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PHYTOCHEMICAL CONSTITUENTS WITH ANTIMICROBIAL ACTIVITY FROM ETHANOLIC EXTRACT OF *LAWSONIA INERMIS* LEAVES AND STEM EXTRACTS

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ABSTRACT

Medicinal plants have been used for decades against microbial diseases. Phytochemicals are used in the pharmacological industry as a natural substitute due to their potential biological activities, including antimicrobial activity. The present study extracted Henna leaves and stems using ethanol and n-hexane. These extracts were assessed for antimicrobial activity using the disc diffusion method and compared with the antibiotic gentamycin as a positive control. Henna extracts were investigated against six clinical bacterial strains, including two Gram-positive strains, *viz. Bacillus cereus* and *Staphylococcus aureus* and four Gram-negative *viz. E. coli, S. typhi, P. aeruginosa, A. baumannii*. The antifungal activity of henna was evaluated against four fungal strains *viz. Aspergillus niger, Aspergillus flavus, Aspergillus paracitus,* and *Rhizopus stolonifer.* The results of antibacterial activity revealed that the ethanol extract of henna leaves and stems reported maximum activity against S. aureus, while minimum activity was shown against *A. baumanni*. The ethanol extract of henna leaves and stems showed remarkable antifungal activity against *A. paracitus* and *R. stolonifer*, respectively. The High-performance liquid chromatography analysis revealed that the ethanol extract of henna was composed of ten phytochemicals. The results of this study showed that henna is potent with the antimicrobial component.

***Keywords*:** Antibiotic susceptibility, Antimicrobial activity, Disk diffusion assay, *Lawsonia inermis*

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1. INTRODUCTION

The therapeutic plants utilized in conventional medications give intriguing outcomes and are still a great unexplored source of creation and modification of new medicines to improve chemotherapy, which may overcome the problem of noxiousness and obstruction against diseases of commercially manufactured antibiotics (Spellberg et al. 2008; Batiha et al. 2023; Youl et al. 2024). The conventional therapeutic strategy, particularly the assumption of pharmaceutical plants in medicines, plays an essential role in covering the fundamental necessities of humans in emerging states (Elebeedy et al. 2022). Consequently, it is of incredible enthusiasm to screen these plants to favor their utilization in society medication and to uncover the vital values by separating and categorizing their elements (Mohiuddin, 2019). Analysts have recently concentrated on expanding human infections caused by microbes and fungi (Elizabeth et al. 2022). Pharmaceutical herbs symbolize an amusing wellspring of antibiotic operators. Since pathogens have created protection from numerous anti-infection agents. Using herbal concentrates and their derivatives, which work like a shield against parasites, has been extended (Hassine et al. 2014; Fatahi-Bafghi et al. 2022). Bacteria and fungi are considered as the root cause of severe infectious diseases. For example, *Staph. aureus, P. aeruginosa,* and *V. cholera* are bacterial species that cause skin diseases, food poisoning, and diarrhea (Baloch et al. 2013). An extraordinary number of therapeutic plants are being utilized to treat microbial diseases, especially in the provincial zones of developing countries where the customary society medicine remains a significant source to fix minor infirmities (El-Fiky et al. 1996; Manuja et al. 2020; Ghazy et al. 2022). With the beginning of 1930, synthetic drugs were replaced by herbal medication. For the first time in history, morphine isolated from plants was used as medicine, followed by cocaine and aspirin (Mahule et al. 2012). These days, the expression "Elective Medicine" has turned out to be exceptionally common in Western culture, it centers on utilizing the plants for a restorative purpose. However, the current convictions that drugs are consumed in holders or pills are the principal

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medications that we can believe and use. To be sure, the vast majority of these medicines we draw and utilize during our regular day-to-day existence originated from plants. Medicinal plants are used as crude materials for a significant part of the time to extract chemical constituents that can be used to formulate various medications (Moutawalli et al. 2023). Similarly, if there ought to be an event of intestinal medications, anticoagulants, against contamination operators, and threatening intestinal infection solutions, it comprises plant extracts. Furthermore, the potent components of Taxol, vincristine, and morphine are isolated from foxglove, periwinkle, yew, and opium poppy, respectively (Rasool Hassan 2012).

Phytochemicals are bioactive synthetic substances of plants. They are generally determined from bark, seeds, flowers, stems, fruits, leaves, roots, etc (Banerjee et al. 2008; Joyroy et al. 2025). They have been considered the reason for traditional medication practiced previously (Sofowora 1996) and presently. The parts of the plant are examined for phytochemicals that may be accessible; the proof of a phytochemical of interest may provoke its additional isolation, cleaning, and characterization. Later it tends to be utilized as a source of another pharmacological element (Revathi et al. 2025). Numerous rustic individuals in Cameroon depend on plant derivatives for essential human needs. In that regard, it seems helpful to get a logical reason for the conceivable utilization of natural medications in treating sicknesses, for example, cancer, infectious illnesses, and those related to oxidative harm (de Dieu Tamokou et al. 2013). The naturally isolating chemical compounds from plants, such as polyphenols, show fascinating restorative properties (Queen and Tollefsbol, 2010). Polyphenols are secondary metabolites of plants and are normally drawn in to protect against ultraviolet radiation or sickness caused by microbes. These polyphenols probably enhance the sharpness, astringency, concealing, flavor, smell, and oxidative strength of food. Towards the completion of the twentieth century, hygienic examinations and associated assessments firmly recommended that prolonged use of diets rich in herbal polyphenols offered some affirmation against the progression of cancerous cell developments, cardiac ailments, diabetes, osteoporosis, and neurodegenerative diseases (Graf et al. 2005). Polyphenols and other phytochemicals are the sources of growing consistent interest, considering their possible health benefits for human prosperity. The assessments focus on the current appreciation of the typical effects of dietary polyphenols and their importance in human health and disease (Arts and Hollman, 2005; Supian et al. 2022; Malaikozhundan et al. 2024). Polyphenols may control microorganisms, such as microbes and fungi, and may be utilized as natural food additives. Restorative and fragrant plant polyphenols accessible in various parts of the plant were effective in controlling foodborne microbes, for example, *Escherichia coli*, *Staphylococcus aureus,* and *Bacillus cereus*; human pathogenic parasites, for example, Candida albicans; and plant pathogenic forms, for example, Penicillium funiculosum (Elansary et al. 2020).

Lawsonia inermis L., ordinarily inferred as henna, has been placed in the Lythraceae family and is the sole species in this genus. It looks like a little fence, like a tree, 2–6 m in height, with needle-slanted offshoots. The leaves are outlined as smooth, inverted, sub-sessile, circularly formed, and altogether lanceolate, having disheartened veins distinguishable on the lower side of the leaves (Chaudhary et al. 2010). The ovary is divided into four parts, comprising an upright style. The tree delivers little, earthy colored natural products containing 32– 49 seeds (Kumar et al. 2005). It is prestigious worldwide because of its corrective use for explaining selective dynamic standards in the leaves. It contains a different assortment of bioactive particles. It is accepted to diminish the internal heat level in circumstances of high fever and give solid hair color. *L. inermis* is developed in different dry tropical and subtropical territories of North Africa, South Asia, South East Asia, and the Middle East (Chung et al. 2002). *L. inermis* leaves were tested for their antimicrobial potential and showed prominent antibacterial action against Gram-negative bacterial strains (Abulyazid et al. 2013).

Very nearly a hundred phytoconstituents, speaking to various classes, have been isolated from all parts of *L. inermis*. Phenolic composites, including coumarins, flavonoids, and naphthoquinones, are especially pervasive in *L. inermis* abstracts. This abundance of normally unique constituents suggests that henna has diversified its compound stockpile during the time to resist the extent of threats to which individuals were exposed (Phirke and Saha, 2013). A wide scope of organic activities has been credited to henna, including antimicrobial, anti-inflammatory, and anticancer properties based on its therapeutic properties (Semwal et al. 2014; Salma et al. 2024).

Here, we report the consequences of studies intended to assess the phytochemical constituents, anticancer, and antimicrobial activities of the chosen L. inermis restorative plant, with recently shown pharmacological activities. This plant was chosen based on recorded ethnobotanical information, proof of its widespread use, and neighborhood accessibility.

2. MATERIALS AND METHODS

2.1. Plant Materials

The stem and leaves of Henna were collected from Jinnah Garden, Lahore, Pakistan, in June 2019 and transferred to the Laboratory of Plant Biotechnology, IMBB, UOL. The samples were washed with distilled water and dried under shade for one week. The dried samples were crushed into a fine powder using an electric blender

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and preserved in airtight jars for further use.

2.2. Preparation of Plant Extract

Ethanol and n-hexane were used as solvents due to their remarkable antimicrobial properties. The cold maceration method was used for sample extraction, for which 30 g of each sample was dissolved in 250mL of ethanol and n-hexane. The mixture was shaken repeatedly for 72h at room temperature and filtered. The extracts were concentrated through a rotary evaporator at 40ºC at 60rpm. The concentrated extracts were preserved in airtight jars to avoid contamination until further analysis.

2.3. Antimicrobial Activity

***2.3.1. Bactericidal Action*:** The bactericidal action of ethanolic and n-hexane extracts of *L. inermis* was assessed against six bacterial strains, namely *Escherichia coli, Salmonella typhi, Staphylococcus aureus, Bacillus cereus, Pseudomonas aeruginosa, and Acinetobacter baumannii,* through the disc diffusion method. Bacterial inoculum was prepared from an overnight-grown bacterial culture to streak dried Muller-Hinton nutrient agar media plates. The bacterial strains were streaked on agar media with the help of sterilized swabs. The sterilized paper discs prepared from Whatman filter paper, having a size of 6mm, were soaked in ethanol, and the n-hexane extracts of Henna leaves and stems were firmly placed onto MH agar media, which was inoculated with clinical bacterial strains. Gentamycin was used as a positive control. The plates were incubated at aerobically at 37ºC for 24h. The test was repeated three times, and the diameter of the zone of inhibition was recorded in mm, and the mean values were determined for bactericidal action.

***2.3.2. Fungicidal Action*:** To evaluate the fungicidal action of ethanol and n-hexane extracts of henna leaves and stems, four fungal strains, *viz. Aspergillus niger, Aspergillus flavus, Aspergillus paracitusa,* and *Rhizopus stolonifer* were used. The Potato Dextrose Agar media was used to study fungi toxic activity of tested plants through the disc diffusion method. The fungal strains were streaked on air-dried agar media with the assistance of sterilized swabs. The paper discs were impregnated with crude extract and put on to the surface of fungal inoculated media plates and incubated for 72 h at 37ºC. The diameter of inhibition zones was recorded after 72h with a mm scale, and average values were calculated to estimate the fungicidal action of henna.

2.4. HPLC Profiling

The mobile phase comprises 1% aqueous acetic acid solution (solvent A) and acetonitrile (Solvent B), the stream rate was changed to 0.7mL/min, the column temperature was maintained at 28°C, and the infusion volume was set at 20µL. A gradient flow was performed by fluctuating the degree of solvent B to solvent A. The gradient elution was altered from 10 to 40% B directly for a range of 28 min, from 40 to 60% B in 39min, from 60 to 90% B in 50min. The mobile phase creation back to the initial condition (solvent B: solvent A: 10: 90) in 55 min besides, allowed to run for another 10 min, before the imbuement of another example. All out-examination period for each experiment was 65min. HPLC chromatograms were identified utilizing a photograph diode cluster UV finder at 270nm wavelength (272, 280, and 310nm) as indicated by the ingestion maxima of the dissected sample. Every complex was distinguished through its maintenance interval and via spiking with norms in similar conditions. The evaluation of the test was finished by the estimation of the coordinated pinnacle territory furthermore, the substance was determined utilizing the alignment bend by plotting top territory against a grouping of the particular norm test. The information was accounted for with a combined limit in triplicate.

3. RESULTS

3.1. Antibacterial Activity Henna

The stem and leaf extracts of henna showed remarkable antibacterial activity against all six bacterial strains, as shown in Fig. 1a, 1b, 1c, and 1d. The ethanol extracts showed higher antibacterial activity than the n-hexane extracts. The most sensitive bacteria towards the plant extracts were *S. aureus* and *P. aeruginosa*, while the most resistant bacterial strain was B. cereus, which showed the least zone of inhibition.

3.2. Antifungal Activity of Henna

The ethanol and n-hexane extracts of stem and leaves of henna showed noteworthy antifungal activity against four fungal strains *viz. A. niger, A. flavus, A. paracitus, and R. stolonifer* demonstrated in Fig. 2a, 2b, 2c and 2d. The ethanol extract showed maximum activity against *A. paracitus,* and minimum activity was reported against *A. flavus,* while n-hexane extract showed extreme activity against *R. stolonifer* and the least activity was stated against *A. paracitus.*

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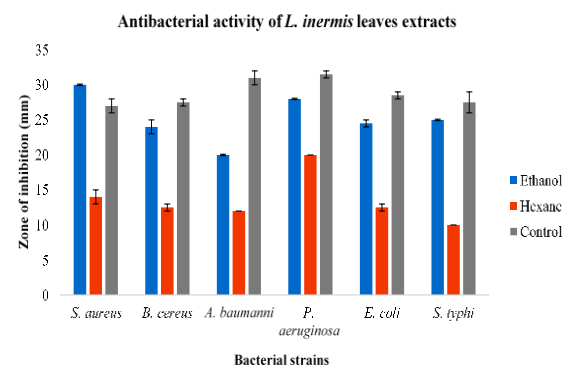

Fig. 1a: Antibacterial activity of *L. inermis* leaves extracts against different clinical bacterial strains.

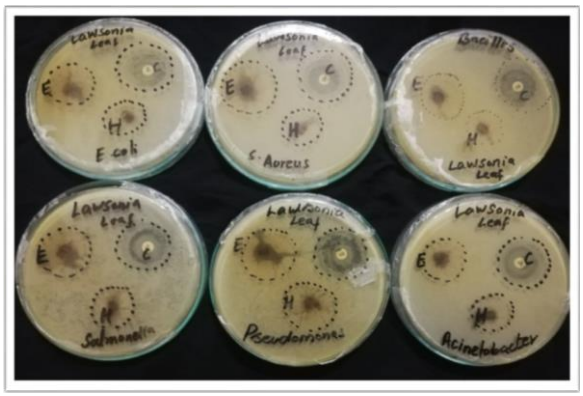

Fig. 1b: Zone of inhibition against six different bacterial strains by Alcoholic plant extract.

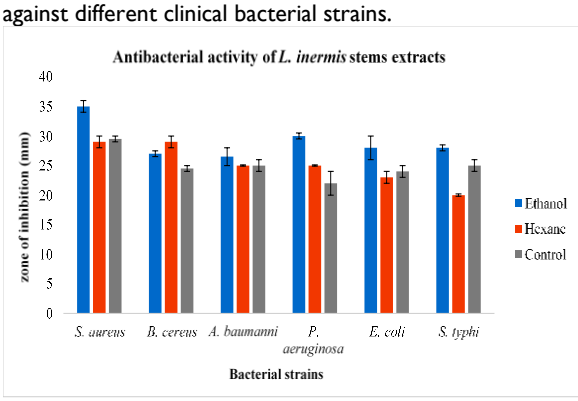

Fig. 1c: Antibacterial activity of *L. inermis* stems extracts against six different clinical bacterial strains.

Fig. 1d: Zone of inhibition against six different bacterial strains by *L. inermis* stems extracts.

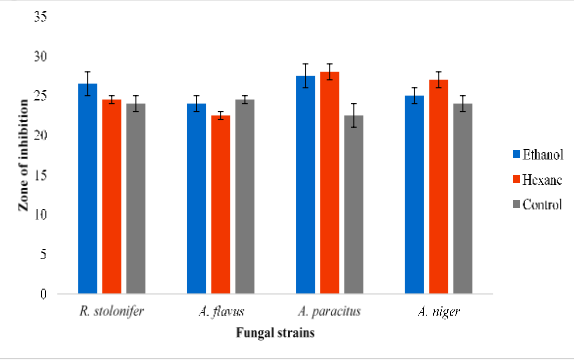

Fig. 2a: Antifungal activity of alcoholic extracts of *L. inermis* leaves against different fungal strains.

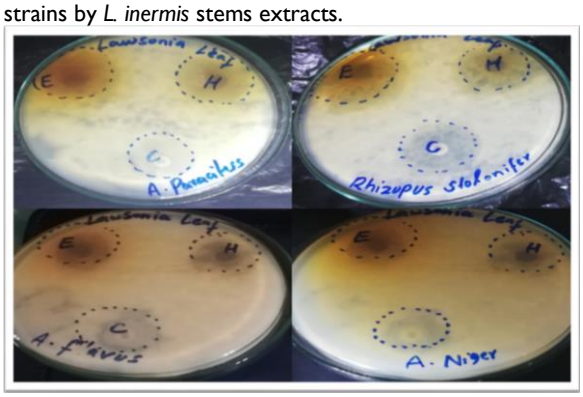

Fig. 2b: Antifungal activity of *L. inermis* leaves extracts against four fungal strains.

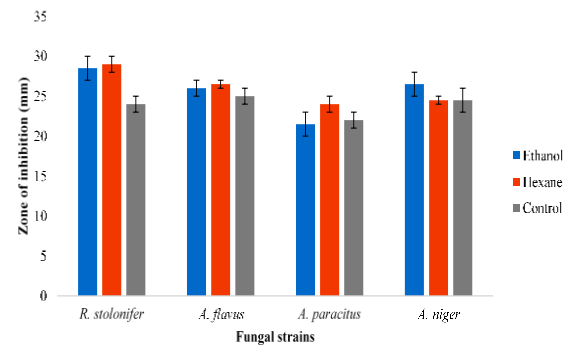

Fig. 2c: Antifungal activity of *L. inermis* stems extracts against different fungal strains.

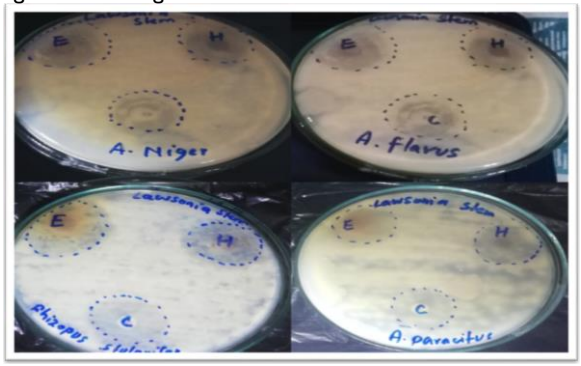

Fig. 2d: Antifungal activity of *L inermis* stems extracts against four fungal strains.

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Table 1: HPLC analysis of ethanol extracts of *L. inermis* leaves

Peak No.	Name	*L. inermis*
1	Ferulic acid	ND
2	Kaempferol	ND
3	Gallic acid	11.4 ppm
4	Benzoic acid	ND

ND = Not detected

3.3 HPLC Analysis

The phenolic acids present in the ethanol extracts of leaves of *L. inermis* were estimated by HPLC analysis. Four different standards were used to identify the phenolic acids in the tested plant samples. The results have revealed the presence of gallic acid in leaves of *L. inermis* (Table 1). The benzoic acid, kaempferol, and gallic acid were not determined in *L. inermis* leaf extracts.

4. DISCUSSION

Cancer and infections brought about by microorganisms and parasites are so far a real danger to the overall population, regardless of the epic progression in Western medicine. In this way, investigating new anticancer and antimicrobial operators from characteristic sources should be continued, remembering the ultimate objective to find novel, progressively effective, and increasingly reasonable prescriptions. The current investigation was led to contemplate the antibacterial movement of *Lawsonia inermis* utilized by Asian people groups to show those helpful properties (Salma et al. 2024). The antibacterial movement was communicated at different degrees with the movement being both strain and portion subordinate. Six microscopic organisms were utilized for antibacterial investigations and four strains for antifungal activity. Restorative plants are being utilized by the huge extent of the Asian populace. It has additionally been broadly watched and acknowledged that the restorative estimation of plants lies in the bioactive photo components present in the plants (Youl et al. 2024). In the current investigation, the outcomes were empowering, as the *Lawsonia inermis* seemed to contain substances that have antimicrobial and anticancer properties. The alcoholic extract of Lawsonia inermis leaves and stems was dynamic against six clinical bacterial and four fungal strains. The Phytochemical study affirmed the presence of phytocompounds in concentrates of the plant. These phytochemical constituents are an acceptable wellspring of antimicrobial and anticancer investigation (Malaikozhundan et al. 2024).

Our antimicrobial results of ethanolic and n-hexane extracts of *L. inermis* are in agreement with a previous study done by (Rahiman et al. 2013), who reported an excellent antibacterial effect of ethanol extract of *L. inermis* leaves against four bacterial strains, namely *S. aureus, P. aeruginosa, B. cereus,* and *E. coli.* Besides, it was previously demonstrated that petroleum ether and methanol extract of henna leaves showed growth-resistant results against both gram-positive bacteria *viz. B. cereus, S. aureus,* and Gram-negative bacteria *viz. K. pneumonia* and *E. coli* (Raja et al. 2013). Previous studies on the antimicrobial effect of *L. inermis* indicated remarkable antifungal activity of the methanolic extract of henna leaves against *Candida albicans,* as reported in the present study (Shinwari and Qaiser, 2011). A modern study on Asian pharmaceutical plants showed that L. inermis is among the plants which have antimicrobial activity i.e. antibacterial, antiviral, antimycotic, and antiparasitic activities against drug-resistant microorganisms (Babu and Subhasree, 2009). Furthermore, our antimicrobial activity result of henna was similar to a previous study done on petroleum ether, benzene, chloroform, acetone, ethanol, and aqueous extracts of L. inermis (Sharma and Sharma, 2011). The results of HPLC analyses have revealed the presence of gallic acid in leaves of *L. inermis*. The benzoic acid, kaempferol and gallic acid were not determined in *L. inermis* leaves extracts (Joyroy et al. 2025).

5. CONCLUSION

The present study was designed to investigate the phytochemical constituents, antimicrobial, and anticancer activities of organic extracts of *L. inermis.* A few phytochemicals were probably identified in the leaf extracts of henna. Without a doubt, further investigations are required to better comprehend the examined plants' total creation. *L. inermis* leaf extracts had antimicrobial effects against most microscopic organisms researched. The ethanolic extracts had higher antimicrobial activity than n-hexane against all microscopic organisms. The most delicate microscopic organisms were *A. paracitus* and *S. aureus*. The current outcomes will shape the reason for determining *L. inermis* species for additional examination in the likely revelation of new normal bioactive compounds. More examinations are expected to confirm the acquired exercises and to clarify the system of activity of the anticancer and antimicrobial action. Besides, further examinations focused on the confinement and structure explanation of further antiproliferative and antimicrobial constituents from these plant species ought to have been conducted. The results of HPLC analyses have revealed the presence of gallic acid in leaves of *L. inermis*. The benzoic acid, kaempferol, and gallic acid were not determined in *L. inermis* leaf extracts.

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Consent for Publication: All of the authors declare their consent for publication in this journal.

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