

IN-VITRO ANTIBACTERIAL POTENTIAL OF GINGER (*ZINGIBER OFFICINALE*) AND HENNA (*LAWSONIA INERMIS*) AGAINST BACTERIAL ISOLATES FROM CUTANEOUS LEISHMANIASIS WOUNDS

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Abstract

Cutaneous leishmaniasis (CL) is a neglected tropical disease that produces chronic ulcerative skin lesions, which may become colonized or secondarily infected by bacteria and consequently complicate wound healing. The present study was conducted to investigate the antibacterial activity of ethanolic extracts of *Lawsonia inermis* (henna) and *Zingiber officinale* (ginger) against bacterial isolates recovered from CL lesions in Kohat, Khyber Pakhtunkhwa, Pakistan. Fresh plant materials were collected locally, authenticated, and processed for ethanolic extraction. Wound swab samples were collected from CL patients and cultured using standard microbiological procedures. The bacterial isolates were identified on the basis of colony morphology, Gram staining, microscopic characteristics, and biochemical tests. The antibacterial activity of the plant extracts was evaluated by the agar well diffusion method at concentrations of 250, 500, 750, and 1000 µl/ml, using penicillin as a positive control and ethanol as a negative control. Two bacterial species, *Escherichia coli* and *Staphylococcus aureus*, were recovered from the examined CL lesions. Both plant extracts exhibited antibacterial activity against the

tested isolates, with inhibition increasing generally with increasing extract concentration. *L. inermis* produced mean inhibition zones of 12.67 ± 1.15 to 21.77 ± 2.89 mm against *E. coli* and 16.53 ± 0.81 to 22.00 ± 1.00 mm against *S. aureus* across the tested concentrations. *Z. officinale* produced mean inhibition zones of 17.90 ± 2.53 to 21.53 ± 0.68 mm against *E. coli* and 22.83 ± 0.38 to 27.60 ± 0.87 mm against *S. aureus*. Overall, *Z. officinale* demonstrated stronger inhibitory activity, particularly against *S. aureus*, while both extracts showed substantial *in vitro* antibacterial activity against the selected wound-associated bacteria. These findings provide preliminary evidence supporting the antibacterial potential of *L. inermis* and *Z. officinale* against bacterial isolates associated with CL lesions. Further studies involving phytochemical characterization, MIC and MBC determination, toxicity assessment, formulation development, and *in vivo* and clinical evaluation are required to determine their potential application in the management of secondary bacterial infections associated with CL wounds.

INTRODUCTION

Leishmaniasis is a vector-borne infectious disease caused by protozoan parasites of the genus *Leishmania*. The disease is transmitted to humans and other mammalian hosts through the bite of infected female phlebotomine sand flies belonging to the genera *Phlebotomus* in the Old World and *Lutzomyia* in the New World (Torres-Guerrero *et al.*, 2017). *Leishmania* are obligate intracellular kinetoplastid protozoa that occur in two major morphological forms during their life cycle: the flagellated and motile promastigote form, which develops within the gut of the sand fly, and the non-flagellated amastigote form, which resides and multiplies within the phagolysosomes of macrophages in the mammalian host (Akhoundi *et al.*, 2016). The genus *Leishmania* comprises approximately 54 recognized species, of which at least 30 infect mammals and at least 21 are known to cause disease in humans. These species are transmitted by more than 100 sand fly vector species distributed across almost all continents except Oceania (New Insights into the Life Cycle review, 2025). Owing to its considerable health, social, and economic impact, the World Health Organization (WHO) has classified leishmaniasis as a neglected tropical disease, reflecting the historically limited investment in its diagnosis,

treatment, and vector control despite its substantial disease burden (WHO, 2020).

The transmission of *Leishmania* involves the parasite, sand fly vector, and susceptible mammalian host. During a blood meal, female sand flies ingest macrophages containing amastigotes from infected hosts. In the midgut, amastigotes transform into procyclic promastigotes and progress through nectomonad and leptomonad stages before becoming infective metacyclic promastigotes, which migrate toward the foregut and stomodeal valve (Tom *et al.*, 2024). During a subsequent blood meal, these promastigotes are inoculated into the host dermis along with salivary proteins that modulate local immunity and facilitate parasite establishment (Leishmaniasis: The Act of Transmission review, 2021).

Pakistan is significantly affected by leishmaniasis, with CL being the predominant clinical form. Two major transmission patterns occur: anthroponotic CL, mainly caused by *Leishmania tropica* and transmitted human-to-human by vectors such as *Phlebotomus sergenti*, and zoonotic CL, primarily caused by *Leishmania major* and maintained in animal reservoirs, particularly rodents. Both forms occur in Khyber Pakhtunkhwa, Balochistan, Punjab, Sindh, and Azad Jammu and Kashmir (Khan *et al.*, 2016). During the past two decades,

CL has also expanded into previously non-endemic areas, with population displacement and migration, particularly of IDPs from tribal and border regions, contributing to the introduction and spread of parasites and vectors (Karak epidemiology study, 2020). Khyber Pakhtunkhwa (KP) is one of Pakistan's major endemic regions for CL, with favourable ecological conditions for sand fly transmission and cross-border population movement from Afghanistan potentially contributing to continued transmission (Lu *et al.*, 2024). Important endemic foci include Peshawar, Bannu, Dir, Karak, Hangu, Swat, and Kohat (Khan *et al.*, 2023). Recent surveillance involving more than 3,000 confirmed patients indicated continued spatial expansion of CL, including transmission in areas previously considered disease-free (Distribution and Risk of CL in KP, 2023). Surveillance in Bajaur, Malakand, and Lower Dir during January 2022–February 2023 also recorded more than 2,000 confirmed cases, with younger age groups particularly affected. Similarly, studies among children reported that over 70% of paediatric CL cases occurred in individuals below 15 years, highlighting the vulnerability of children in rural and agrarian communities with greater exposure to sand flies (Ullah *et al.*, 2025).

Kohat District in southern Khyber Pakhtunkhwa is a well-documented endemic focus of CL. An epidemiological and molecular study conducted in 13 villages during April 2015–May 2016 examined 26,250 local residents and 3,615 IDPs, identifying 1,359 cases among locals (5.17%) and 140 among IDPs (3.86%). Active lesions were more common than healed scars, with children aged 1–15 years most affected, while slightly higher prevalence among males was linked to greater outdoor exposure to sand flies during agricultural and pastoral activities (Hussain *et al.*, 2017). In Surgul village, PCR detected CL in 87.6% of clinical samples, compared with 54.0% by microscopy and 46.9% by culture, highlighting its greater diagnostic sensitivity (PCR and Microscopic Identification study, Kohat, 2014). Recent investigations across the wider Kohat division also

reported substantial CL prevalence and poor community awareness of sand fly vectors, transmission, complications, and preventive measures (Knowledge, Attitude and Practices survey, 2022).

Clinically, leishmaniasis occurs mainly in three forms: cutaneous (CL), mucocutaneous (MCL), and visceral leishmaniasis (VL), with CL being the most common and widely distributed form (WHO, 2010). CL is broadly classified into Old World CL (OWCL), prevalent in South and Central Asia, the Middle East, and parts of Africa, and New World CL (NWCL), mainly found in Central and South America. OWCL is associated primarily with *L. major*, *L. tropica*, *L. aethiopica*, *L. donovani*, and *L. infantum*, whereas NWCL is mainly caused by species including *L. braziliensis*, *L. panamensis*, *L. guyanensis*, *L. amazonensis*, *L. mexicana*, and *L. peruviana* (Kevric *et al.*, 2015). CL typically begins as an erythematous papule that enlarges and may ulcerate over several weeks; lesions may remain localized or become multiple and disseminated (WHO, 2015). Although *L. major* lesions may heal spontaneously within 3–18 months, infections caused by *L. tropica* and, to a lesser extent, *L. major* and *L. aethiopica* may persist and cause extensive scarring, particularly on exposed sites (Burza *et al.*, 2018). MCL, mainly associated with *L. braziliensis*, can cause progressive destruction of nasopharyngeal mucosa, whereas VL, primarily caused by *L. donovani* and *L. infantum*, involves systemic infection, fever, hepatosplenomegaly, pancytopenia, and cachexia and can be fatal if untreated (PAHO/WHO, 2025).

Secondary bacterial infection is an important complication of ulcerative CL lesions because disruption of the skin barrier facilitates bacterial invasion, potentially delaying healing, prolonging inflammation, and increasing tissue damage (Sadeghian *et al.*, 2011). Bacteria such as *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Klebsiella* spp. have been isolated from CL lesions, with one study reporting secondary bacterial infection in 20.7% of patients

and *S. aureus* as the most frequently isolated pathogen (Mokni *et al.*, 2013; Sadeghian *et al.*, 2011). Management is further complicated by antimicrobial resistance (AMR), which was associated with an estimated 4.71 million deaths in 2021, including 1.14 million directly attributable to resistant infections, with approximately 39 million cumulative AMR-attributable deaths projected for 2025–2050 if current trends persist (Naghavi *et al.*, 2024).

Medicinal plants offer potential sources of alternative antibacterial agents, with their activity influenced by phytochemical composition, extraction solvent, plant part, and bacterial cell-wall characteristics. Flavonoids, tannins, and terpenoids are among the major phytochemical groups associated with antibacterial effects (Zouine *et al.*, 2024), while combinations of bioactive compounds may produce synergistic effects through multiple antimicrobial mechanisms (Wagner & Ulrich-Merzenich, 2009). Ginger (*Zingiber officinale*) contains bioactive compounds such as gingerols, shogaols, paradols, and zingerone, which contribute to its antimicrobial, anti-inflammatory, and antioxidant properties (Ali *et al.*, 2008). Henna (*Lawsonia inermis*), traditionally used for skin and wound-related applications, contains lawsone (2-hydroxy-1,4-naphthoquinone), flavonoids, tannins, phenolic acids, and other bioactive compounds (Kotb *et al.*, 2025). Both plants have therefore

attracted interest as sources of natural antibacterial compounds (Newman & Cragg, 2020).

Despite these reported antibacterial properties, limited research has specifically examined *Z. officinale* and *L. inermis* against bacteria isolated from CL lesions. Although bacterial infections of CL wounds have been documented in Kohat, their antibacterial activity against locally isolated CL-associated pathogens remains insufficiently investigated. The present study therefore evaluates the *in vitro* antibacterial activity of *Lawsonia inermis* and *Zingiber officinale* extracts against bacterial pathogens isolated from CL wounds, including bacterial isolation and identification, preparation of plant extracts, assessment of antibacterial activity using standard microbiological methods, and comparison of the effectiveness of both extracts.

Materials And Methods

2.1. Plant Collection and Preparation

2.1.1. Identification of Plant Materials

Fresh leaves of *Lawsonia inermis* and rhizomes of *Zingiber officinale* were collected from local areas of Kohat (Figure 1). The plant materials were identified using standard botanical keys and morphological characteristics in the Department of Botany, Kohat University of Science and Technology. Proper identification and authentication of the plant materials were ensured before their use in the experimental study.



Figure 2.1. Fresh plant materials used in the study: (a) fresh leaves of *Lawsonia inermis* (henna); (b) fresh rhizome of *Zingiber officinale* (ginger).

2.1.2. Rinsing of Plant Materials

The collected plant materials were thoroughly washed with running tap water and subsequently rinsed with sterile distilled water to remove dust, soil particles, and other surface contaminants. This step was performed to minimize microbial contamination and prevent possible interference during the antibacterial assays (Pandey & Tripathi, 2014).

2.1.3. Shade Drying

The washed leaves of *Lawsonia inermis* were air-dried under shade at room temperature in a clean and well-ventilated environment. Shade drying was employed to preserve thermolabile phytochemicals, including flavonoids, phenolic compounds, and essential oils, which may contribute to the antimicrobial activity of the plant (Parekh & Chanda, 2017).

2.1.4. Grinding

After complete drying, the plant material was ground into a fine powder using a sterile electric grinder. Powdering increased the surface area of the plant material, thereby facilitating greater contact between the plant material and extraction solvent and improving the extraction of bioactive compounds (Gallina *et al.*, 2022).

2.1.5. Crushing of *Zingiber officinale*

The washed rhizomes of *Zingiber officinale* were peeled using a sterile stainless-steel knife and cut into small pieces. The pieces were then crushed using a sterile electric blender to obtain fresh ginger juice. The resulting juice was filtered through sterile muslin cloth and subsequently through Whatman No. 1 filter paper to remove coarse particles (Kumar *et al.*, 2023).

2.2. Preparation of Crude Ethanolic Extract of *Lawsonia inermis*

Twenty grams (20 g) of powdered *Lawsonia inermis* leaves were transferred into a sterile 500 mL conical flask. Subsequently, 200 mL of 95% analytical-grade ethanol was added to achieve a 1:10 (w/v) ratio (Figure 2). The flask was tightly sealed with aluminum foil and parafilm to minimize contamination and prevent solvent evaporation (Azwanida, 2015).

2.2.1. Preparation of Crude Ethanolic Extract of *Zingiber officinale*

Exactly 20 mL of freshly prepared *Zingiber officinale* juice were transferred into a sterile 500 mL conical flask, followed by the addition of 200 mL of 95% analytical-grade ethanol (Figure 2). The flask was tightly sealed with aluminum foil and parafilm to prevent solvent evaporation and contamination during the extraction process (Azwanida, 2015).



Figure 2.2. Crude ethanolic extract of *Lawsonia inermis* and *Zingiber officinale*

2.3. Shaking Process

The conical flasks containing the plant materials and extraction solvent were placed on an orbital shaker operating at 150 rpm for 72 hours at room temperature ($25 \pm 2^\circ\text{C}$). The flasks were tightly sealed with aluminum foil and parafilm throughout the extraction period to prevent contamination and solvent evaporation. Continuous agitation facilitated solvent penetration into the plant tissues, enhanced the transfer of phytochemicals into the extraction solvent, and improved extraction efficiency (Azwanida, 2015).

2.4. Filtration

After completion of the shaking process, the extracts were filtered using Whatman No. 1 filter paper to remove residual plant material. The resulting filtrates were considered crude ethanolic

extracts containing the soluble bioactive constituents responsible for the antibacterial activity (Sasidharan *et al.*, 2011).

2.5. Rotary Evaporation

The filtered extracts were concentrated using a rotary evaporator under reduced pressure at 40°C to remove ethanol while minimizing the thermal degradation of heat-sensitive phytochemicals (Figure 3). The application of reduced pressure lowers the boiling point of the solvent, allowing efficient solvent removal and concentration of the extracts while minimizing potential degradation of bioactive compounds. The resulting concentrated semi-solid crude extracts were collected in sterile amber-colored bottles and stored at 4°C until further use (Lama-Muñoz and Contreras, 2022).



Figure 2.3. Rotary evaporation of extract

2.6. Collection of Bacterial Samples

2.6.1. Prior Informed Consent

Before sample collection, written informed consent was obtained from all patients diagnosed with cutaneous leishmaniasis who attended KDA Hospital, Kohat. The purpose of the study and the sample collection procedure were explained to the participants. Ethical approval was obtained from the relevant institutional ethical review committee before commencement of the study.

2.6.2. Swab Collection Method

Wound swab samples were collected aseptically from cutaneous leishmaniasis lesions using sterile cotton swabs. Before sampling, the lesion surface was gently cleaned with sterile normal saline to remove superficial debris (Figure 4). The sterile swab was then gently rotated over the active margin and base of the lesion to collect wound exudate and associated bacterial flora. Strict aseptic techniques were maintained throughout the procedure to minimize contamination (Cheesbrough, 2018).



Figure 2.4. Clinical presentation of cutaneous leishmaniasis (CL) lesions: (a) arm ulcer with typical appearance caused by cutaneous leishmaniasis; (b) diffuse arm ulcer associated with a cutaneous leishmaniasis lesion.

2.6.3. Preservation of Samples

Immediately after collection, each swab was placed in a sterile transport tube containing Amies transport medium and appropriately labeled with a sample identification number. The transport medium helped maintain the viability of bacterial isolates during transportation to the laboratory (Forbes *et al.*, 2022).

2.6.4. Transportation to the Laboratory

The collected specimens were transported to the Microbiology Laboratory of Kohat University of Science and Technology under aseptic conditions in an insulated transport box. All samples were processed within two hours of collection to maintain bacterial viability and obtain reliable culture results (Cheesbrough, 2018).

2.7. Culture of Bacteria

Nutrient Agar and MacConkey Agar media were prepared according to standard microbiological procedures under aseptic conditions (Cheesbrough, 2018). The required amount of dehydrated Nutrient Agar powder, approximately 28 g/L, was weighed and dissolved in distilled water with continuous heating and stirring until a clear solution was obtained. Similarly, MacConkey Agar powder, approximately 50 g/L depending on the manufacturer's instructions, was dissolved in

distilled water and mixed thoroughly until completely dissolved (Forbes *et al.*, 2022).

Both media were sterilized in an autoclave at 121°C for 15 minutes at 15 psi pressure to ensure sterilization (Forbes *et al.*, 2022). After sterilization, the media were allowed to cool to approximately 45–50°C before being poured into sterile Petri dishes inside a laminar airflow cabinet (Cheesbrough, 2018). Approximately 15–20 mL of each medium was dispensed into sterile Petri plates and allowed to solidify. The plates were then inverted and stored at 4°C until further use (Forbes *et al.*, 2022).

2.8. Isolation of Bacteria

For bacterial isolation, the collected swab samples were inoculated onto Nutrient Agar and MacConkey Agar plates using the streak plate method to obtain isolated colonies (Forbes *et al.*, 2022). A sterile inoculating loop was first sterilized by flaming and allowed to cool before use (Cheesbrough, 2018). The clinical sample was then streaked over the first quadrant of the agar surface. The loop was subsequently sterilized, cooled, and used to transfer a small portion of the sample from the first quadrant into the second quadrant, thereby reducing the bacterial load (Cheesbrough, 2018). The process was repeated across the third and fourth quadrants to

progressively dilute the bacterial population and facilitate the formation of isolated colonies (Forbes *et al.*, 2022).

The inoculated plates were incubated aerobically at 37°C for 24 hours (Forbes *et al.*, 2022). Following incubation, bacterial growth was examined, and colonies exhibiting distinct morphological characteristics were selected and sub-cultured onto fresh media to obtain pure isolates (Cheesbrough, 2018). All procedures were performed using strict aseptic techniques to minimize contamination and ensure reliable culture results (Forbes *et al.*, 2022).

2.9. Identification of Bacterial Isolates

Bacterial isolates obtained from pure cultures were identified on the basis of microscopic characteristics, Gram-staining reactions, colony morphology, and biochemical properties following standard microbiological procedures (Forbes *et al.*, 2022). These methods provided information regarding the staining characteristics, cellular morphology, enzymatic activities, and metabolic properties of the isolates and were used collectively for their identification.

2.9.1. Microscopic Examination

Microscopic examination was performed as a preliminary step in the identification of bacterial isolates. Freshly prepared Gram-stained smears were examined under a bright-field light microscope using the 100× oil-immersion objective. The bacterial size, shape, cellular arrangement, and staining characteristics were recorded. The observed morphological forms included cocci and bacilli, with arrangements such as clusters, chains, pairs, or individual cells. These microscopic characteristics provided preliminary information that guided subsequent biochemical identification tests (Madigan *et al.*, 2021).

2.9.2. Gram Staining

Gram staining was performed as a differential staining technique to distinguish bacteria into Gram-positive and Gram-negative groups based on differences in their cell wall structures. A bacterial

smear was prepared on a clean glass slide, heat-fixed, and sequentially stained with crystal violet, iodine solution, alcohol as a decolorizing agent, and safranin as a counterstain. The stained slide was examined under oil immersion using the 100× objective lens.

Gram-positive bacteria retained the crystal violet-iodine complex and appeared purple because of their thick peptidoglycan layer, whereas Gram-negative bacteria lost the primary stain during decolorization and appeared pink or red after taking up safranin. Bacterial cell shape and arrangement, including cocci in clusters and bacilli occurring singly or in pairs, were also recorded to support preliminary identification (Cheesbrough, 2018).

2.9.3. Catalase Test

The catalase test was performed to detect the presence of the catalase enzyme, which breaks down hydrogen peroxide into water and oxygen. A small amount of bacterial colony was placed on a clean glass slide, followed by the addition of a drop of 3% hydrogen peroxide. Immediate formation of bubbles indicated a positive reaction due to the release of oxygen, whereas the absence of bubbling indicated a negative reaction. The test was used to differentiate catalase-positive organisms, such as *Staphylococcus* species, from catalase-negative organisms, such as *Streptococcus* species (Forbes *et al.*, 2022).

2.9.4. Coagulase Test

The coagulase test was used to determine the ability of bacterial isolates, particularly *Staphylococcus aureus*, to produce the coagulase enzyme, which causes plasma clotting. In this test, bacterial colonies were mixed with plasma and examined for clumping or clot formation. A positive reaction indicated coagulase production, whereas the absence of clot formation indicated a negative reaction (Forbes *et al.*, 2022).

2.9.5. Indole Test

The indole test was performed to determine the ability of bacteria to produce indole from the

amino acid tryptophan through the action of the enzyme tryptophanase. Following incubation in tryptophan-rich broth, Kovac's reagent was added. Formation of a red or pink ring at the surface of the medium indicated a positive reaction, whereas the absence of color development indicated a negative reaction. The test is commonly positive for *Escherichia coli* (Cheesbrough, 2018).

2.9.6. Citrate Utilization Test

The citrate utilization test was used to determine whether bacterial isolates could utilize citrate as their sole carbon source. The organisms were inoculated onto Simmons citrate agar containing bromothymol blue as an indicator. Growth accompanied by a color change from green to blue indicated a positive reaction, whereas the absence of growth and color change indicated a negative reaction. This test is useful for differentiating members of the Enterobacteriaceae family (Forbes *et al.*, 2022).

2.9.7. Oxidase Test

The oxidase test was performed to detect the presence of cytochrome c oxidase in bacterial isolates. An oxidase reagent, such as tetramethyl-p-phenylenediamine, was applied to the bacterial colony. Development of a dark purple color within a few seconds indicated a positive reaction, whereas the absence of color development indicated a negative reaction. The test is useful for distinguishing oxidase-positive bacteria from oxidase-negative members of the Enterobacteriaceae family (Forbes *et al.*, 2022).

2.9.8. Urease Test

The urease test was used to determine the ability of bacterial isolates to hydrolyze urea into ammonia and carbon dioxide through the action of the urease enzyme. The organism was inoculated into a urea-containing medium with phenol red as an indicator. A positive reaction was indicated by a pink color change resulting from alkaline conditions, whereas no color change indicated a negative reaction. This test is

particularly useful for identifying organisms such as *Proteus* species (Cheesbrough, 2018).

Based on the microscopic, staining, and biochemical characteristics, the isolates were identified as *Escherichia coli* and *Staphylococcus aureus*, both of which are commonly associated with wound infections and secondary complications in chronic lesions (Forbes *et al.*, 2022).

2.9.9. Standardization of Inoculum

The bacterial suspensions were standardized to a 0.5 McFarland turbidity standard, corresponding to approximately 1.5×10^8 CFU/mL. Standardization was performed to ensure a uniform bacterial density and reproducible conditions for subsequent antibacterial susceptibility testing (Balouiri *et al.*, 2016).

2.10. Antibacterial Activity Assay

The antibacterial activity of the plant extracts was evaluated using the agar well diffusion method. Mueller-Hinton agar plates were prepared and sterilized at 121°C for 15 minutes. The standardized bacterial inoculum was evenly spread over the surface of the agar plates using sterile cotton swabs. Wells measuring 6 mm in diameter were prepared using a sterile cork borer and filled with the respective plant extracts. Ethanol was used as a negative control. The plates were incubated at 37°C for 24 hours, after which the zones of inhibition were measured in millimeters. The agar well diffusion method is widely used for the preliminary assessment of antimicrobial activity of plant extracts (Khan *et al.*, 2020).

2.10.1. Controls Used

A standard broad-spectrum antibiotic disc of penicillin was used as the positive control in the susceptibility assays to validate assay performance and provide a comparative reference for the antibacterial activity of the plant extracts. Ethanol was used as the negative control because it was the solvent employed for preparation of the plant extracts. The negative control was included to

determine whether the solvent itself produced any inhibitory effect on bacterial growth.

2.10.2. Measurement of Antibacterial Activity

Antibacterial activity was assessed by measuring the diameter of the inhibition zones surrounding the wells in millimeters. All experiments were performed in triplicate, and the results were expressed as mean $\pm$ standard deviation (CLSI, 2024).

2.11. Statistical Analysis

The experimental data were analyzed using the Statistical Package for the Social Sciences (SPSS), version 22.

RESULTS

3.1 Bacterial Isolates Recovered from Cutaneous Leishmaniasis Wound Samples

Wound swab samples collected from patients with cutaneous leishmaniasis (CL) were cultured on appropriate bacteriological media for the recovery and identification of bacterial pathogens. The bacterial isolates were characterized on the basis of colony morphology, Gram staining, microscopic characteristics, and biochemical tests. Two bacterial species were identified from the examined wound samples, namely *Escherichia coli* and *Staphylococcus aureus*. *E. coli* was identified as a Gram-negative bacillus, whereas *S. aureus* was identified as Gram-positive cocci. These confirmed bacterial isolates were subsequently used to evaluate the antibacterial activity of the ethanolic extracts of *Lawsonia inermis* and *Zingiber officinale*.

3.2 Antibacterial Activity of Ethanolic Extract of *Lawsonia inermis* Against *Escherichia coli*

The antibacterial activity of the ethanolic extract of *Lawsonia inermis* (henna) against *Escherichia coli* isolated from CL lesions was evaluated by the agar well diffusion method (Figure 3.1). The negative control showed no zone of inhibition, with a mean value of 0.00 ± 0.00 mm, indicating that the solvent used as the negative control did not produce detectable antibacterial activity against *E. coli*. In comparison, positive control produced a mean inhibition zone of 7.27 ± 0.87 mm. The ethanolic extract of *L. inermis* demonstrated a clear concentration-dependent inhibitory effect against *E. coli*. At a concentration of 250 $\mu\text{l/ml}$, the extract produced inhibition zones of 12.0, 14.0, and 12.0 mm in the three experimental trials, giving a mean inhibition zone of 12.67 ± 1.15 mm. At 500 $\mu\text{l/ml}$, inhibition zones of 13.6, 17.0, and 15.6 mm were recorded, with a mean of 15.40 ± 1.70 mm. Further enhancement of antibacterial activity was observed at 750 $\mu\text{l/ml}$, where the recorded inhibition zones were 15.6, 20.0, and 19.0 mm, resulting in a mean inhibition zone of 18.20 ± 2.19 mm. The highest activity was observed at 1000 $\mu\text{l/ml}$, with inhibition zones of 20.0, 25.0, and 20.3 mm and a mean value of 21.77 ± 2.89 mm. The inhibition zone increased progressively with increasing extract concentration, from 12.67 ± 1.15 mm at 250 $\mu\text{l/ml}$ to 21.77 ± 2.89 mm at 1000 $\mu\text{l/ml}$. The reported p-values for the tested extract concentrations were below 0.05, indicating statistically significant differences according to the statistical analysis performed. The greatest inhibition was recorded at the highest concentration tested (Table 3.1).

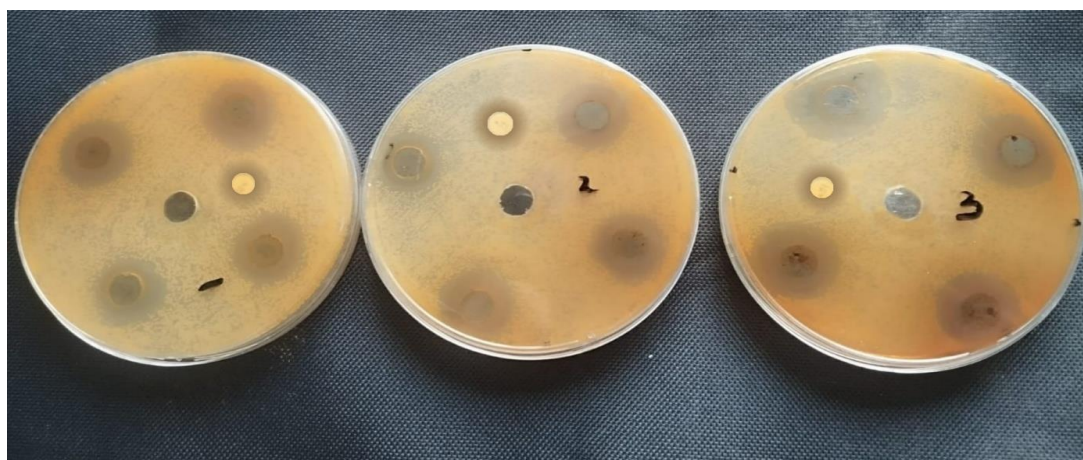


Figure 3.1. Antibacterial activity of *Lawsonia inermis* against *E. coli* using well diffusion method

Table 3.1. Antibacterial activity of ethanolic extract of *Lawsonia inermis* against *Escherichia coli* isolated from cutaneous leishmaniasis lesions

Concentration (µl/ml)	Trial 1 (mm)	Trial 2 (mm)	Trial 3 (mm)	Mean ± SD (mm)	P value	Lower	Upper
Negative control	0.0	0.0	0.0	0.00 ± 0.00	NA	—	—
Positive control	7.5	8.0	6.3	7.27 ± 0.87	0.0048	5.0963	9.437
250	12.0	14.0	12.0	12.67 ± 1.15	0.0028	9.7982	15.535
500	13.6	17.0	15.6	15.40 ± 1.70	0.0041	11.155	19.645
750	15.6	20.0	19.0	18.20 ± 2.19	0.0053	12.47	23.93
1000	20.0	25.0	20.3	21.77 ± 2.89	0.0055	14.801	28.733

3.2.1 Concentration-Dependent Inhibition of *Escherichia coli* by *Lawsonia inermis*

The concentration-response pattern further demonstrated the increasing antibacterial activity of *L. inermis* against *E. coli*. The mean inhibition zone increased from 12.67 ± 1.15 mm at 250 µl/ml to 15.40 ± 1.70 mm at 500 µl/ml, followed by 18.20 ± 2.19 mm at 750 µl/ml and 21.77 ± 2.89 mm at 1000 µl/ml.

Thus, an overall increase of approximately 9.10 mm in the mean inhibition zone was observed between the lowest and highest concentrations tested. This corresponds to an approximately 72% increase in the mean inhibition-zone diameter relative to the value recorded at 250 µl/ml. The results indicate a pronounced concentration-dependent inhibitory response of the henna

extract against *E. coli* under the experimental conditions of the study.

3.3 Antibacterial Activity of Ethanolic Extract of *Zingiber officinale* Against *Escherichia coli*

The antibacterial activity of the ethanolic extract of *Zingiber officinale* (ginger) against *Escherichia coli* isolated from CL lesions was determined using the agar well diffusion method (Figure 3.2). No inhibition was observed with the negative control, which produced a mean inhibition zone of 0.00 ± 0.00 mm. The positive control produced a mean inhibition zone of 5.13 ± 1.02 mm. The ethanolic ginger extract produced substantially larger inhibition zones than the positive control at all tested concentrations. At 250 µl/ml, the three trials produced inhibition zones of 19.0, 19.7, and 15.0 mm, corresponding to a mean inhibition

zone of 17.90 ± 2.53 mm. At 500 $\mu\text{l/ml}$, inhibition zones of 19.7, 20.4, and 19.0 mm were recorded, giving a mean of 19.70 ± 0.70 mm. At 750 $\mu\text{l/ml}$, the recorded inhibition zones were 21.0, 21.0, and 20.5 mm, resulting in a mean inhibition zone of 20.83 ± 0.29 mm. The maximum inhibition was observed at 1000 $\mu\text{l/ml}$, where inhibition zones of 21.3, 22.3, and 21.0 mm were recorded, giving a mean of 21.53 ± 0.68 mm. The results therefore

demonstrated a progressive increase in antibacterial activity with increasing concentration of the ginger extract. The reported p-values for the tested concentrations were below 0.05. The highest mean inhibition zone was recorded at 1000 $\mu\text{l/ml}$, indicating the strongest inhibitory response against *E. coli* at the highest concentration evaluated (Table 3.2).

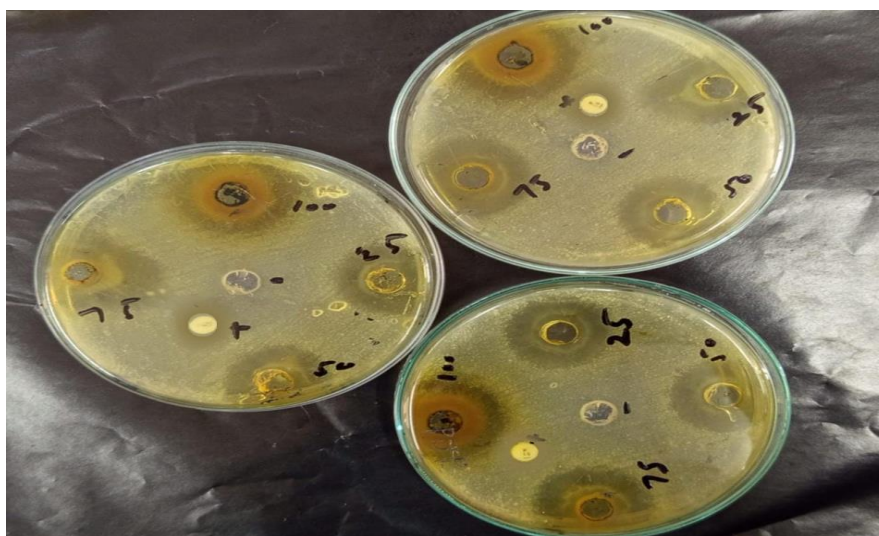


Figure 3.2. Antibacterial activity of *Zingiber officinale* against

Table 3.2. Antibacterial activity of ethanolic *Zingiber officinale* extract against *Escherichia coli* isolated from cutaneous leishmaniasis lesions

Concentration ($\mu\text{l/ml}$)	Trial 1 (mm)	Trial 2 (mm)	Trial 3 (mm)	Mean $\pm$ SD (mm)	P value	Lower	Upper
Negative control	0.0	0.0	0.0	0.00 ± 0.00	NA	—	—
Positive control	6.0	5.4	4.0	5.13 ± 1.02	0.0131	2.5838	7.6829
250	19.0	19.7	15.0	17.90 ± 2.53	0.0066	11.601	24.199
500	19.7	20.4	19.0	19.70 ± 0.70	0.0004	17.961	21.439
750	21.0	21.0	20.5	20.83 ± 0.29	0.0001	20.116	21.55
1000	21.3	22.3	21.0	21.53 ± 0.68	0.0003	19.842	23.224

3.3.1 Concentration-Dependent Inhibition of *Escherichia coli* by *Zingiber officinale*

The concentration-dependent response of *E. coli* to the ethanolic extract of *Z. officinale* is illustrated in Figure 3.4. The mean inhibition zone increased progressively from 17.90 ± 2.53 mm at 250 $\mu\text{l/ml}$ to 19.70 ± 0.70 mm at 500 $\mu\text{l/ml}$. At 750 $\mu\text{l/ml}$,

the mean inhibition zone increased further to 20.83 ± 0.29 mm, reaching 21.53 ± 0.68 mm at 1000 $\mu\text{l/ml}$. The relatively small variation among the three replicates at the higher concentrations, particularly at 750 $\mu\text{l/ml}$, indicates consistency of the observed inhibitory response under the experimental conditions. Overall, the findings

demonstrate an increasing antibacterial effect of the ginger extract with increasing concentration, with the maximum mean inhibition recorded at 1000 µl/ml.

3.4 Antibacterial Activity of Ethanolic Extract of *Zingiber officinale* Against *Staphylococcus aureus*

The antibacterial activity of ethanolic *Zingiber officinale* extract against *Staphylococcus aureus* isolated from CL lesions was evaluated at four concentrations using the agar well diffusion method (Figure 3.3). The negative control showed no inhibition, with a mean inhibition zone of 0.00 ± 0.00 mm, confirming the absence of detectable antibacterial activity from the solvent alone. The positive control produced a mean inhibition zone of 18.57 ± 0.60 mm. The ginger extract demonstrated substantial antibacterial activity

against *S. aureus* at all tested concentrations. At 250 µl/ml, inhibition zones of 22.4, 23.1, and 23.0 mm were observed in the three trials, producing a mean inhibition zone of 22.83 ± 0.38 mm. At 500 µl/ml, the recorded inhibition zones were 24.6, 24.1, and 24.7 mm, giving a mean of 24.47 ± 0.32 mm. At 750 µl/ml, inhibition zones of 25.6, 27.0, and 26.0 mm were observed, resulting in a mean inhibition zone of 26.20 ± 0.72 mm. The maximum inhibition was recorded at 1000 µl/ml, with individual inhibition zones of 26.6, 28.0, and 28.2 mm and a mean of 27.60 ± 0.87 mm. The inhibition zone increased progressively with increasing extract concentration. The reported p-values at all tested concentrations were below 0.05. Notably, the mean inhibition zones produced by the ginger extract were greater than that of the positive control at each tested concentration (Table 3.3).

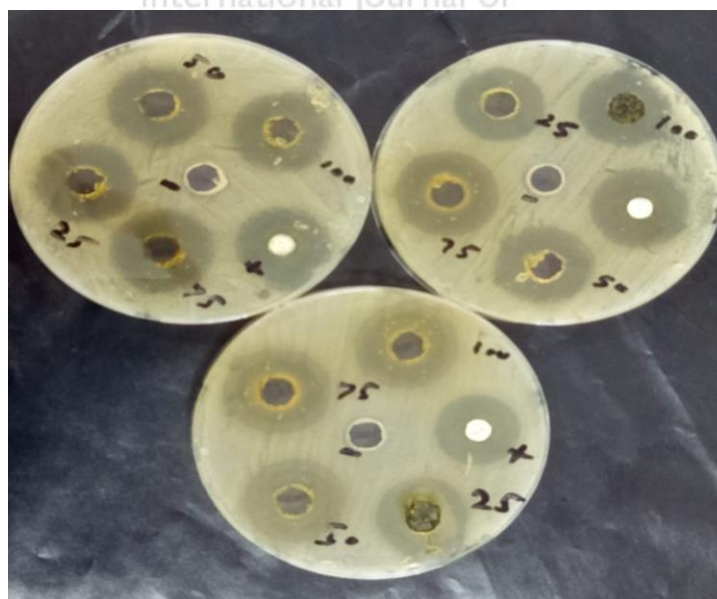


Figure 3.3. Antibacterial activity of *Zingiber officinale* against *Staphylococcus aureus*

Table 3.3. Antibacterial activity of ethanolic *Zingiber officinale* extract against *Staphylococcus aureus*

Concentration (µl/ml)	Trial 1 (mm)	Trial 2 (mm)	Trial 3 (mm)	Mean $\pm$ SD (mm)	P value	Lower	Upper
Negative control	0.0	0.0	0.0	0.00 ± 0.00	NA	—	—
Positive control	19.2	18.5	18.0	18.57 ± 0.60	0.0004	17.069	20.064
250	22.4	23.1	23.0	22.83 ± 0.38	0.0001	21.893	23.774
500	24.6	24.1	24.7	24.47 ± 0.32	0.0001	23.668	25.265

750	25.6	27.0	26.0	26.20 ± 0.72	0.0003	24.409	27.991
1000	26.6	28.0	28.2	27.60 ± 0.87	0.0003	25.434	29.766

3.4.1 Concentration-Dependent Inhibition of *Staphylococcus aureus* by *Zingiber officinale*

A clear concentration-dependent antibacterial response was observed for the ethanolic ginger extract against *S. aureus*. The mean inhibition zone increased from 22.83 ± 0.38 mm at 250 µl/ml to 24.47 ± 0.32 mm at 500 µl/ml and further to 26.20 ± 0.72 mm at 750 µl/ml. At 1000 µl/ml, the inhibition zone reached a maximum mean value of 27.60 ± 0.87 mm. The increase was more pronounced between 250 and 750 µl/ml, whereas the increase between 750 and 1000 µl/ml was comparatively smaller. This pattern suggests that the inhibitory response increased with concentration but began to show a relatively smaller increment at the highest concentration tested. The ginger extract produced larger inhibition zones than the positive control at all four concentrations. The maximum mean inhibition zone produced by the extract, 27.60 mm, was approximately 1.5 times the mean inhibition zone produced by the positive control (18.57 mm) under the conditions of this experiment.

3.5 Antibacterial Activity of Ethanolic Extract of *Lawsonia inermis* Against *Staphylococcus aureus*

The antibacterial activity of ethanolic *Lawsonia inermis* extract against *Staphylococcus aureus* isolated from CL lesions was assessed at four concentrations (Figure 3.4). The negative control produced no detectable inhibition, with a mean inhibition zone of 0.00 ± 0.00 mm. The positive control produced a mean inhibition zone of 8.47 ± 0.50 mm. The ethanolic *L. inermis* extract demonstrated increasing antibacterial activity with increasing concentration. At 250 µl/ml, the inhibition zones recorded in the three trials were 15.6, 17.0, and 17.0 mm, resulting in a mean inhibition zone of 16.53 ± 0.81 mm. At 500 µl/ml, inhibition zones of 16.6, 18.0, and 17.2 mm were recorded, giving a mean of 17.27 ± 0.70 mm. At 750 µl/ml, the recorded inhibition zones were 19.0, 20.0, and 20.7 mm, corresponding to a mean inhibition zone of 19.90 ± 0.85 mm. The highest antibacterial activity was observed at 1000 µl/ml, where inhibition zones of 21.0, 23.0, and 22.0 mm were recorded, resulting in a mean inhibition zone of 22.00 ± 1.00 mm. The reported p-values for all tested concentrations were below 0.05. The progressive increase in inhibition-zone diameter across the concentration range indicates a concentration-dependent inhibitory effect of the ethanolic *L. inermis* extract against *S. aureus* (Table 3.4).



Figure 3.4. Antibacterial Activity of Ethanolic Extract of *Lawsonia inermis* against *Staphylococcus aureus*

Table 3.4. Antibacterial activity of ethanolic *Lawsonia inermis* extract against *Staphylococcus aureus*

Concentration (µl/ml)	Trial 1 (mm)	Trial 2 (mm)	Trial 3 (mm)	Mean $\pm$ SD (mm)	P value	Lower	Upper
Negative control	0.0	0.0	0.0	0.00 $\pm$ 0.00	NA	—	—
Positive control	8.0	9.0	8.4	8.47 $\pm$ 0.50	0.0012	7.2163	9.717
250	15.6	17.0	17.0	16.53 $\pm$ 0.81	0.0008	14.525	18.541
500	16.6	18.0	17.2	17.27 $\pm$ 0.70	0.0006	15.522	19.011
750	19.0	20.0	20.7	19.90 $\pm$ 0.85	0.0006	17.778	22.022
1000	21.0	23.0	22.0	22.00 $\pm$ 1.00	0.0007	19.516	24.484

3.5.1 Concentration-Dependent Inhibition of *Staphylococcus aureus* by *Lawsonia inermis*

The concentration-response analysis showed a progressive increase in the antibacterial activity of the ethanolic *L. inermis* extract against *S. aureus*. The mean inhibition zone increased from 16.53 $\pm$ 0.81 mm at 250 µl/ml to 17.27 $\pm$ 0.70 mm at 500 µl/ml, followed by 19.90 $\pm$ 0.85 mm at 750 µl/ml and 22.00 $\pm$ 1.00 mm at 1000 µl/ml. The difference between the lowest and highest concentrations was 5.47 mm, demonstrating a measurable increase in inhibitory activity across the tested concentration range. The extract produced inhibition zones greater than those observed with positive control at every concentration tested. The highest inhibition was recorded at 1000 µl/ml. The relatively narrow variation among replicate measurements indicates a consistent antibacterial response across the

experimental trials. Overall, the results demonstrate that the ethanolic extract of *L. inermis* inhibited the growth of *S. aureus*, with the magnitude of inhibition increasing as the extract concentration increased.

3.6 General Pattern of Antibacterial Activity

The results demonstrated antibacterial activity of both plant extracts against the two bacterial isolates recovered from CL lesions. Both *Lawsonia inermis* and *Zingiber officinale* produced measurable inhibition zones against *E. coli* and *S. aureus*, whereas the negative control produced no inhibition. For *E. coli*, the mean inhibition produced by *L. inermis* increased from 12.67 $\pm$ 1.15 mm at 250 µl/ml to 21.77 $\pm$ 2.89 mm at 1000 µl/ml. In comparison, *Z. officinale* produced mean inhibition zones ranging from 17.90 $\pm$ 2.53 mm to 21.53 $\pm$ 0.68 mm over the same

concentration range. Against *S. aureus*, *Z. officinale* produced mean inhibition zones ranging from 22.83 ± 0.38 mm at 250 μ l/ml to 27.60 ± 0.87 mm at 1000 μ l/ml, while *L. inermis* produced mean inhibition zones ranging from 16.53 ± 0.81 mm to 22.00 ± 1.00 mm. Across both bacterial isolates, the inhibition-zone diameters generally increased with increasing extract concentration. The greatest inhibition observed in the study was produced by the ethanolic extract of *Z. officinale* against *S. aureus* at 1000 μ l/ml, with a mean inhibition zone of 27.60 ± 0.87 mm. These findings demonstrate measurable and concentration-dependent antibacterial activity of both plant extracts against bacterial isolates recovered from CL lesions under the conditions of the present study.

DISCUSSION

The recovery of *Escherichia coli* and *Staphylococcus aureus* from wound swabs collected from patients with cutaneous leishmaniasis (CL) indicates the presence of secondary bacterial colonization or infection within the examined lesions. This finding is consistent with previous studies reporting bacterial involvement in CL wounds from different endemic settings (Sadeghian *et al.*, 2013; Al-Bayati *et al.*, 2010; Mokni *et al.*, 2013). The ulcerative and exposed nature of CL lesions, particularly when lesions persist for prolonged periods, may provide favorable conditions for the establishment and persistence of bacterial populations. Delayed wound healing can further extend the period during which lesions remain susceptible to bacterial colonization (Bittencourt *et al.*, 2017; Teles *et al.*, 2017). The detection of *S. aureus* is particularly relevant because this organism is commonly associated with the human skin microbiota and is frequently implicated in secondary infections of wounds. In contrast, *E. coli* is primarily an enteric organism and its recovery from open skin lesions may reflect contamination or colonization associated with environmental exposure and hygiene conditions. Such exposure may be particularly relevant in rural and resource-limited settings where CL represents

an important public-health problem (Hussain *et al.*, 2017; Khan *et al.*, 2016). The presence of secondary bacterial organisms in CL lesions is therefore of clinical importance because bacterial involvement may complicate wound management and potentially influence the healing process. Previous investigations have suggested that secondary bacterial infection of CL lesions may delay epithelialization and prolong wound healing. Bacterial involvement has also been reported to potentially influence the response to antileishmanial treatment, including therapy with glucantime (Sadeghian *et al.*, 2011). Consequently, the identification of bacterial organisms associated with CL lesions is important not only for understanding the microbiological characteristics of the wounds but also for considering appropriate strategies for their management.

The present study demonstrated that the ethanolic extract of *Lawsonia inermis* exhibited antibacterial activity against *E. coli* isolated from CL lesions. The inhibition zones increased progressively with increasing extract concentration, from 12.67 ± 1.15 mm at 250 μ l/ml to 21.77 ± 2.89 mm at 1000 μ l/ml. This concentration-dependent response indicates that increasing the concentration of the crude ethanolic extract was associated with greater inhibition of *E. coli* growth under the experimental conditions. The observed activity is consistent with previous investigations of the antimicrobial properties of *L. inermis*. The antibacterial effects of henna have been associated with several phytochemical constituents, particularly lawsone (2-hydroxy-1,4-naphthoquinone), together with tannins, flavonoids, and coumarins. Lawsone is a biologically active naphthoquinone that has been proposed to contribute to antimicrobial activity through interactions with cellular components, while other phenolic compounds may additionally affect bacterial membranes and enzymes (Dash *et al.*, 2025). Previous studies have also reported inhibitory effects of henna extracts against both *E. coli* and *S. aureus*, supporting the antibacterial

potential observed in the present study (Singh *et al.*, 2015).

The progressive increase in inhibition with increasing concentration observed in the present investigation is biologically plausible because higher concentrations of the crude extract provide a greater quantity of potentially active phytochemicals available to interact with bacterial cells. However, because the present study evaluated a crude extract rather than individual purified compounds, the contribution of specific constituents to the observed inhibition cannot be established directly.

The ethanolic extract of *L. inermis* also demonstrated considerable antibacterial activity against *S. aureus*. The mean inhibition zone increased from 16.53 ± 0.81 mm at 250 $\mu\text{l/ml}$ to 22.00 ± 1.00 mm at 1000 $\mu\text{l/ml}$, demonstrating a concentration-dependent inhibitory response. The inhibition observed at the tested concentrations was greater than that produced by the positive control under the experimental conditions. The activity against *S. aureus* is consistent with reports describing the antibacterial properties of henna extracts. The phytochemical constituents of *L. inermis*, particularly lawsone and other phenolic compounds, have been proposed to contribute to antimicrobial activity through multiple interactions with bacterial cellular components (Dash *et al.*, 2025). Singh *et al.* (2015) similarly reported antibacterial activity of henna-derived preparations against bacterial species including *S. aureus*. The comparatively greater inhibition of *S. aureus* than *E. coli* at corresponding concentrations may be related, at least in part, to differences in bacterial cell-envelope structure. Gram-positive bacteria possess a thick peptidoglycan layer but lack the outer membrane characteristic of Gram-negative bacteria. This difference may influence the interaction and diffusion of certain plant-derived antimicrobial compounds into bacterial cells. Similar patterns of greater susceptibility of Gram-positive bacteria to some crude plant extracts have been described in the medicinal-plant literature (Parekh & Chanda, 2017).

The ethanolic extract of *Zingiber officinale* demonstrated marked antibacterial activity against *E. coli*. The mean inhibition zone increased from 17.90 ± 2.53 mm at 250 $\mu\text{l/ml}$ to 21.53 ± 0.68 mm at 1000 $\mu\text{l/ml}$, showing a progressive increase in inhibitory activity with increasing extract concentration. The antibacterial activity of ginger observed in this study is consistent with its established phytochemical profile. Ginger contains several biologically active compounds, particularly gingerols and shogaols, which have been associated with antimicrobial effects (Mao *et al.*, 2019). These compounds may affect bacterial cells through interactions with cellular membranes and other essential cellular processes. The presence of such bioactive constituents provides a plausible explanation for the inhibitory activity observed against *E. coli* in the present investigation.

An important observation was that the ginger extract produced larger inhibition zones than the positive control at each of the tested extract concentrations. At 1000 $\mu\text{l/ml}$, the mean inhibition zone reached 21.53 ± 0.68 mm, compared with 5.13 ± 1.02 mm for the positive control. Although inhibition-zone diameters provide useful comparative information within the experimental system, this finding should not be interpreted as demonstrating that the crude extract is therapeutically superior to the antibiotic. Differences in diffusion characteristics, concentration, formulation, and assay conditions can influence inhibition-zone measurements.

The strongest antibacterial response observed in the present study was produced by the ethanolic extract of *Z. officinale* against *S. aureus*. The mean inhibition zone increased from 22.83 ± 0.38 mm at 250 $\mu\text{l/ml}$ to 27.60 ± 0.87 mm at 1000 $\mu\text{l/ml}$. The response was consistently greater than that observed with the positive control, which produced a mean inhibition zone of 18.57 ± 0.60 mm. The concentration-dependent increase in inhibition suggests that the antibacterial activity of the ginger extract was enhanced as the amount of extract applied to the bacterial culture increased. A comparatively smaller increase was observed

between 750 µl/ml and 1000 µl/ml than across some of the lower concentration intervals, suggesting that the inhibitory response may begin to approach a plateau at higher concentrations. Nevertheless, the maximum inhibition was recorded at 1000 µl/ml. The observed activity is consistent with the antimicrobial properties attributed to ginger-derived bioactive compounds. Gingerols and shogaols are important phenolic constituents of ginger and have been investigated for their effects on bacterial growth and cellular processes (Mao *et al.*, 2019). The substantial inhibition of *S. aureus* observed in the present study therefore supports the potential of the crude ethanolic ginger extract as a source of antibacterial activity against this wound-associated organism.

Comparison of the two plant extracts demonstrated differences in their antibacterial activity against the bacterial isolates examined in this study. In general, *Z. officinale* produced larger inhibition zones than *L. inermis*, particularly against *S. aureus*. At the highest concentration tested (1000 µl/ml), ginger produced a mean inhibition zone of 27.60 ± 0.87 mm against *S. aureus*, whereas henna produced 22.00 ± 1.00 mm. Against *E. coli*, the two extracts produced relatively similar inhibition at the highest concentration. *L. inermis* produced a mean inhibition zone of 21.77 ± 2.89 mm, while *Z. officinale* produced 21.53 ± 0.68 mm. At lower concentrations, however, ginger generally produced greater inhibition against *E. coli* than henna. The differences in antibacterial activity may reflect differences in the phytochemical composition of the two plants. Ginger contains phenolic compounds such as gingerols and shogaols, whereas henna contains lawsone and other phenolic and related compounds associated with antimicrobial activity (Dash *et al.*, 2025; Mao *et al.*, 2019). Because the present investigation used crude extracts, the observed antibacterial activity is likely to represent the combined effects of multiple constituents rather than the activity of a single compound.

The greater inhibition observed against *S. aureus* for both plant extracts may also be related to

differences in the cell-envelope structures of Gram-positive and Gram-negative bacteria. Gram-negative bacteria such as *E. coli* possess an additional outer membrane that can act as a permeability barrier to certain antimicrobial compounds. In contrast, Gram-positive bacteria lack this outer membrane, potentially allowing greater access of some plant-derived compounds to cellular targets (Parekh & Chanda, 2017). Such structural differences may contribute to the susceptibility pattern observed in the present study.

Kohat district and the wider Khyber Pakhtunkhwa region have been reported as important endemic areas for cutaneous leishmaniasis, with recurrent transmission and a substantial burden of CL lesions (Khan *et al.*, 2023). Within this epidemiological context, bacterial colonization of chronic and open lesions represents an additional consideration in wound management. The antibacterial activity demonstrated by *L. inermis* and *Z. officinale* against bacterial isolates recovered directly from CL lesions provides experimental evidence supporting further investigation of these locally available medicinal plants. Their accessibility and traditional use provide a rationale for studying their potential role in supportive wound-care approaches, particularly in resource-limited settings (Breijyeh & Karaman, 2024). However, the present findings should be considered as *in vitro* evidence of antibacterial activity rather than evidence of clinical efficacy. The inhibition of bacterial growth in an agar diffusion assay does not by itself establish that application of these extracts to CL lesions would be safe, effective, or capable of improving wound healing in patients. Further investigations involving standardized extracts, toxicity assessment, appropriate formulation studies, and controlled clinical evaluation would be required before therapeutic applications could be considered.

5.1 Conclusion

This study provides a preliminary report on the antibacterial potential of ethanolic extracts of

Lawsonia inermis (henna) and *Zingiber officinale* (ginger) against *Escherichia coli* and *Staphylococcus aureus* isolated from cutaneous leishmaniasis (CL) lesions in Kohat. The findings demonstrated that both plant extracts exhibited concentration-dependent antibacterial activity against the tested bacterial isolates. The ethanolic extract of *Lawsonia inermis* showed progressive increases in inhibition zones with increasing concentrations against both *E. coli* and *S. aureus*. Similarly, *Zingiber officinale* demonstrated concentration-dependent antibacterial activity, with the greatest inhibitory effect observed against *S. aureus*. Overall, *Z. officinale* produced larger inhibition zones than *L. inermis*, particularly against *S. aureus*, whereas both extracts showed comparable inhibitory activity against *E. coli* at the highest tested concentration. The findings therefore support the objectives of the study and provide in vitro evidence that these locally available medicinal plants contain constituents with antibacterial activity against selected bacteria associated with CL wounds. However, the observed inhibition zones should be interpreted as evidence of in vitro antibacterial activity rather than proof of clinical efficacy or therapeutic superiority over conventional antibiotics. Further investigations involving phytochemical characterization, isolation of active compounds, determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC), toxicity assessment, formulation development, and in vivo studies are required before their potential clinical application in the management of secondary bacterial infections associated with CL lesions can be established.

5.2 Recommendations

Further research should focus on the isolation, identification, and characterization of the bioactive phytochemical compounds responsible for the antibacterial activity observed in the ethanolic extracts of *Lawsonia inermis* and *Zingiber officinale*.

Comparative studies should be conducted using different extraction methods and solvents to determine the most effective extraction procedure for obtaining the antibacterial constituents of these medicinal plants.

The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of both plant extracts should be determined against the bacterial isolates to provide a more comprehensive quantitative assessment of their antibacterial potency.

Further studies should evaluate the antibacterial activity of these extracts against a broader range of bacterial species that may be associated with secondary infection or colonization of cutaneous leishmaniasis lesions.

Appropriate in vitro and in vivo toxicity studies should be conducted to establish the safety and tissue compatibility of the extracts before their therapeutic application is considered.

In vivo studies should be undertaken to evaluate the antibacterial efficacy, wound-healing potential, and safety of *Lawsonia inermis* and *Zingiber officinale* extracts under biological conditions.

The development of suitable topical formulations, including creams, gels, or ointments containing standardized extracts of *Lawsonia inermis* and *Zingiber officinale*, should be explored as potential complementary approaches for managing bacterial complications associated with cutaneous leishmaniasis wounds.

Well-designed clinical studies should be conducted to determine the safety, tolerability, and therapeutic effectiveness of standardized preparations of these plant extracts in patients with cutaneous leishmaniasis-associated secondary bacterial infections.

Future studies should also establish appropriate standardization and quality-control parameters for the plant materials and extracts to ensure consistency and reproducibility of their antibacterial activity.

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