

ANTIBACTERIAL ACTIVITY OF *Leea indica* LEAF EXTRACT AGAINST *Escherichia coli* ATCC 25922

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Abstract

Background: *Leea indica*, a large herbaceous plant traditionally used to treat diarrhea, has been studied for its medicinal potential. However, research on the antibacterial activity of its extract against *Escherichia coli* has not yet been widely reported.

Objective: This research was conducted to assess the antibacterial potential of *Leea indica* leaf extract against *E. coli* ATCC 25922 and to identify its phytochemical profile.

Methods: Leaves of *Leea indica* were subjected to maceration using 96% ethanol, followed by solvent removal to obtain a crude extract. Antibacterial activity was evaluated through the agar well diffusion technique using extract concentrations of 20%, 10%, and 5%. The diameters of inhibition zones were recorded to assess antibacterial strength. Phytochemical screening was carried out qualitatively to identify secondary metabolite compounds.

Results: The extract at a concentration of 20% showed the widest inhibition zone, measuring 27.26 mm, indicating very strong antibacterial activity. Phytochemical tests revealed the presence of flavonoids, alkaloids, tannins, phenolic compounds, steroids, and saponins.

Conclusions: The findings indicate that *Leea indica* leaf extract has significant antibacterial activity against *E. coli* ATCC 25922. The detected secondary metabolites may contribute to its antibacterial effect and support its traditional application in diarrhea management.

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INTRODUCTION

Diarrhea is a disease commonly caused by infection with microorganisms. It is defined as the passage of abnormally loose or liquid stools, occurring three or more times within a 24-hour period (1). Among the causative agents, *Escherichia coli* is one of the main pathogenic bacteria responsible for diarrhea. *E. coli* is classified as a Gram-negative bacterium that naturally inhabits the digestive systems of humans and animals, where harmless strains contribute to normal digestive functions. However, pathogenic strains of *E. coli* can invade the intestinal mucosa, attach to epithelial cells, and trigger an inflammatory response that may cause bleeding (2).

Diarrheal diseases cause over 1 million deaths annually, with children under 5 years being the most affected group. In Indonesia, the occurrence of diarrhea in children below five years of age remains persistently elevated, as indicated by national survey data showing prevalence rates ranging from 11% to 12,3% in recent years. Mortality rates in Indonesian children aged 12–59 months due to diarrhea are reported at 4.55%. Regional data show significant variation, with some provinces like South Kalimantan reaching nearly 20% prevalence, while Bali reports around 10.5%(1).

Treatment of gastrointestinal infections caused by *Escherichia coli* primarily focuses on supportive care and maintaining proper hydration status. Most patients are treated with drugs from various therapeutic groups, such as antimotility drugs, adsorbent agents, antisecretory medications, antibiotics, and preparations containing beneficial intestinal microorganisms (3). However, inappropriate or excessive use of antibiotics can lead to the development of resistance (4). One of the most important factors influencing bacterial infections and antibiotic resistance is the ability of *E. coli* to form biofilms, which enhances bacterial survival and makes treatment more difficult. Biofilm formation can increase resistance to antimicrobial therapy by up to 100–1000 times (5).

Based on these considerations, researchers are interested in utilizing natural materials as alternative antibiotics. The use of medicinal plants as an alternative treatment for bacterial infections offers several advantages, including fewer side effects compared to synthetic drugs, wide availability, and lower cost. *Leea indica* (locally known as leaf girang-girang) is one of the potential plants that can be developed as an alternative treatment for bacterial infections. This plant is known to have antibacterial potential by inhibiting bacterial growth. Previous research demonstrated that *L. indica* leaf extract demonstrated inhibitory effects on the growth of *Staphylococcus aureus* and *Salmonella typhi* (6). The antimicrobial properties of this plant are associated with the presence of biologically active constituents. Phytochemical studies have reported that *L. indica* contains several bioactive metabolites, including alkaloids, carotenoids, coumarins, dihydrochalcones, fatty acids, polyphenols, flavonoids, tannins, steroids, glycosides, terpenoids, and saponins (7). Scientific studies that specifically assess the antibacterial properties of *Leea indica* leaf extract toward *Escherichia coli* ATCC 25922 remain limited, particularly in the context of diarrhea-causing bacteria. This lack of empirical evidence creates a knowledge gap regarding the possibility of *Leea indica* being developed as a scientifically supported source of natural antibacterial compounds.

MATERIALS AND METHODS

Material

Materials used in the study are leaf girang-girang (*L.indica*) obtained from JL. Palapa IV, Sesetan, South Denpasar, Denpasar City, Bali 80223, while material chemicals used is Aquades, Ethanol 96% (E Merck), Nutrient Agar (Oxoid), Hydrochloric acid (HCl) Merck, magnesium (Mg), glacial acetic acid, concentrated sulfuric acid (H₂SO₄) Merck 1.00731.2500, ammonia (NH₃) Merck 105432, Dragendorff Merck, 2,2, Ciprofloxacin 1%

Method

This study employed both quantitative and qualitative experimental methods. *Leea indica* leaves (1 kg) were air-dried at ambient for 3 days and then ground into powder using a blender (8). A total of 100 g of the powdered leaves was subjected to maceration in 1000 mL of 96 percent ethanol at a ratio of 1:10 (w/v) (9). The solution was stored in a sealed container at room temperature for 48 hours, after which it was filtered through Whatman filter paper with a pore diameter of 2.5 µm. The resulting filtrate was then concentrated by means of a rotary evaporator to produce the crude extract material of *L. indica* leaves (10).

The antibacterial effect of *Leea indica* leaf crude extract on *E. coli* ATCC 25922 was evaluated using the well agar well diffusion technique (diffusion method). A total of 15 mL of Nutrient Agar (NA) was dispensed into sterile Petri plates and left to solidify. After the medium had solidified, a suspension of *E. coli* ATCC 25922 was applied to the agar surface using a sterile cotton swab. Agar wells measuring 5 mm in diameter were subsequently prepared. Each well received 20 µL of the leaf extract at different concentrations (5%, 10%, 15%, and 20% v/v), along with positive and negative controls. The plates were maintained under incubation at 37 °C for 24 hours. Antibacterial activity was demonstrated by the appearance of a clear inhibition zone around the wells. The inhibition zone diameters were measured in millimeters and classified as follows: ≥ 20 mm (very strong activity), 10–20 mm (strong activity), 5–10 mm (moderate activity), and < 5 mm (low or no activity) (11).

An extract volume of 2 mL sample was transferred into a test tube and allowed to extract with 5 mL of 2N HCl, then heated. After cooling, the solution was divided equally into three separate reaction tubes (1 mL each). Each tube was then treated with a different reagent. A positive reaction with Wagner's reagent produced a brown precipitate as a positive response. A positive result with Mayer's reagent was shown by the formation of a white precipitate, while Dragendorff's reagent produced an orange precipitate as a positive indication of alkaloids (11).

For the total flavonoid test, 2 g of the extract was subjected to heating for no more than 5 minutes, then 0.1 g of powdered magnesium (Mg) was added, followed by the dropwise addition of concentrated HCl. The color change was then observed using the Wilstätter method. The appearance of yellow, orange, or red coloration indicated a positive result for flavonoids (12). For steroids and terpenoids test, an aliquot of 2 mL extract was combined with the addition of 10 drops of glacial acetic acid (CH₃COOH) and 2 drops of concentrated sulfuric acid (H₂SO₄) as Liebermann-Burchard reagent. The solution was gently shaken and allowed to remain undisturbed for several minutes. Terpenoids are identified by the appearance of red or purple coloration, whereas steroids

are indicated by a green or blue coloration (13). Phenolic compounds was examined by reacting the extract with 5% FeCl₃ solution, where a change in a color change to green, red, purple, blue-black, or greenish-black indicated a positive result (14). Tannins were detected by boiling 1 mL of leaf extract with 10 mL of water for a duration of 5 minutes, followed by filtration, and the filtrate was then treated with 1% FeCl₃ solution; the appearance of a greenish-brown, blue, or greenish-black color confirmed the occurrence of tannins (15). Saponins were tested by placing 0.3 g of leaf extract in a test tube, followed by the addition of 10 mL distilled water and vigorous shaking for a period of 30 to 60 seconds; the formation of stable foam approximately 3 cm in height that persisted for more than 30 minutes was considered a positive result (16).

RESULTS AND DISCUSSION

Extraction of leaf samples yielded 2 g of crude extract. The antibacterial effect of the leaf extract on *Escherichia coli* ATCC 25922 is presented in Table 1. The suppressive effect of the extract on bacterial proliferation was demonstrated by the formation of a clear inhibition zone, as shown in Figure 1.

Table 1 Inhibitory zone of Leaf Extract on *Escherichia coli* ATCC 25922

Concentration Extract (w/v)	Inhibition Zone Diameter (mm)**	Category Power Resistor
Control Positive	27.26 ± 0.40 ^a	Very Strong
20%	22.54 ± 0.69 ^b	Very Strong
15%	20.29 ± 0.12 ^c	Strong
10%	15.52 ± 0.25 ^d	Currently
5%	14.37 ± 0.27 ^e	Currently
Control Negative	0.00 ± 0.00 ^f	-

** The values in Table 1 shows the mean values ± standard deviation obtained from four replicates. Values sharing the same letter in the same column indicate no significant difference ($p > 0.05$) based on Duncan's Multiple Range Test (DMRT), following analysis of variance (ANOVA).

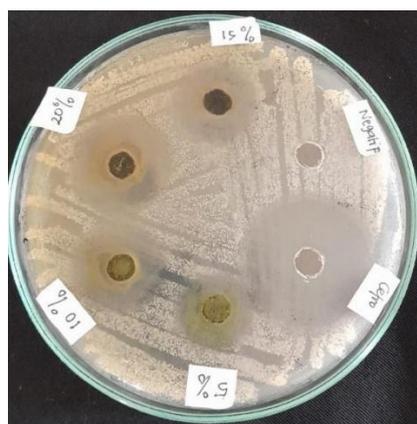


Figure 1. Inhibitor zone of Leaf Extract on *Escherichia coli* ATCC 25922

The data in Table 1 show that the antibacterial effect of the leaf extract on *E. coli* ATCC 25922 was highest at the 20 percent concentration, with a mean inhibition zone diameter of 22.54 mm, which is classified as strong. The lowest activity was observed at a concentration of 5%, resulting in a mean inhibition zone diameter of 14.37 mm, classified

as moderate. The negative control using 96 percent ethanol exhibited no inhibition zone, indicating a lack of antibacterial effect. In contrast, the positive reference control (ciprofloxacin) produced an inhibition zone with an average diameter of 27.26 mm, classified as very strong.

Phytochemical screening of the ethanolic crude leaf extract showed positive findings for multiple classes of secondary metabolites, including alkaloids, flavonoids, tannins, phenolic compounds, saponins, and steroids.

Table 2. Test Results Phytochemicals Extract Rough Leaf Excited

Group Compound	Reagent	Change Color	Results
Alkaloid	Mayer	Cloudy and Formed Sediment	+
Flavonoid	Mg + HCl	Formed color yellow	+
Steroid	CH ₃ COOH glacial + H ₂ SO ₄ concentrated	Formed color green, red , blue	+
Phenol	FeCl ₃	Formed color chocolate green Black	+
Tannin	FeCl ₃	Formed color chocolate greenish Black	+
Saponin	Aquadest , shaken	Formed foam	+

Information :

+ = Reaction Positive

- = Reaction Positive

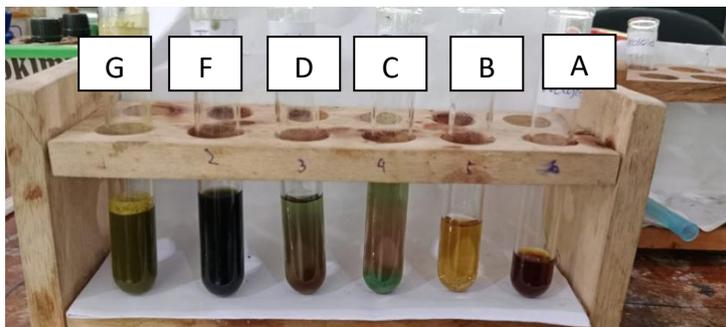


Figure 2. Test Results Phytochemicals Compound Leaf Excited (A) Alkaloids, (B) Flavonoids, (C) Steroids (D) Phenols , (E) (B) Tannin , (F) Saponin.

The ethanolic leaf extract exerted a significant effect ($p < 0.05$) on the growth of *E. coli* ATCC 25922 (Table 1), as evidenced by differences in the inhibition zone diameters observed surrounding the wells (Figure 1). These results are in agreement with earlier reports reporting that *Leucaena indica* leaf extract exhibits antibacterial activity against *Staphylococcus epidermidis* and *Salmonella typhi*, the causative agents of nosocomial infections and typhoid fever (17). Methanol extracts of *L. indica* leaves have shown antimicrobial effects against *E. coli* and *S. aureus*, with minimum lethal concentration

values (MLC) as low as 16 µg/mL for both bacteria. The active compound identified, cytoside, was effective against these pathogens, indicating the potential of *L. indica* as a potential source of antimicrobial compounds (30). *Leea indica* leaf extract demonstrates marked antibacterial activity across various bacterial species, including *Staphylococcus epidermidis* and *Salmonella typhi* species, which are known to cause nosocomial (hospital-acquired) infections and typhoid fever, respectively (17) In laboratory tests, the ethanolic extract derived from *Leea indica* leaves produced measurable inhibitory zones against Gram-positive as well as Gram-negative bacteria including *S. typhi* strains (3). Increasing the concentration of antimicrobial extracts amplifies their ability to penetrate bacterial cells, disrupt membranes, and damage metabolic systems, leading to more effective bacterial inhibition and cell death. This dose-dependent effect is well-supported across various natural extracts and bacterial targets (26)

The phytochemical test results of *Leea indica* leaf extract showed positive reactions for several groups of active compounds (Table 2). This finding is consistent with the study of Rahman (2013), who reported that *L. indica* leaf extract contains alkaloids, flavonoids, tannins, saponins, steroids, and terpenoids. Alkaloids were detected in the alkaloid group assay and are widely recognized for their antibacterial and antifungal activities (29). Their mechanism of action involves inhibition of DNA and RNA synthesis by targeting DNA gyrase and topoisomerase enzyme systems, which are essential for cellular replication and transcription processes (21,22). Flavonoids exert inhibitory actions by damaging the bacterial cell wall structure, inactivating enzymes, and disrupting the cellular membrane. The β-ring structure and hydroxyl (-OH) groups of flavonoids play an essential role in antibacterial function (24).

Steroids, as antibacterial agents, can induce liposome leakage. Due to their lipophilic nature, steroids interact with cell membrane phospholipids, leading to decreased membrane integrity and morphological alterations that result in cell lysis (25). Phenolic compounds are characterized by broad antibacterial effects against Gram-positive as well as Gram-negative bacteria (26). Their mode of action includes protein coagulation, which causes membrane lysis; once the membrane is disrupted, phenols penetrate the cells and interfere with cellular metabolic processes (27).

Tannins, which are derivatives of phenols, are widely distributed in wood, stems, leaves, and fruits. Their antimicrobial activity is attributed to interactions with cell membranes and inactivation of genetic material. In addition, tannins form complexes with proteins, causing protein denaturation and thereby disrupting cellular metabolism (27). More broadly, phenolic compounds act as antibacterials by denaturing proteins and inhibiting nucleic acid synthesis (28). Saponins, another active compound, exert detergent-like effects by reducing interfacial tension, thereby increasing membrane permeability and leading to the leakage of intracellular constituents, which ultimately results in membrane disruption and cellular lysis (23, 28).

CLINICAL IMPLICATION

The clinical implication of this study is to provide preliminary information on the secondary metabolites of the traditional plant *Leea indica* leaves, further in vivo and clinical studies are necessary to confirm their potential therapeutic applicability.

LIMITATIONS

The limitation of this study is use of a single extraction solvent may have restricted the diversity of secondary metabolites obtained, potentially affecting the observed antibacterial activity. In addition, antibacterial testing was limited to one bacterial strain, *Escherichia coli* ATCC 25922, which constrains the generalizability of the findings. As the the study was performed under in vitro experimental conditions, the findings should be interpreted as preliminary. Future research should involve multiple extraction solvents, a broader range of bacterial pathogens, and in vivo studies to better validate the antibacterial capability of *Leea indica* leaf-derived extract.

CONCLUSIONS

This study demonstrates that the ethanol-based extract obtained from *Leea indica* leaves exhibits antibacterial activity observed under in vitro conditions against *Escherichia coli* ATCC 25922. The presence of multiple secondary metabolites suggests a potential contribution to the observed inhibitory effect. These results offer initial evidence supporting the antibacterial potential of *Leea indica* leaves and highlight the need for further studies to clarify the mechanism of action and to validate the activity through broader and more advanced experimental approaches.

CONFLICT OF INTEREST

The authors state that this study was carried out without any commercial or financial affiliations that might be interpreted as a conflict of interest.

AUTHOR CONTRIBUTIONS

II was responsible for study design and implementation, data analysis and interpretation, and manuscript preparation. IBD and NLS provided manuscript review and overall supervision.

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DECLARATION OF ARTIFICIAL INTELLIGENCE USE

No artificial intelligence (AI) tools were used in the preparation of this manuscript.

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