 10 ml of sterile NB medium contained in a test tube. The suspension was incubated at 35 °C for approximately 3 hours. The turbidity of the bacterial suspension was subsequently adjusted by comparing it with the McFarland standard equivalent to 10^8 CFU/ml. The same procedure was also applied to colonies of *Escherichia coli*.

Preparation of Variations in Concentration of Methanol, Ethyl Acetate, and *n*-Hexane Extracts from *Barringtonia asiatica* Fruit Peel

Each extract obtained using methanol, ethyl acetate, and *n*-hexane was weighed at 500 mg and subsequently dissolved in 1 ml of dimethyl sulfoxide (DMSO) to prepare a stock solution with a concentration of 500 mg/ml. From this stock solution, a series of dilutions was then prepared to obtain concentrations of 400 mg/ml, 300 mg/ml, 200 mg/ml, and 100 mg/ml.

Antibacterial Activity Test of Ethyl Acetate, Methanol, and *n*-Hexane Extracts of *Barringtonia asiatica* Fruit Peel

Place the MHA medium in a sterile petri dish at a temperature of 45-50 °C, then leave it until it solidifies. Take a sterile cotton swab, dip it into the *S. aureus* bacterial inoculum, then streak it onto the solidified MHA medium. Sterile paper discs that had been impregnated with methanol, ethyl acetate, and *n*-hexane extracts derived from the fruit peel of *Barringtonia asiatica* at different concentrations were carefully placed onto petri dishes inoculated with *Staphylococcus aureus*. The plates were then incubated at 35 °C for approximately 18–24 hours. After incubation, the clear inhibition zones formed around each disc were measured using a caliper to determine their diameters. The same experimental procedure was also applied to cultures of *Escherichia coli*.

Result and Discussion

Phytochemical Composition of Methanol Extracts from *Barringtonia asiatica* Fruit Peel

The methanol extract obtained from the fruit peel of *Barringtonia asiatica* was subjected to phytochemical screening in order to identify the presence of several secondary metabolites, including alkaloids, flavonoids, tannins, saponins, and terpenoids. The results of this analysis are presented in Table 1.

Table 1. Phytochemical Composition of Methanol Extracts from *Barringtonia asiatica* Fruit Peel

No	Phytochemical	Reagent	Methanol Extract
1	Alkaloid	Bouchardat	–
		Meyer's	–
		Dragendorff's	–
2	Flavonoid	$FeCl_3$	+
3	Saponin	Distilled water	–
4	Tannin	$FeCl_3$ 5%	+
5	Terpenoid	$CeSO_4$ 1%	+

Legend: (+) Present in Traceable Amount, (–) Absent

Phytochemical screening represents an initial step in plant-based research that aims to identify the major groups of chemical constituents present in plant materials (Maheshwaran et al, 2024). The results of the screening indicated that the methanol extract derived from the fruit peel of *Barringtonia asiatica* contains several classes of secondary metabolites, particularly terpenoids, flavonoids, and tannins (Kong et al, 2020) (Tanor et al, 2014). The detection of these compounds suggests that the methanol solvent was able to effectively

extract bioactive constituents from the plant material. Methanol is considered a versatile solvent because it possesses both polar (–OH) and non-polar (–CH_3) functional groups, allowing it to dissolve compounds with different polarity characteristics (Lee et al, 2024) (Maheshwaran et al, 2024) (Tanor et al, 2014) (Umaru et al, 2018). This property enables methanol to extract a wide range of phytochemicals from plant tissues. The identification of flavonoids in the methanol extract was carried out using the $FeCl_3$ reagent. During the test, the appearance of a reddish-yellow coloration indicated a positive reaction for flavonoids in the extract of *B. asiatica* fruit peel. Flavonoids occur in various structural forms, either as free compounds known as aglycones or as glycoside derivatives bound to sugar molecules. Polymethoxylated aglycones tend to be non-polar, while polyhydroxylated aglycones are generally semi-polar. In contrast, flavonoid glycosides are more polar because they contain multiple hydroxyl groups and sugar moieties. Due to this structural diversity, flavonoids can interact well with methanol, which is widely recognized as an effective universal solvent for phytochemical extraction (Harborne & Williams, 2000) (Klangmanee & Athipornchai, 2019) (Shaikh & Patil, 2020). Terpenoid compounds were identified based on their ability to produce a characteristic color reaction following the addition of 1% $CeSO_4$ in 10% H_2SO_4. In this study, the formation of a brownish-red color confirmed the presence of terpenoid compounds in the extract (Dubale et al, 2023) (Kong et al, 2020) (Shaikh & Patil, 2020). The detection of tannins was performed using a 5% $FeCl_3$ reagent. When this reagent was added, hydrolyzable tannins reacted with ferric ions, resulting in the formation of a dark or black coloration. This color change occurs due to the interaction between $FeCl_3$ and the hydroxyl groups present in the tannin structure (Shaikh & Patil, 2020) (Shriwas & Singh, 2023). These results collectively confirm the presence of several important bioactive compounds in the methanol extract of *B. asiatica* fruit peel.

Antibacterial Activity of Methanol, Ethyl Acetate, and *n*-Hexane Extracts from *Barringtonia asiatica* Fruit Peel

The methanol, ethyl acetate, and *n*-hexane extracts obtained from the fruit peel of *Barringtonia asiatica* demonstrated antibacterial effects against the tested microorganisms, namely *Staphylococcus aureus* and *Escherichia coli*. This activity was indicated by the formation of inhibition zones around the paper discs containing the respective extracts. These clear zones represent areas where bacterial growth was suppressed due to the presence of antibacterial compounds in the extracts. The diameters of the inhibition zones formed around the discs were measured to evaluate the level of antibacterial activity. The results of these measurements for the methanol, ethyl acetate, and *n*-hexane extracts against *S. aureus* and *E. coli* are presented in Table 2.

Table 2. Antibacterial Activity Test of *Barringtonia asiatica* Fruit Peel Extract

Concentration (mg/ml)	Inhibition Zone Diameter (mm)					
	Methanol Extract		Ethyl Acetate Extract		*n*-Hexane Extract	
	S. aureus	*E. coli*	*S. aureus*	*E. coli*	*S. aureus*	*E. coli*
Blank	–	–	–	–	–	–
100	6.34	6.00	9.14	6.81	6.05	6.00
200	6.90	6.84	11.03	7.27	6.19	6.09
300	7.58	7.20	11.80	8.12	6.50	6.22
400	9.25	8.33	12.24	9.38	7.26	7.11
500	13.30	10.63	16.42	11.37	7.45	7.32

Legend: Blank = Disc Paper Soaked in DMSO

According to the criteria established by the Clinical and Laboratory Standards Institute (2012), the effectiveness of antibacterial activity based on inhibition zone diameter can be classified as follows: a diameter of ≥ 20 mm is considered highly effective, 15–19 mm is categorized as effective, and ≤ 14 mm is regarded as less effective. Referring to the data presented in Table 2, the ethyl acetate extract derived from the fruit peel of *Barringtonia asiatica* exhibited stronger antibacterial activity against the Gram-positive bacterium *Staphylococcus aureus* at a concentration of 500 mg/ml, producing an inhibition zone with a diameter of 16.42 mm. In comparison, its activity against the Gram-negative bacterium *Escherichia coli* at the same concentration resulted in a smaller inhibition zone of 11.37 mm. The data also indicate that increasing the concentration of the extract led to a larger inhibition zone diameter, suggesting a directly proportional relationship between extract concentration and antibacterial activity. This trend is further illustrated in Figures 1 and 2.

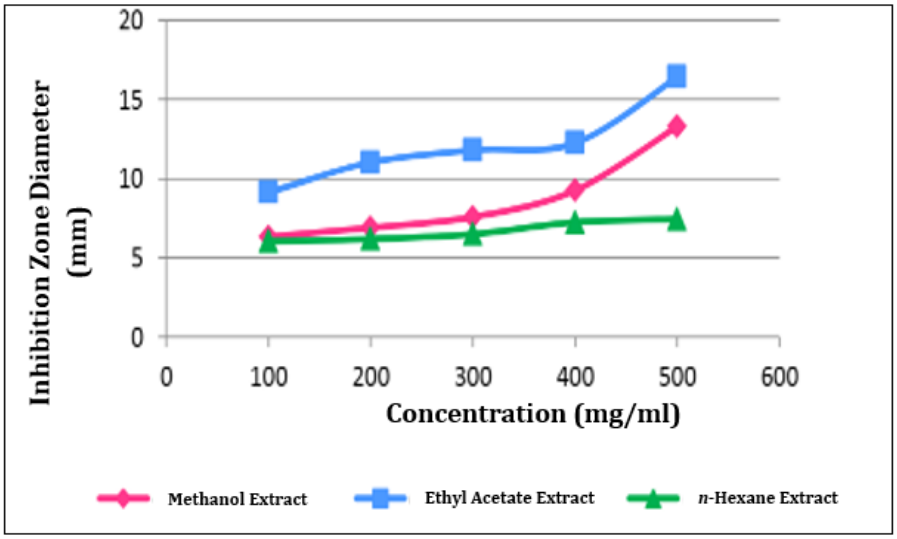

Figure 1. Graph of the Inhibitory Zone Diameter of *Staphylococcus aureus* Bacteria in Methanol, Ethyl Acetate, and *n*-Hexane Extracts

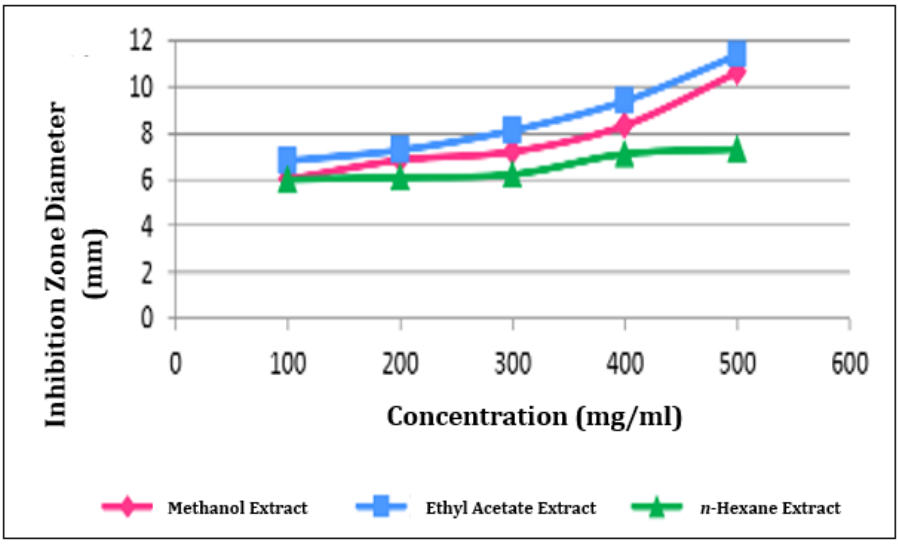

Figure 2. Graph of the Inhibitory Zone Diameter of *Escherichia coli* Bacteria in Methanol, Ethyl Acetate, and *n*-Hexane Extracts

The antibacterial assay indicated that extracts obtained from the fruit peel of *Barringtonia asiatica* were capable of suppressing bacterial growth, although with different levels of effectiveness depending on the solvent used. The methanol extract demonstrated inhibitory activity against *Staphylococcus aureus* and *Escherichia coli* at a concentration of 500 mg/ml, producing inhibition zones of 13.30 mm and 10.63 mm, respectively. A stronger antibacterial effect was observed for the ethyl acetate extract at the same concentration, which generated inhibition zones measuring 16.42 mm against *S. aureus* and 11.37 mm against *E. coli*. In contrast, the *n*-hexane extract exhibited relatively weaker activity, with inhibition zone diameters of 7.45 mm for *S. aureus* and 7.32 mm for *E. coli*. These findings suggest that the extracts of *B. asiatica* fruit peel tend to inhibit the growth of the Gram-positive bacterium *S. aureus* more effectively than the Gram-negative bacterium *E. coli*. Previous comparative studies of plant-derived antibacterial agents have also reported that Gram-positive bacteria are generally more susceptible to botanical extracts than Gram-negative bacteria, a difference largely attributed to variations in cell envelope structure (Gregory & Langland, 2025). The distinct structural organization of bacterial cell walls plays a crucial role in determining sensitivity to antimicrobial substances. The cell wall of Gram-positive bacteria consists of a thick peptidoglycan layer with relatively low lipid content, typically around 1–4%, and lacks an outer membrane (Sarjono et al, 2024). In contrast, Gram-negative bacteria possess a more complex envelope structure with a higher lipid content, approximately 11–12%, and an outer membrane composed of lipopolysaccharides, lipoproteins, and phospholipids (Breijyeh et al, 2020) (Sarjono et al, 2024). This outer membrane functions as an additional permeability barrier, limiting the penetration of many antibacterial compounds and thereby reducing susceptibility to antimicrobial agents (Saxena et al, 2023). The antibacterial activity observed in this study is consistent with the phytochemical screening results, which revealed that the methanol extract of *B. asiatica* fruit peel contains several bioactive constituents, including flavonoids, terpenoids, and tannins.

Numerous recent investigations have reported that methanol extracts of plants commonly contain these groups of secondary metabolites, which are frequently associated with antibacterial properties and are often detected through qualitative phytochemical analyses (Mambo et al, 2025). The presence of tannins and flavonoids, which are phenolic compounds, indicates that *Barringtonia asiatica* fruit peel extract has antibacterial activity. Natural phenolic compounds, including flavonoids and tannins, are widely recognized for exhibiting antibacterial activity against pathogenic bacteria by interacting with bacterial cell structures and proteins (Dembińska et al, 2025). Phenolic compounds work by denaturing cell proteins, causing the proteins in bacteria to lose their biological activity. Phenolic compounds have been shown to interact with bacterial proteins and cellular components, leading to protein denaturation, enzyme inhibition, and disruption of cellular processes that collectively inhibit bacterial growth (Mambo et al, 2025). As a result of the disruption of bacterial cell permeability, the bacteria undergo lysis (cell breakdown), which leads to bacterial cell death. Disruption of membrane integrity and increased permeability caused by phenolic interactions with the lipid bilayer and membrane proteins can result in leakage of cellular contents, loss of homeostasis, and eventually bacterial cell lysis and death (Lobiuc et al, 2023) (Pandey & Vavilala, 2025). Terpenoid compounds present in the methanol extract of *Barringtonia asiatica* fruit peel are known to inhibit bacterial growth primarily through their interaction with the bacterial cell membrane, which functions as a protective barrier and regulates the exchange of substances between the cell and its environment. Due to their lipophilic nature, terpenoids are able to penetrate the phospholipid bilayer of bacterial membranes, leading to disruption of membrane integrity, increased permeability, and interference with membrane-associated proteins. This membrane damage results in leakage of intracellular components, loss of cellular homeostasis, and impairment of essential physiological processes, ultimately causing bacterial growth inhibition and cell death (Fink, 2023) (Khanam et al, 2025).

Conclusion

Phytochemical screening indicated that the methanol extract derived from the fruit peel of *Barringtonia asiatica* contains several classes of secondary metabolites, particularly flavonoids, tannins, and terpenoids. Evaluation of antibacterial activity using the agar diffusion technique showed that the ethyl acetate extract produced the strongest inhibitory effect on bacterial growth. At a concentration of 500 mg/ml, this extract generated inhibition zones measuring 16.42 mm against *Staphylococcus aureus* and 11.37 mm against *Escherichia coli*. In comparison, the methanol extract produced inhibition zones of 13.30 mm and 10.63 mm against *S. aureus* and *E. coli*, respectively. Meanwhile, the *n*-hexane extract demonstrated weaker antibacterial activity, with inhibition zones of 7.45 mm for *S. aureus* and 7.32 mm for *E. coli*. Further investigations are recommended to isolate and characterize the specific bioactive compounds responsible for the antibacterial activity of *B. asiatica* fruit peel extracts. Additional studies should also determine important pharmacological parameters, including the minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), toxicity profile, and in vivo antibacterial efficacy. Moreover, future

research may explore the formulation of pharmaceutical or herbal products based on the most active extract fraction to support the utilization of *B. asiatica* fruit peel as a potential natural antibacterial agent while enhancing the value of plant-derived waste as a beneficial bioresource.

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