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Phytochemical Screening And Antibacterial Activity Of *N*-Hexane, Ethyl Acetate, And Ethanol Extracts Of Malacca Fruit (*Phyllanthus Emblica* L.) Against *Escherichia Coli* And *Staphylococcus Aureus*

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ABSTRACT

Phyllanthus emblica L. (Malacca fruit) is a tropical plant traditionally used for its medicinal properties. This study aimed to identify the secondary metabolite content through phytochemical screening and to evaluate the antibacterial activity of *n*-hexane, ethyl acetate, and ethanol extracts of Malacca fruit against *Escherichia coli* and *Staphylococcus aureus*. The simplicia was extracted using graded maceration. Phytochemical screening revealed the presence of flavonoids, terpenoids, steroids, saponins, and tannins. Antibacterial testing was performed using the agar well diffusion method at concentrations of 3.125–100 mg/mL. Results showed that all three extracts exhibited antibacterial activity against both bacteria. The ethanol extract demonstrated the highest inhibition, with zones of 23.78 mm and 21.63 mm against *E. coli* and *S. aureus* at 100 mg/mL, respectively. Inhibition zones increased with increasing extract concentration. The ethanol extract exhibited superior antibacterial activity compared to ethyl acetate and *n*-hexane extracts, likely due to its ability to extract a broader range of polar bioactive compounds.

Keywords: *Phyllanthus emblica*, Antibacterial activity, Phytochemical screening, Agar well diffusion

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INTRODUCTION

Indonesia possesses remarkable biodiversity with over 20,000 medicinal plant species, yet only approximately 1,300 species have been documented for traditional medicine use (Ningrum et al., 2016). Among these, *Phyllanthus emblica* L., commonly known as Malacca fruit or amla, has long been recognized for its pharmacological potential due to its rich bioactive compound content (Bhagat, 2016; Pathak, 2003). The fruit contains various secondary metabolites including flavonoids, terpenoids, steroids, saponins, and tannins, which contribute to its diverse biological activities (Kumar et al., 2012).

The emergence of antibiotic-resistant bacteria has become a major global health concern, prompting researchers to explore alternative antimicrobial agents from natural sources (Davies & Davies, 2010). *Escherichia coli*, a Gram-negative bacterium, is a leading cause of diarrheal diseases, particularly in immunocompromised individuals (Mufti, 2017). *Staphylococcus aureus*, a Gram-positive bacterium, is responsible for various skin infections, respiratory disorders, and can act as an opportunistic pathogen (Yunika, 2017). The exploration of plant-based antibacterial agents offers a promising approach to combat these pathogenic bacteria.

Previous studies have demonstrated the antibacterial potential of *P. emblica*. Singh and Sarika (2011) reported that various extracts of Malacca fruit exhibited inhibitory activity against *Klebsiella pneumoniae*, *Micrococcus luteus*, and *S. aureus*, with inhibition zones ranging from 17 to 26 mm. Similarly, Dahl and Muggle (2011) demonstrated that leaf extracts exhibited antibacterial activity against several pathogenic bacteria including *E. coli* and *S. aureus*. However, limited studies have systematically

compared the antibacterial efficacy of different polarity-based fruit extracts of *P. emblica* against these clinically relevant pathogens.

The selection of extraction solvents with varying polarities is critical in phytochemical studies, as it determines the types of bioactive compounds extracted (Mariana et al., 2018). The *n*-hexane (non-polar), ethyl acetate (semi-polar), and ethanol (polar) solvents were chosen to enable a comprehensive extraction of compounds with different polarities through graded maceration. This approach facilitates the identification of which polarity fraction exhibits the most potent antibacterial activity, thereby providing valuable information for further pharmaceutical development. This study aimed to conduct phytochemical screening of *n*-hexane, ethyl acetate, and ethanol extracts of Malacca fruit (*P. emblica* L.) and to evaluate their antibacterial activity against *E. coli* and *S. aureus* using the agar well diffusion method.

MATERIALS AND METHODS

Plant Material Collection and Identification

Malacca fruits were collected from Desa Pamuntara, Padang Bolak Hulu, Gunung Tua, North Sumatra, Indonesia. Taxonomic identification was performed at the Herbarium Medanense (MEDA), Laboratory of Plant Systematics, Universitas Sumatera Utara, confirming the species as *Phyllanthus emblica* L. of the family Phyllanthaceae.

Simplicia Preparation

A total of 15 kg of fresh Malacca fruits were cleaned, separated from seeds, yielding 10 kg of fruit flesh. The material was dried in a drying cabinet at 40–50°C until brittle, then ground into powder using a blender, producing 1.6 kg of dried simplicia powder.

Graded Maceration Extraction

Six hundred grams of simplicia powder were subjected to graded maceration using solvents of increasing polarity: *n*-hexane p.a., ethyl acetate p.a., and ethanol p.a. The powder was first macerated with 4.5 L of *n*-hexane in a dark, sealed container for 3 days with intermittent stirring. The macerate was filtered, and the residue was re-macerated with 1.5 L of fresh solvent until the filtrate was clear. The combined filtrate was evaporated using a rotary evaporator at 68–70°C. The dried residue was then sequentially macerated with ethyl acetate (evaporated at 77–78°C) and ethanol (evaporated at 78–80°C) following the same procedure. Each extract was further concentrated over a water bath to obtain viscous extracts.

Phytochemical Screening

Phytochemical screening was performed on all three extracts following standard procedures (Depkes RI, 2014). Tests included alkaloid detection (Mayer, Bouchardat, and Dragendorff reagents), flavonoid identification (Mg-HCl test, $FeCl^3$ test), terpenoid and steroid analysis (Liebermann-Burchard and Salkowski reagents), saponin detection (foam test), and tannin identification ($FeCl^3$ 1% test).

Bacterial Strains and Culture Preparation

Clinical isolates of *E. coli* and *S. aureus* were obtained from the Universitas Sumatera Utara Hospital. Stock cultures were maintained on Nutrient Agar (NA) slants at 35±2°C for 18–24 hours. Inocula were prepared by transferring colonies into Mueller Hinton Broth (MHB), incubating at 35°C for 18–24 hours, and adjusting turbidity to McFarland standard No. 3.

Antibacterial Activity Assay

The antibacterial activity was determined using the agar well diffusion method. Mueller Hinton Agar (MHA) was poured as a base layer (10 mL) into sterile Petri dishes. After solidification, 0.1 mL of bacterial suspension was added, followed by a second layer of MHA (25 mL). Metal borer (6 mm diameter) was used to create wells. Each well was filled with 0.1 mL of extract at concentrations of 100, 50, 25, 12.5, 6.25, and 3.125 mg/mL. Chloramphenicol (10 mg/mL) served as positive control, and DMSO with the respective solvent (6:4) as negative control. Plates were incubated at 35°C for 24

hours. Inhibition zone diameters were measured using a vernier caliper and corrected by subtracting the well diameter (6 mm).

RESULTS AND DISCUSSION

Extraction Yield

The graded maceration of 600 g simplicia yielded 10 g *n*-hexane extract (1.67%), 50 g ethyl acetate extract (8.33%), and 90 g ethanol extract (15.00%). The increasing yield with higher solvent polarity indicates that Malacca fruit contains predominantly polar compounds. The substantially higher ethanol extract yield reflects the abundance of polar secondary metabolites such as flavonoids, tannins, and saponins that are efficiently extracted by polar solvents (Mariana et al., 2018).

Phytochemical Screening

The phytochemical screening results are presented in Table 1. The *n*-hexane extract contained only terpenoids and steroids, consistent with their non-polar nature. The ethyl acetate extract showed a broader profile, containing flavonoids, steroids, terpenoids, saponins, and tannins. The ethanol extract exhibited a similar profile to ethyl acetate, with flavonoids, steroids, terpenoids, saponins, and tannins detected. No alkaloids were found in any of the three extracts.

Table 1. Phytochemical screening results of Malacca fruit extracts

Compound Class	*n*-Hexane	Ethyl Acetate	Ethanol
Alkaloid	–	–	–
Flavonoid	–	+	+
Terpenoid	+	+	+
Steroid	+	+	+
Saponin	–	+	+
Tannin	–	+	+

Note: (+) = detected; (–) = not detected

The differential compound distribution across extracts aligns with the principle of polarity-based extraction, where non-polar solvents extract lipophilic compounds (terpenoids, steroids), while polar solvents extract hydrophilic compounds (flavonoids, tannins, saponins) (Kiswandono, 2011). The presence of diverse secondary metabolites in the ethyl acetate and ethanol extracts suggests their potential for broader biological activity.

Antibacterial Activity

The antibacterial activity results are summarized in Table 2 and illustrated in Figures 1 and 2. All three extracts demonstrated concentration-dependent antibacterial activity against both *E. coli* and *S. aureus*. The ethanol extract consistently exhibited the largest inhibition zones at all tested concentrations, followed by ethyl acetate and *n*-hexane extracts.

Table 2. Inhibition zone diameters (mm) of Malacca fruit extracts against *E. coli* and *S. aureus*

Conc. (mg/mL)	*Escherichia coli*			*Staphylococcus aureus*		
	n-Hex	EtOAc	EtOH	n-Hex	EtOAc	EtOH
K(+)	19.13	20.47	22.53	18.54	20.15	22.24
K(–)	0.00	0.00	0.00	0.00	0.00	0.00
100	13.92	15.06	23.78	13.43	13.49	21.63
50	10.03	9.56	19.98	8.53	8.31	18.86
25	3.35	4.92	15.43	2.96	3.65	14.48
12.5	0.00	0.00	9.07	0.00	0.00	8.39

6.25	0.00	0.00	5.73	0.00	0.00	5.03
3.125	0.00	0.00	3.79	0.00	0.00	3.29

Note: K(+) = chloramphenicol 10 mg/mL; K(–) = DMSO + solvent (6:4); n-Hex = n-hexane; EtOAc = ethyl acetate; EtOH = ethanol. Values represent mean inhibition zone diameter (mm) corrected by subtracting well diameter (6 mm).

At the highest concentration (100 mg/mL), the ethanol extract exhibited the largest inhibition zones of 23.78 mm and 21.63 mm against *E. coli* and *S. aureus*, respectively. These values exceeded the positive control chloramphenicol (22.53 mm and 22.24 mm, respectively), suggesting a potent antibacterial potential. The ethyl acetate extract produced moderate inhibition zones (15.06 mm and 13.49 mm), while the *n*-hexane extract showed the lowest activity (13.92 mm and 13.43 mm) at the same concentration.

The *n*-hexane and ethyl acetate extracts lost detectable antibacterial activity below 25 mg/mL, whereas the ethanol extract maintained activity at all tested concentrations, reaching minimum inhibition zones of 3.79 mm and 3.29 mm at 3.125 mg/mL against *E. coli* and *S. aureus*, respectively. This sustained activity likely results from the ethanol extract's diverse phytochemical composition, including flavonoids, tannins, and saponins, which act synergistically through multiple antimicrobial mechanisms.

The superior activity of the ethanol extract can be attributed to its richer secondary metabolite profile. Flavonoids disrupt cell membrane integrity and inhibit nucleic acid synthesis (Ani, 2018). Tannins exert their antibacterial effect by precipitating proteins and inhibiting cell wall chitin synthesis (Ani, 2018). Saponins exhibit surfactant properties that compromise bacterial membrane permeability (Novitasari & Putri, 2016). The combined action of these compounds provides a multi-target antimicrobial strategy, explaining the enhanced activity of polar extracts.

The results also indicated that all extracts were slightly more effective against *E. coli* (Gram-negative) compared to *S. aureus* (Gram-positive). Although Gram-positive bacteria typically exhibit greater susceptibility to plant antimicrobials due to the absence of an outer membrane (Radji & Manurung, 2011), the observed pattern suggests that the specific bioactive compounds in Malacca fruit may interact more effectively with the membrane components of Gram-negative bacteria. However, the differences were relatively small, and both bacteria demonstrated dose-dependent susceptibility to all three extracts.

The negative control (DMSO with respective solvents) showed no inhibition zones, confirming that the observed antibacterial activity was solely attributable to the plant-derived bioactive compounds and not the solvent system used for extract preparation.

CONCLUSION

Phytochemical screening of Malacca fruit (*P. emblica* L.) extracts revealed the presence of flavonoids, terpenoids, steroids, saponins, and tannins, with variation depending on solvent polarity. All three extracts (*n*-hexane, ethyl acetate, and ethanol) demonstrated antibacterial activity against both *E. coli* and *S. aureus* in a concentration-dependent manner. The ethanol extract exhibited the highest antibacterial activity, surpassing chloramphenicol at the highest tested concentration, likely due to its comprehensive secondary metabolite profile. These findings support the potential of *P. emblica* L. fruit as a natural source of antibacterial agents, warranting further investigation into its bioactive compound isolation, mechanism of action, and development as a standardized herbal preparation.

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CONFLICT OF INTEREST

The authors declare no conflict of interest regarding the publication of this manuscript.

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