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Antibacterial Potential and Phytochemical Profiling of *Lawsonia Inermis* Ethanolic Extract against Skin-Associated Bacterial Pathogens

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ABSTRACT

Lawsonia inermis (henna) has long been recognized in traditional medicine for its bioactive nature, particularly its antimicrobial and anti-inflammatory effects. These properties highlight its potential as a natural therapeutic agent for wound infection control and healing, mitigating inflammation and soothing diverse skin conditions. Considering its medicinal attributes *L. inermis*, was used in the current study as a natural agent for the control of skin infections causing pathogens. Notable bacterial pathogens associated with skin diseases include *Staphylococcus aureus, Escherichia coli*, and *Pseudomonas aeruginosa*. The ethanolic extract of *L. inermis* extract exhibiting alkaloids, saponins, phenols, steroids, terpenoids, anthraquinones and tannins. The antibiotic susceptibility test revealed that most bacterial species were sensitive to commonly used antibiotics. Bacterial cultures revealed a dose dependent relationship using the agar well diffusion assay. Isolates of *S. aureus* displayed the largest zone (22 ± 0.81 mm) at a concentration of 1.5 mg/ml followed by *P. aeruginosa* (21 ± 0.81 mm) and *E. coli* (17 ± 1.63 mm). The *L. inermis* extract displayed MIC of 0.375 mg/ml for ATCC and clinical isolates of *E. coli* and *S. aureus*, and 0.188 mg/ml for *P. aeruginosa* strains, with MBC of 0.75 mg/ml recorded for all tested strains. These findings indicate that ethanolic *L. inermis* extract has promising in-vitro antibacterial activity against selected skin-associated bacterial pathogens and further suggests its potential as a natural agent for combating skin infections.

Keywords: *Antibacterial Agent, Antibiotic, Lawsonia Inermis, Phytochemicals, Pathogens, Skin Diseases.*

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INTRODUCTION

Skin infections are known to pose significant challenges in the healthcare sector due to their increasing anti-microbial resistance, prevalence, and associated morbidity[1]. Such infections may arise due to bacteria, viruses, parasites and fungi varying in severity, based on superficial skin infections to deep tissue infections which could potentially lead to life threatening conditions. Currently available treatments may lead to antimicrobial resistance. Delayed diagnosis may also result in severe clinical consequences. Skin infections and its outcomes are more common in individuals with compromised immune systems particularly those suffering from diabetes mellitus[2], HIV/AIDS[3] and those receiving immunosuppressive therapy, hence the need for effective therapeutic and preventive strategies increases. Bacterial skin infections are the most prevalent type of infections accounting for 5.7% to 8.4% of reported cases in a dermatology outpatient department[4] and are commonly caused by bacteria acquired from external sources or those residing on the normal skin microbiota[5].

There are several ways through which bacteria can enter the body, through disruptions in the skin barrier some of which are through a hair follicle, cut, abrasion or an open wound that is not treated properly[6]. Once established, they either remain localized in an area or progress to deeper skin tissue concerns. Many anatomical surfaces, particularly those near the nose, mouth, genitals, and anus, can support the growth of opportunistic bacteria by serving as important reservoirs[7]. Furthermore, sharing contaminated personal care objects like towels and brushes may also contribute to propagation of the infection from one person to the other[8].

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Lawsonia inermis, generally recognized as Henna, is attributed to the Lythraceae taxon and is classified as a shrub with 2- 6 meters in height[9]. It has been extensively used in traditional medicine for its diverse therapeutic properties. *L. inermis* has gained popularity among scientists because of its pharmacological significance such as antifungal, anti-inflammatory, antibacterial, and is also capable of accelerating wound healing[10,11]. These biological effects are due to the presence of rich phyto-constituents such as lawsone, flavonoids, steroids, alkaloids and tannins[12]. In addition, DPPH assays, displaying radical quenching activity is significantly suppressed by the plant displaying anti-oxidant properties highlighting its importance particularly in tissue repair.

Traditionally, different parts of the plant such as the roots, leaves and seeds[13], have been explored to manage several dermatological conditions such as fungal infections, nail disorders and other inflammatory conditions and for its use as a natural dye in cosmetic applications[14]. Several studies have reported the inhibition of *Enterobacter aerogenes, Escherichia coli, Staphylococcus aureus, Enterococcus faecalis, Pseudomonas aeruginosa,* and *Streptococcus* by the methanolic extracts of *L. inermis*[15]. Furthermore, aqueous, alcoholic and oily extracts of the plant have been proven to be comparable alternatives to commercially available antibiotics[16].

Traditional medicinal plants along with their natural bio active compounds have been explored for their broad-spectrum antimicrobial properties and chemical composition particularly as sources of novel anti-microbial agents. Despite its therapeutic potential, it remains challenging to incorporate *L inermis* into clinical applications due to concerns with standardization, formulation, and dosage. This study provides insight into analyzing the effect of *L. inermis* ethanolic extract against clinical skin pathogens, through phytochemical screening, antimicrobial activity followed by the quantitative analysis for the depiction of MIC and MBC.

METHODOLOGY

Sample Collection

Forty wound swabs of skin infection were taken from the clinically diagnosed inpatients and outpatients, aged 18-60 years at Ziauddin hospital, Karachi, Pakistan. The patients not administered with antibiotic treatment in the last 48 hours were included. The samples exhibiting no bacterial growth or mixed cultures were excluded from the study. Following culture identification, 9 *E. coli*, 10 *S. aureus*, and 6 *P. aeruginosa* were isolated from the wound swabs. Standard reference strains of American Type Culture Collection (ATCC) were used including *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, and *Staphylococcus aureus* ATCC 25923. To obtain fresh cultures, the strains were sub-cultured in TSB and placed under incubation for 24 hours at 37°C.

Sample Processing and Analysis

To collect wound sample, the area of wound infection was cleaned with normal saline. The swabs were then obtained from the wound bed of the infected patient by rubbing the sterile cotton swabs over the exudate-rich areas and placed in Amies transport medium. The samples were transferred to the laboratory on ice to reduce the growth of microorganisms and maintain sample integrity during transportation. The swab samples were inoculated on Blood agar, MacConkey agar and Mannitol salt agar, for the isolation and differentiation of pathogenic bacteria. These plates were incubated for 24 hours at 37°C. Automated microbial identification system was used to identify colonies on the basis of biochemical profiling, gram staining and colony morphology.

Preparation of the extract

Fresh leaves of *L. inermis* were procured from a local nursery in Karachi, Pakistan. The leaves were botanically identified and authenticated based on standard taxonomic and morphological characteristics. The extract was prepared following the Joyroy N et al, 2025[16] method, with slight modifications. The leaves were washed using distilled water and were then allowed to dry at room temperature until constant weight was achieved. Dried leaves were crushed into fine powder using a mechanical grinder and kept in a sealed container. Plant extract was prepared using absolute ethanol. A total of 10 g of the powdered leaves were macerated in 100 mL of ethanol using 1:10 (w/v) ratio and the suspension was agitated continuously at 500 rpm on an orbital shaker for 72 hours. After maceration, the mixture was filtered using Whatman filter paper (No. 1) and the filtrate was then concentrated in a rotary evaporator at 50° C under reduced pressure. The crude extract was dissolved in 99.99 % dimethyl sulfoxide and stored at 4°C to preserve stability. The schematic representation of extract preparation is illustrated in (Figure 1).

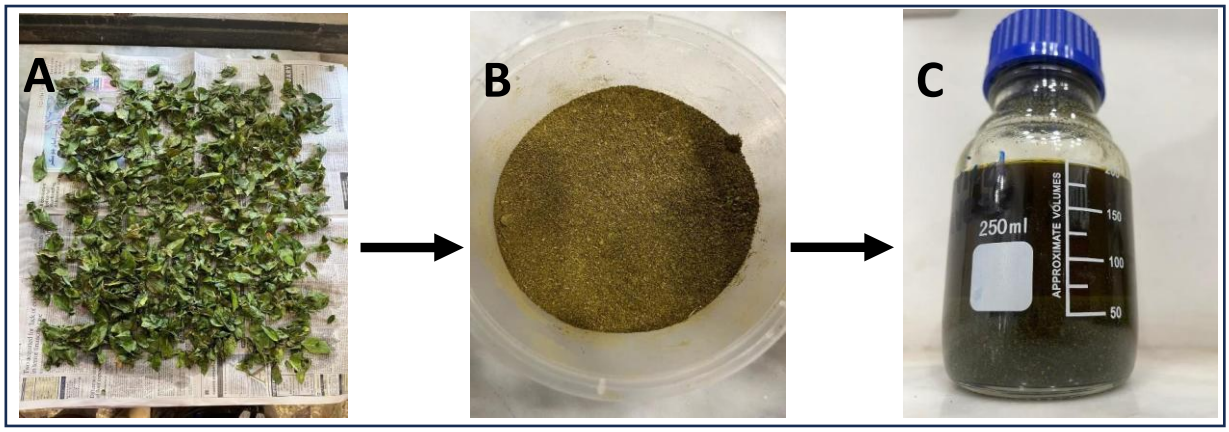


Figure-1 Schematic preparation of L. inermis extract, illustrating sequential steps: A: Dried plant leaves; B: Pulverized fine powder; C: Obtained extract after filtration through Whatman filter paper No 1.

Antibiotic Susceptibility Test

The capacity of bacterial cultures to be resistant against antibiotics was investigated by the Kirby-Bauer disk diffusion method, a technique approved by CLSI[17]**.** The bacterial suspensions were inoculated onto MHA plates using the sterile cotton swab, producing a standardized microbial lawn. Commercial antibiotic discs including multiple classes of antibiotics – Colistin (CS), Meropenem (MEM), Fosfomycin (FOS), Ciprofloxacin (CIP), Piperacillin and tazobactam (TZP), Nitrofurantoin (F), Trimethoprim-Sulfamethoxazole (S*T), Aztreonam (ATM), Ceftriaxone (CRO), Ampicillin (AMP), Cefalexin (CN), Cefixime (CMF), and Amikacin (AK) were chosen for the test.

The diameter of inhibition zones was measured in mm after incubation period under standard conditions. Zones were then compared with CLSI breakpoints corresponding to a particular antibiotic and bacterial isolate to interpret results and to classify isolates into the categories of susceptibility (S) or resistance (R).

Phytochemical Screening

Lawsonia inermis leaves were preliminarily analyzed to determine the presence of major groups of bioactive secondary metabolites. The yield % of ethanolic extract was determined using the following w/w% formula:

$$yield\ \% = \frac{weight\ of\ extract\ after\ drying}{weight\ of\ dried\ leaf\ powder} \times 100$$

The screening was done using the standard procedure outlined by Ndezo Bisso B et al, 2022[18] but slight adjustments were made to confirmatory tests for each class of compound screened.

a. Test for Alkaloids

The *L. inermis* extract was first acidified by dissolving it in dilute hydrochloric acid (HCl) and the alkaloids were protonated to form water soluble salts to screen for their presence. The mixture was then filtered to eliminate the plant debris and the filtrate was split for parallel testing.

Mayer's Test: A few drops of Mayer's were added to an aliquot of the acidic filtrate. When cream colored, yellowish or white precipitate was formed, it was taken as a positive sign for alkaloids.

Dragendorff's Test: Dragendorff's reagent was added to the filtrate, which is formed by dissolving bismuth subnitrate in dilute acid and adding a concentrated potassium iodide solution. The protonated tertiary amine group of alkaloids will form an insoluble orange-red precipitate with the bismuth complex. The presence of this characteristic precipitate gave additional confirmation of the presence of alkaloids in the extract.

b. Test for Saponins

To test for saponins, about 2 mL of the *L. inermis* extract was poured into a graduated test tube, followed by an equal volume of distilled water. The mixture was vortexed for 5 minutes and then left to stand still. The formation of a persistent and stable froth or foam which persisted for over 10 minutes was noted as the positive result for the presence of saponins.

c. Test for Phenols

Crude extract of *L. inermis* was prepared in a suitable solvent and 2% $FeCl_3$ solution was added dropwise. The color of the complex being blue-green, deep blue, violet or black,

was a positive indication of the presence of phenolic compounds.

d. Test for Tannins

Distilled water was added to the plant extract and filtered to get a clear aqueous solution. The filtrate was then treated with ferric chloride reagent. The presence of tannins was confirmed by the formation of blue-green, blue-black or brownish-green precipitate indicating positive result.

e. Test for Anthraquinones

The crude *L. inermis* extract was vigorously shaken with chloroform for about 5 minutes for the detection of free anthraquinones. It was then filtered and the filtrate was mixed with an equal volume of concentrated ammonia solution NH_3 (100%). The ammoniacal layer (lower aqueous phase) was carefully observed when the container was upright. A pink, violet or red coloration was noted in this lower layer and was taken as a positive response to the presence of free anthraquinones.

f. Test for Terpenoids

Chloroform was added to the extract and concentrated sulfuric acid (H_2SO_4) was carefully poured down the side of the test tube so that there is a clear interface between the two immiscible liquids. This reddish-brown discoloration at the boundary of the two layers was taken as a positive sign of the existence of terpenoids.

g. Test for Steroids

The crude extract was dissolved in (1:5 v/v) chloroform and used for screening of steroids. It was then subjected to acetic anhydride ($C_4H_6O_3$) followed by concentrated sulfuric acid (H_2SO_4) that was poured carefully along the inner wall of the test tube. The appearance of blue-green color at the acid/organic interface was considered as a positive result for steroids.

h. Test for Flavonoids

The extract was mixed with a few drops of sodium hydroxide (NaOH), forming a yellow color solution. Addition of dilute acid changes the color to colorless indicating presence of flavonoids.

Determination of Antimicrobial Activity

The antibacterial efficacy of the extract was assessed by the agar well diffusion assay, following the Nigussie D, et al, 2021[19] methods. Tryptic Soy Broth (TSB) was selected as the growth medium. The broth was prepared according to the recommendations of the manufacturer and autoclaved at 121°C, 15 psi for 20 minutes to avoid contamination. The test organisms were introduced into TSB and incubated at 37°C for 30 minutes to enter the early logarithmic growth phase. 30 µL of the bacterial inoculum was evenly applied to the surface of Tryptic Soy Agar (TSA) plates with the help of the sterile spreader. Wells with the diameter of 6 mm were aseptically punched out from the agar using sterile borer. 100 µL of each of the seven serial two-fold dilutions (1.5, 0.75, 0.375, 0.188, 0.094, 0.047, and 0.024 mg/mL) of the plant extract was added into each well, allowing to evaluate the antibacterial activity and determining the MIC range. The plates were then left for 30 minutes at ambient temperature and were incubated for 24 hours at 37°C under aerobic conditions. After incubation, the zone of inhibition surrounding each well was quantified in millimeters with a ruler.

Determination of Minimum Inhibitory Concentration

Broth micro dilution method was employed in a 96 well microtiter plate to determine the MIC and MBC of the extract. Fresh cultures were acquired by sub-culturing test bacteria in TSB at 37°C for 24 hours. Crude plant extract was dissolved in 99.99% dimethyl sulfoxide (DMSO). 4.5 mg/ml stock solution of the extract was then subjected to two-fold serial dilution in the microtiter plate, by maintaining a bacterial suspension of 5×10^5 CFU/mL resulting in the formation of the decreasing concentration gradient needed to determine MIC.

To perform the test, 100 µL of sub-cultured cell suspension was pipetted to the well, and 50 µL of two-fold serially diluted crude extract was added to wells 3 through 9, thus creating the concentration gradient of the extract. Column 1 was used as the negative control, containing TSB broth and crude extract but not the bacterial inoculum. Growth control (positive control) contained TSB broth and bacteria, but no extract and was placed in Column 2. Sterility control contained only TSB broth and was placed in the last well of the plate. All plates were incubated under aseptic conditions at 37°C for 24 hours. Turbidity in the well indicated microbial growth. The lowest concentration of the extract with no detectable growth was identified as MIC.

Determination of Minimum Bactericidal Concentration

Wells with no visible growth at and above MIC value were sub-cultured and incubated for 24 hours at 37°C. The lowest concentration

depicting no yield of bacteria on the plates was classified as MBC.

Statistical Significance

The results were performed as mean ± standard deviation in the replicate measurement (n=3). Two-way ANOVA was performed for the experimental analysis using SPSS software (IBM® SPSS®). Statistical significance was measured with a p value ($P<0.05$).

Ethical Considerations

This study was conducted following the ethical standards for research involving human participants. Approval was granted by the Ethical Review Committee of Ziauddin University (Reference No. ERC/ZUFESTM/0041). The wound swab samples were collected after informed consent was received from the patients.

RESULTS

Antibiotic Susceptibility Test

Following the antibiotic susceptibility test, isolates displayed varying resistance patterns. The bacterial strains exhibited resistance to more than one antibiotic class, indicating multidrug resistance. Among 33 bacterial isolates, 18 (54.54%) were sensitive and 15 (45.45%) were resistant. The susceptibility interpretations categorized as resistant and sensitive are presented in (Figure 2).

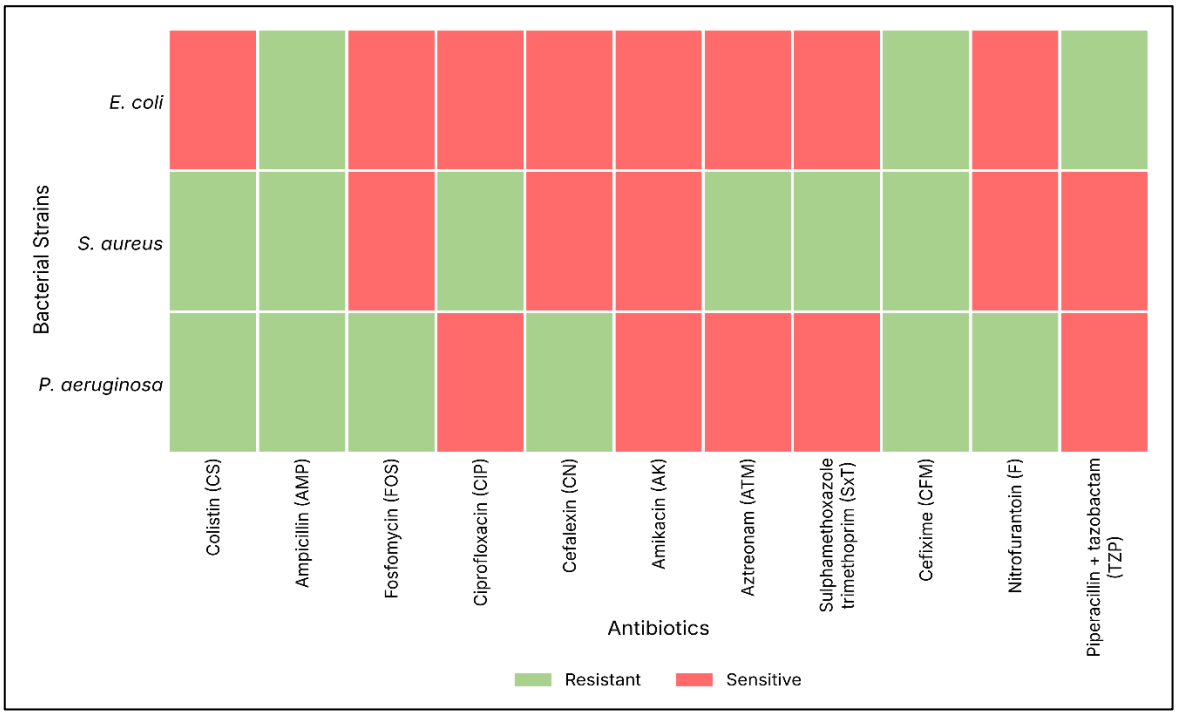


Figure-2 Antibiotic susceptibility test against bacterial isolates

Phytochemical Screening of Plant Extract

Preliminary qualitative analysis of the ethanolic extract of *L. inermis* leaves revealed the presence of several phytoconstituents, including steroids, tannins, phenolic compounds, saponins, anthraquinones, terpenoids, and alkaloids. However, the extract tested negative for flavonoids (Table 1). The percentage yield of *L. inermis* ethanolic extract was calculated to be 36% (w/w%).

Antimicrobial Assay

The ATCC reference strains and isolates recovered from skin infections were tested against ethanolic extract of *L. inermis* to

Table 1. Preliminary Screening of Phytochemicals in *L. inermis*

No.	Secondary metabolites	*L. inermis*
1	Tannins	+
2	Steroids	+
3	Alkaloids	+
4	Flavonoids	-
5	Saponins	+
6	Terpenoids	+
7	Phenols	+
8	Anthraquinones	+

**Key: Present (+), Absent (-)*

evaluate the antibacterial activity of the plant. The extract was highly effective at displaying prominent zones of inhibition, with a progressive increase in diameters of the inhibition zones with increasing concentrations demonstrating dose- dependent efficacy. Among clinical cultures, activity against *S. aureus* was the highest (22 mm ± 0.81) at 1.5 mg/ml (Figure 3a), followed *by P. aeruginosa* (21 mm ± 0.81), whereas *E. coli* exhibited lowest activity (17 mm ± 1.63) at the same concentration (Figure 3a). ATCC and clinical isolates of *S. aureus* and *E. coli* showed no activity at concentrations 0.188, 0.094, 0.047, and 0.024 mg/ml respectively, (Figure 4). Overall, all clinical isolates were less susceptible to the ethanolic extract in comparison to ATCC cultures across the concentration range. ATCC and clinical isolates of *E. coli* and *S. aureus* ceased to display any zones from 0.188 mg/ml onwards as concentration decreased, indicating loss of antibacterial activity. Whereas, *P. aeruginosa* continued to display prominent antibacterial activity at lower concentrations. The lowest zone by *P. aeruginosa* was observed by its clinical isolate at 0.047 mg/ml.

Furthermore, DMSO that served as a negative control showed no zones against any bacterial strain, confirming the antibacterial activity of *L. inermis.* The zones of inhibition of bacterial species at different concentrations are displayed in (Figure 3), while the concentration dependent antibacterial activity of ATCC cultures and clinical isolates is summarized in (Figure 4,5) and (Table 2).

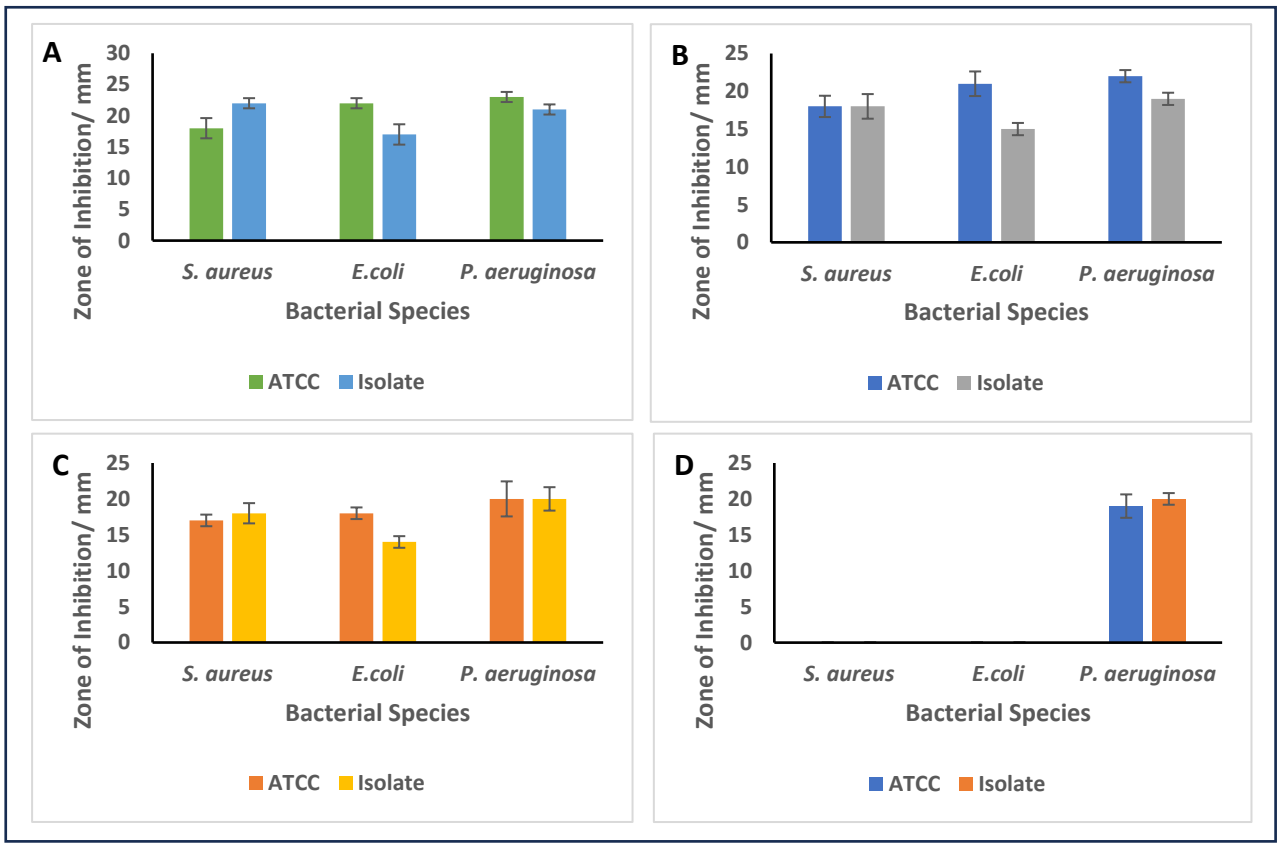


Figure-3 Zones of ATCC and Clinical Isolates at varying concentrations. A: Zones at 1.5 mg/ml; B: Zones at 0.75 mg/ml; C: Zones at 0.375 mg/ml; D: Zones at 0.188 mg/ml

Table 2. Zone of Inhibition of ATCC and Clinical Isolates using ethanolic *L. inermis* extract

Dilution	Concentration (mg/ml)	*S. aureus* (mm)		*E. coli* (mm)		*P. aeruginosa* (mm)	
		ATCC	Isolate	ATCC	Isolate	ATCC	Isolate
1:02	1.5	18 ± 1.62	22 ± 0.81	22 ± 0.81	17 ± 1.63	23 ± 0.81	21 ± 0.81
1:04	0.75	18 ± 1.41	18 ± 1.63	21 ± 1.63	15 ± 0.81	22 ± 0.81	19 ± 0.81
1:08	0.375	17 ± 0.81	18 ± 1.41	18 ± 0.81	14 ± 0.81	20 ± 2.44	20 ± 1.63
1:16	0.188	-	-	-	-	19 ± 1.63	20 ± 0.81

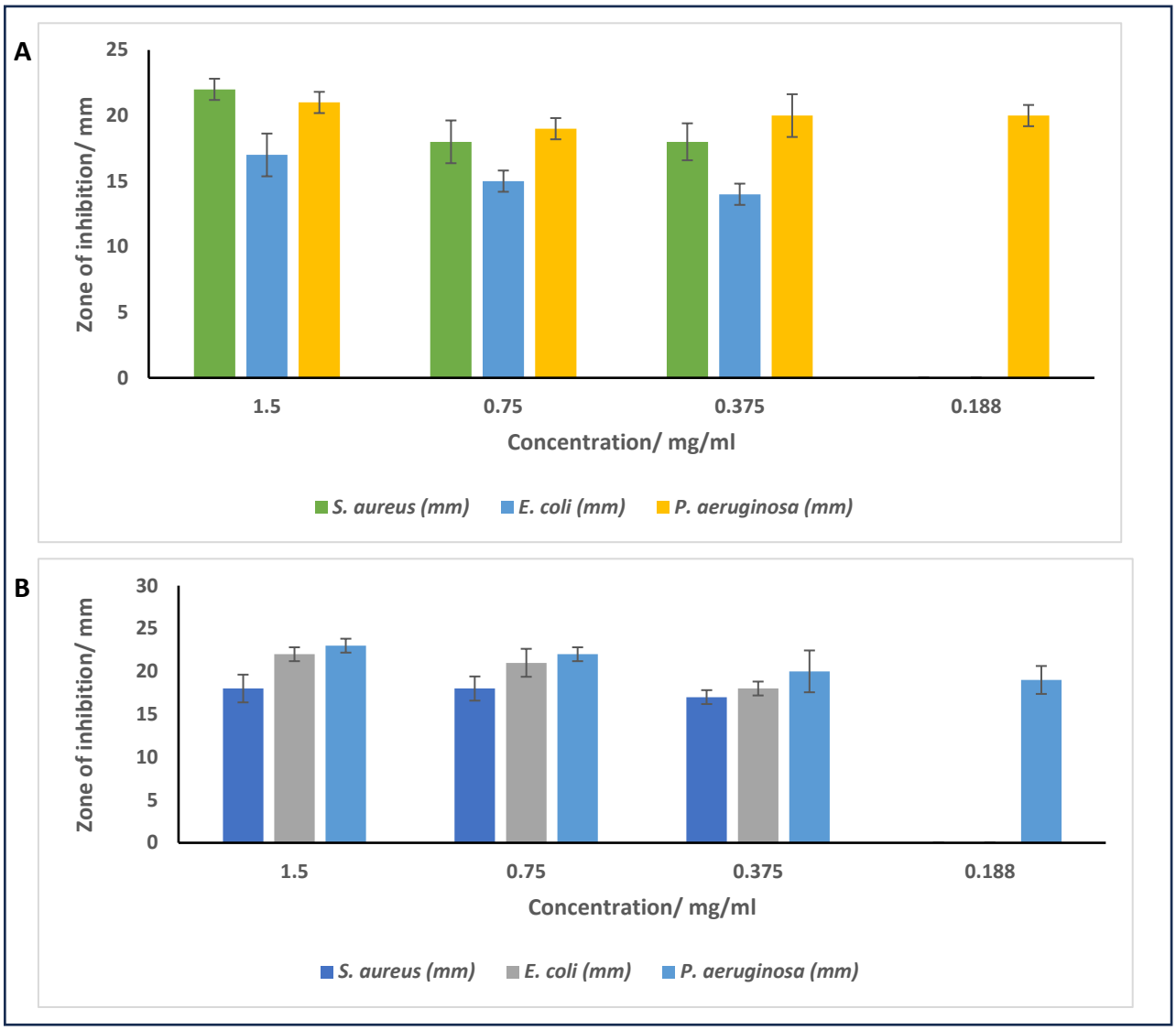


Figure-4 Comparative analysis between Clinical and ATCC cultures, A: Clinical Isolates; B: ATCC Cultures

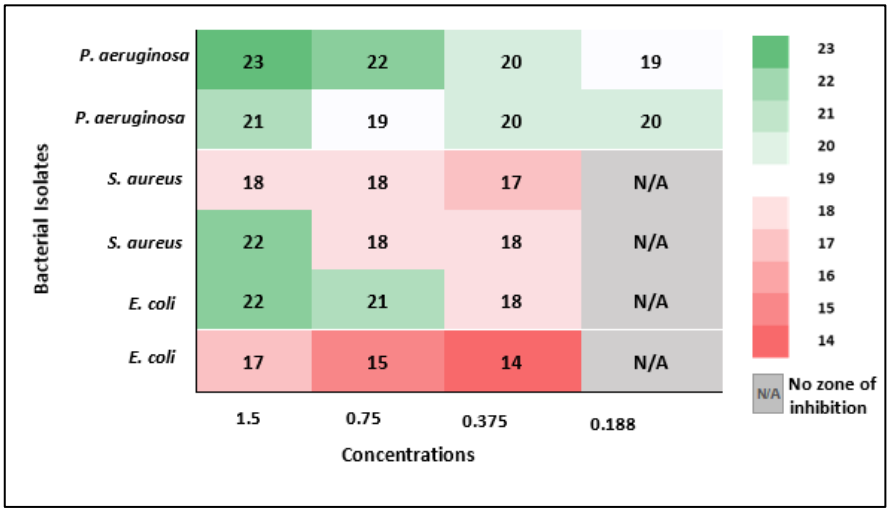


Figure 5- Zone of Inhibition of ATCC and clinical isolates using ethanolic L. inermis extract

Minimal Inhibitory and Bactericidal Concentration

The broth microdilution displayed dose dependent inhibitory growth of three tested bacterial strains against *L. inermis* extract within the concentration ranges of 1.5 to 0.024 mg/ml as displayed in Figure 6. On the plate, column 8, served as sterility control (TSB broth), whereas, column 1 served as a negative control (TSB broth and plant extract). The MIC value for ATCC and clinical isolated of *E. coli* and *S. aureus* were found to be 0.375 mg/ml demonstrating that apparent bacterial growth was suppressed at and above this concentration. However, *P. aeruginosa* displayed MIC at a lower concentration of 0.188 mg/ml indicating that a reduced concentration may work well at inhibiting the specie. The MBC was found to be consistent across all 3 bacterial species, at a value of 0.75 mg/ml, indicating that a lower concentration was required for the complete bactericidal activity.

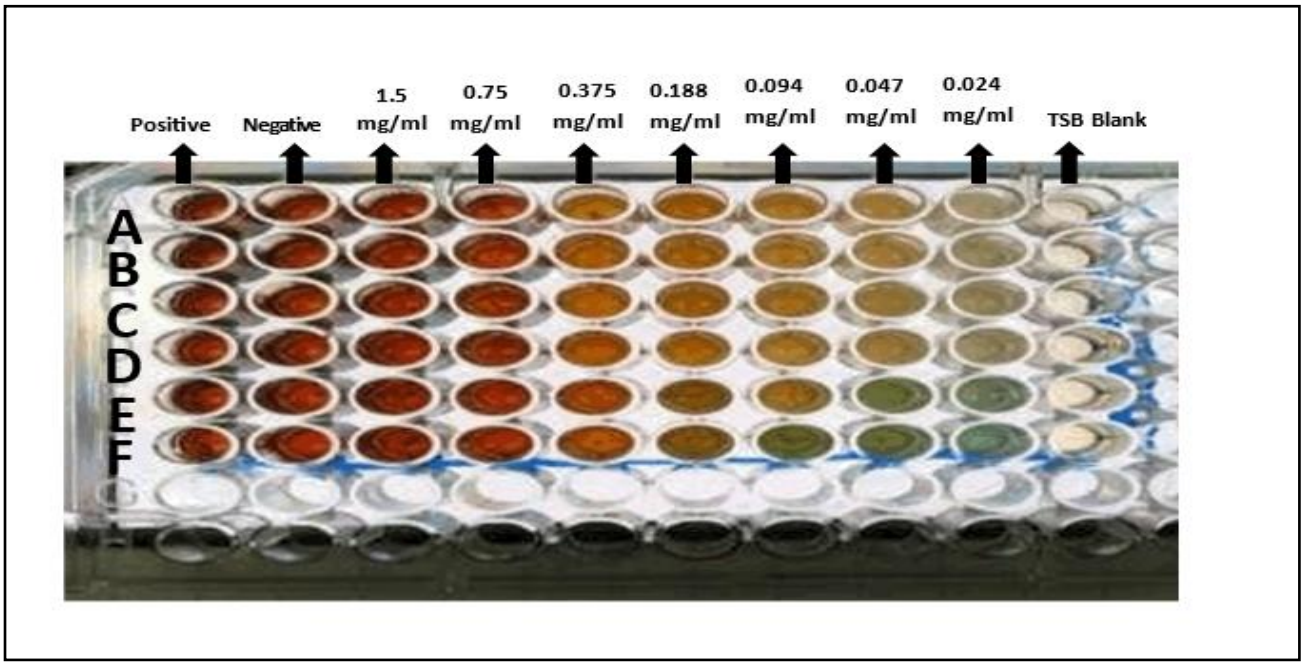


Figure-6 MIC of Skin pathogens. A: ATCC E. coli; B: Isolate E. coli; C: ATCC S. aureus; D: Isolate S. aureus; E: ATCC P. aeruginosa; F: Isolate P. aeruginosa

DISCUSSION

The increasing prevalence of antimicrobial resistance has diminished the effectiveness of antibiotics thus shifting the focus towards the advancement of alternative therapeutic strategies. Herbal remedies have gained acceptance for their medicinal properties over pharmaceutical drugs. They have been believed to have fewer side effects, lower costs, higher availability, and a promising safety profile[20]. Medicinal plants such as *L. inermis* contain bioactive compounds which exhibit anti-diabetic, anti-inflammatory, antibacterial, and anti-oxidative properties[21]. In the current study the ethanolic extraction of *L. inermis* demonstrates the presence of saponins, tannins, steroids, alkaloids, phenols, anthraquinones and terpenoids while, flavonoids were not detected.

Several studies have reported the use of different aqueous and organic solvents for the extraction process, such as ethanol, methanol, chloroform, petroleum ether, among which organic solvents have been proven to isolate the maximum quantity of bioactive compounds[22,23]. The yield generated from methanol and ethanol is approximately the same, due to similar polarities of the solvent[16], hence, the findings of preliminary phytochemical qualitative assessment by Nigussie et al., 2021[24,19] on methanolic extract, is consistent with our findings on ethanolic extract.

organs, possessing high quantities of polar constituents such as phenolic compounds, pigments, and flavonoids, whereas seeds function as storage organs containing neutral lipids, therefore, polar solvents effectively extract phyto-chemicals from leaves, whereas non-polar solvents are better at extraction from seeds[13].

Antibacterial activity displayed by the plant is due to the presence of several bioactive compounds present in the plant extract. The phyto-chemicals

work by inhibiting the synthesis and development of the cell along with its vital organelles[24]. Lawsone, a major constituent of *L. inermis* is known to display microbicidal and mycotic activity against several pathogens[25] driven by the presence of the ketone group on positions 1 and 4 of the lawsone molecule or due to the capability of the free hydroxyl group to merge with both carbohydrates and protein molecules in the bacterial cell wall leading to bacterial growth inhibition.

Decreasing the concentration of the extract also limited the bactericidal activity by the plant, demonstrating dose dependent effect. The antibacterial activity was observed up to 0.375 mg/ ml for *S. aureus* and *E. coli* with no inhibition detected at 0.188 mg/ml. This suggests that 0.188 mg/ ml lies below the minimum effective concentration that can inhibit bacterial growth. Although *P. aeruginosa* is generally regarded as a resistant pathogen due to its lower permeability outer membrane[26] followed by bio-film forming ability, which makes Gram-negative bacteria more resistant to antibiotics than Gram-positive bacteria. Our findings revealed ATCC and clinical isolates of *P. aeruginosa* to be susceptible even at the lowest concentration, exhibiting sustained antibacterial activity of the extract. These results were in agreement with[27], where multi-drug-resistant *P. aeruginosa* strains were effectively inhibited by ethanolic extracts of *L. inermis.* While this contradicts some research findings, as Gram-positive bacteria comprising of thick peptidoglycan layer (20-80 nm)[28], are more susceptible to antimicrobial agents compared to Gram-negative bacteria possessing thin peptidoglycan layers (7-8 nm), enhanced antibacterial activity against *P. aeruginosa* may be due to the synergistic effect of phytochemicals[29], extracted using ethanol such as phenolic compounds, tannins, and lawsone which have the potential to induce oxidative stress and disrupt microbial cell membranes[30] .

The minimum inhibitory concentration (MIC) is regarded as the lowest concentration at which the growth of microorganisms is inhibited after the incubation period, whereas the minimum bactericidal concentration (MBC) is the lowest concentration at which the microorganism undergoes microbial death after sub-culturing in antibiotic-free media. The lower value of MIC seen in the case *of P. aeruginosa* (0.188 mg/ml), suggests the constant susceptibility of the bacteria towards the extract, while the higher values of MIC for *S. aureus* and *E. coli* (0.375 mg/ml), and show that higher concentrations of the extract need to be used to achieve similar antimicrobial activities.

The present findings support potential use of ethanolic *L. inermis* extract as a strong antibacterial agent against broad spectrum of bacterial species as it was highly active against *P. aeruginosa*, remaining sensitive to the lowest tested concentration, while *S. aureus* and *E. coli* showed concentration-dependent inhibitory activity. These findings highlight the possibility of using *L. inermis* as a natural source of antibacterial compounds and require possible research on the isolation of secondary metabolites from the plant followed by exploring the synergic action with traditional antibiotics followed by using antibiotics as a positive control for agar well diffusion assay.

CONCLUSION

The current study demonstrates the in-vitro antibacterial activity by ethanolic *L. inermis* extract against ATCC cultures and clinical pathogens isolated from skin infections. The extract proved its dose dependent efficacy and bactericidal effect against all bacterial cultures. Notably, the largest zone of 22 mm ± 0.81 was exhibited by the isolate of *S. aureus* at 1.5 mg/ml, followed by clinical isolates of *P. aeruginosa* and *E. coli* at decreasing concentrations respectively. These in-vitro findings support and propose it as a natural source of antibacterial agents. The observed activity is likely due to the presence of bio-constituents such as phenols, tannins, saponins, anthraquinones, alkaloids and lawsone. Additionally, further studies are required to evaluate the activity of extract against standard antibiotics and the active constituents in the plant followed by their safety and cytotoxicity profiles before they can be introduced into clinical applications.

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Author Contributions
SF: Conception & Design, Data Collection, Data Analysis & Interpretation, Manuscript Writing, and Critical Revision.
SBZ: Conception & Design, Critical Revision, and Final approval.
TBF: Critical Revision and Final Approval.
MSK: Data Collection and Data Analysis & Interpretation.
MR: Conception & Design and Data Collection.
AY: Data Collection and Data Analysis & Interpretation.

Conflict of Interest
The authors declare that they have no conflict of interest.

Disclosure and Funding Source
There is no disclosure or funding source, as applicable.

Ethical Approval
The study was reviewed and approved by the Ethical Review Committee (ERC) of Ziauddin University, Karachi, Pakistan (Reference No.: ERC/ZUFESTM/0041).

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