COMPARATIVE STUDY OF ANTIBACTERIAL ACTIVITY OF HERBAL EXTRACTS AGAINST MULTIDRUG RESISTANT BACTERIA

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ABSTRACT
The rapid emergence of multidrug resistant (MDR) bacteria has become a major global health concern due to the reduced effectiveness of conventional antibiotics. Medicinal plants are considered promising alternative sources of antimicrobial compounds because of their rich phytochemical constituents and traditional therapeutic importance. The present study aimed to comparatively evaluate the antibacterial activity of selected herbal extracts against MDR bacterial pathogens. Leaf extracts of *Azadirachta indica* (Neem), *Ocimum sanctum* (Tulsi), *Curcuma longa* (Turmeric), and bulb extract of *Allium sativum* (Garlic) were prepared using ethanolic extraction method. Antibacterial activity was assessed against MDR strains of *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae* using the agar well diffusion method. Zones of inhibition were measured after 24 h incubation at 37^0C. Among the tested extracts, garlic extract exhibited the highest antibacterial activity with inhibition zones ranging from 18–24 mm, followed by neem extract (15–21 mm), tulsi extract (12–18 mm), and turmeric extract (10–16 mm). *Staphylococcus aureus* was found to be the most susceptible organism, whereas *Pseudomonas aeruginosa* showed comparatively higher resistance. The study demonstrated significant variation in antibacterial efficacy among different herbal extracts. The findings suggest that medicinal plants possess considerable antibacterial potential against MDR pathogens and may serve as natural alternatives for the development of novel antimicrobial agents. Further studies involving purification and characterization of active phytoconstituents are recommended for future therapeutic applications.

Keywords: Herbal extracts, Multidrug resistant bacteria, Antibacterial activity, Medicinal plants, Zone of inhibition

2. INTRODUCTION

2.1 Background

Antibiotic resistance has emerged as one of the most serious global public health challenges of the 21st century. The extensive and irrational use of antibiotics in human medicine, veterinary practices, and agriculture has accelerated the development of resistant microbial strains. Multidrug resistant (MDR) bacteria are microorganisms that exhibit resistance to multiple classes of antibiotics, making infections difficult to treat and increasing morbidity, mortality, and healthcare costs. According to the World Health Organization (WHO), antimicrobial resistance occurs when microorganisms no longer respond to antimicrobial drugs, resulting in prolonged illness and increased risk of death.

MDR pathogens such as *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae* are increasingly associated with hospital-acquired and community-acquired infections. These resistant bacteria possess several defense mechanisms including enzymatic degradation of antibiotics, efflux pump systems, target site modification, and biofilm formation, which significantly reduce the effectiveness of conventional antimicrobial therapies. The rapid spread of MDR bacteria has created an urgent need for the discovery of novel and effective antimicrobial agents. In recent years, medicinal plants have gained considerable scientific attention as potential alternatives due to their diverse bioactive constituents and broad-spectrum antimicrobial properties.

2.2 Importance of Medicinal Plants

Medicinal plants have been used since ancient times in traditional systems of medicine such as Ayurveda, Siddha, Unani, and traditional Chinese medicine for the treatment of infectious diseases. Plant-derived remedies are considered valuable because they contain naturally occurring secondary metabolites including alkaloids, flavonoids, tannins, terpenoids, phenolics, and essential oils, which exhibit antimicrobial, antioxidant, and anti-inflammatory activities. These phytochemicals can inhibit microbial growth through multiple mechanisms such as disruption of cell membranes, inhibition of protein synthesis, interference with nucleic acid synthesis, and suppression of biofilm formation.

Compared to synthetic antibiotics, herbal medicines are generally considered safer, biodegradable, easily available, and cost-effective. Furthermore, medicinal plants may provide a reduced risk of adverse side effects and can serve as complementary or alternative therapeutic agents against resistant pathogens. Recent studies have demonstrated promising antibacterial activity of several herbal extracts against MDR bacterial strains, highlighting their importance in future antimicrobial drug development.

2.3 Selected Herbal Plants

In the present study, four commonly used medicinal plants were selected based on their traditional therapeutic applications and reported antimicrobial properties.

Neem (*Azadirachta indica*)

Neem is a well-known medicinal plant widely used in Ayurveda for its antibacterial, antifungal, antiviral, and anti-inflammatory properties. Its bioactive compounds such as azadirachtin, nimbin, and quercetin contribute to its strong antimicrobial activity.

Tulsi (*Ocimum sanctum*)

Tulsi, also known as holy basil, possesses significant medicinal importance due to the presence of eugenol, ursolic acid, and flavonoids. It has been traditionally used for respiratory disorders, skin infections, and microbial diseases because of its antibacterial and antioxidant properties.

Garlic (*Allium sativum*)

Garlic has long been recognized for its therapeutic and antimicrobial benefits. The sulfur-containing compound allicin is mainly responsible for its antibacterial activity against several Gram-positive and Gram-negative bacteria, including resistant strains.

Turmeric (*Curcuma longa*)

Turmeric is extensively used in traditional medicine owing to the presence of curcumin, a polyphenolic compound with potent antimicrobial, antioxidant, and anti-inflammatory activities. Turmeric extracts have shown inhibitory effects against various pathogenic microorganisms.

2.4 Problem Statement

The increasing prevalence of multidrug resistant bacteria and the declining effectiveness of conventional antibiotics have become major concerns in modern healthcare systems. Many infectious diseases are becoming difficult to manage due to antibiotic failure and the limited availability of new antimicrobial drugs. Therefore, there is a pressing need to explore alternative antimicrobial sources that are effective, economical, and safer for therapeutic use. Medicinal plants represent a promising reservoir of bioactive compounds; however, systematic scientific evaluation of their antibacterial efficacy against MDR pathogens remains essential.

3. MATERIALS AND METHODS

3.1 Collection of Plant Material

Fresh and healthy plant materials of Neem (*Azadirachta indica*), Tulsi (*Ocimum sanctum*), Garlic (*Allium sativum*), and Turmeric (*Curcuma longa*) were collected from local medicinal plant gardens and herbal markets of Pune during the study period. Neem and Tulsi leaves, garlic bulbs, and turmeric rhizomes were selected based on their traditional medicinal importance and reported antimicrobial properties. The collected plant materials were washed thoroughly with distilled water to remove dust and other contaminants. Plant samples were shade dried at room temperature for 7–10 days until complete removal of moisture.

The plant specimens were identified and authenticated by a botanist from the Department of Botany, affiliated local college/university. Voucher specimens were preserved for future reference.

3.2 Preparation of Herbal Extracts

The dried plant materials were separately powdered using a sterile electric grinder to obtain fine powder. Approximately 25 g of each powdered sample was transferred into separate conical flasks containing 250 mL of ethanol solvent. Ethanol was selected due to its efficient extraction of bioactive phytochemicals.

The mixtures were kept on a rotary shaker at room temperature for 48 h for proper extraction. After extraction, the solutions were filtered using Whatman No. 1 filter paper. The filtrates were concentrated using a water bath at 40–45^{0}C until semi-solid crude extracts were obtained. The extracts were stored in sterile airtight containers at 4^{0}C until further use.

For antibacterial assays, the crude extracts were dissolved in dimethyl sulfoxide (DMSO) to prepare the required concentrations.

3.3 Test Microorganisms

The antibacterial activity of the herbal extracts was evaluated against clinically important multidrug resistant (MDR) bacterial strains obtained from a microbiology laboratory culture collection. The bacterial isolates used in the present study included:

- *Escherichia coli*
- *Staphylococcus aureus*
- *Pseudomonas aeruginosa*
- *Klebsiella pneumoniae*

These bacterial pathogens are commonly associated with hospital-acquired infections and exhibit resistance to multiple antibiotics. Pure cultures were maintained on nutrient agar slants at 4°C throughout the experimental work.

3.4 Culture Media

The following microbiological media were used for cultivation and antibacterial testing of bacterial isolates:

Mueller-Hinton Agar (MHA)

Mueller-Hinton agar was used for antibacterial susceptibility testing because of its excellent reproducibility and suitability for diffusion assays.

Nutrient Broth

Nutrient broth was used for preparation and enrichment of bacterial inoculum before antibacterial analysis.

All media were prepared according to the manufacturer's instructions and sterilized by autoclaving at 121^{0}C for 15 min.

3.5 Antibacterial Assay

Agar Well Diffusion Method

The antibacterial activity of the herbal extracts was determined using the agar well diffusion method under aseptic conditions.

Preparation of Inoculum

A loopful of each bacterial culture was inoculated into sterile nutrient broth and incubated at 37^{0}C for 18–24 h. The turbidity of the bacterial suspension was adjusted to match 0.5 McFarland standard, corresponding to approximately 1×10^8 CFU/mL.

Inoculation of Plates

Sterile cotton swabs were dipped into the prepared bacterial suspension and uniformly spread over the surface of Mueller-Hinton agar plates to obtain confluent bacterial growth.

Addition of Herbal Extracts

Using a sterile cork borer, wells of approximately 6 mm diameter were made in the agar plates. Different concentrations of herbal extracts were introduced into the respective wells using micropipettes. DMSO served as negative control, while a standard antibiotic disc was used as positive control.

Incubation

The inoculated plates were incubated at 37^{0}C for 24 h under aerobic conditions.

Measurement of Zone of Inhibition

After incubation, antibacterial activity was evaluated by measuring the diameter of the clear zone of inhibition around each well using a sterile ruler or digital caliper. The results were recorded in millimeters (mm).

3.6 Statistical Analysis

All experiments were carried out in triplicate, and the obtained results were expressed as Mean ± Standard Deviation (SD). Statistical analysis was performed using one-way Analysis of Variance (ANOVA) to determine significant differences among the antibacterial activities of different herbal extracts. A p-value less than 0.05 were considered statistically significant.

4. RESULTS

The antibacterial activity of selected herbal extracts against multidrug resistant (MDR) bacterial pathogens was evaluated using the agar well diffusion method. The results demonstrated considerable variation in antibacterial efficacy among the tested medicinal plants. The diameter of inhibition zones was measured in millimeters (mm), and all experiments were performed in triplicate.

Among the tested herbal extracts, *Allium sativum* (Garlic) exhibited the highest antibacterial activity against all selected bacterial strains. The maximum zone of inhibition was observed against *Staphylococcus aureus* (24 mm), followed by *Escherichia coli* (22 mm). *Azadirachta indica* (Neem) also showed significant antibacterial activity with inhibition zones ranging from 12–20 mm. Tulsi and turmeric extracts demonstrated moderate antibacterial effects against the tested organisms.

Among the bacterial pathogens, *Pseudomonas aeruginosa* showed the highest resistance toward most herbal extracts, whereas *Staphylococcus aureus* was found to be the most susceptible organism. The variation in antibacterial activity may be attributed to differences in phytochemical composition among the selected medicinal plants.

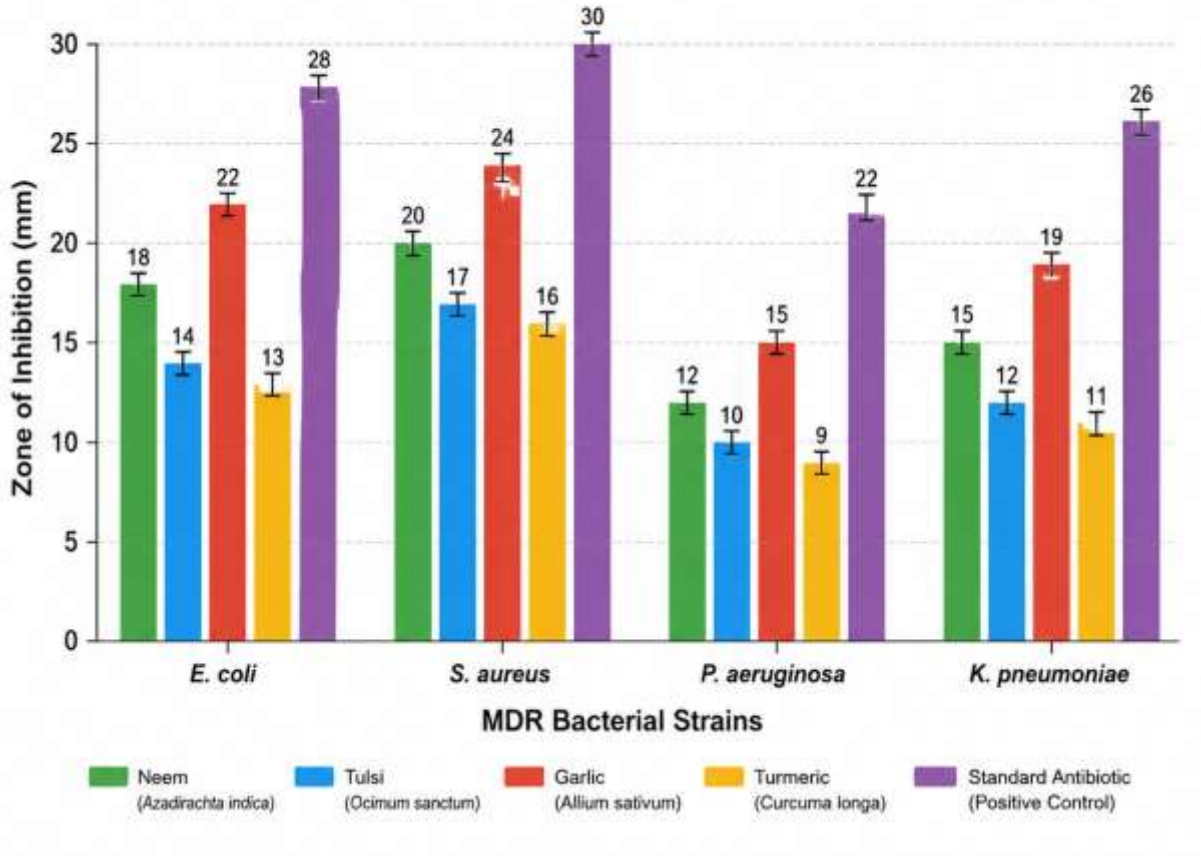
Table 1. Selected Herbal Extracts Used in the Study

Plant Name	Scientific Name	Part Used	Solvent Used
Neem	*Azadirachta indica*	Leaves	Ethanol
Tulsi	*Ocimum sanctum*	Leaves	Methanol
Garlic	*Allium sativum*	Bulb	Ethanol
Turmeric	*Curcuma longa*	Rhizome	Methanol

Table 2. Zone of Inhibition (mm) of Herbal Extracts Against MDR Bacteria

Herbal Extract	*E. coli*	*S. aureus*	*P. aeruginosa*	*K. pneumoniae*
Neem	18 ± 0.5	20 ± 0.6	12 ± 0.4	15 ± 0.5
Tulsi	14 ± 0.4	17 ± 0.5	10 ± 0.3	12 ± 0.4
Garlic	22 ± 0.7	24 ± 0.8	15 ± 0.5	19 ± 0.6
Turmeric	13 ± 0.3	16 ± 0.4	9 ± 0.2	11 ± 0.3
Standard Antibiotic	28 ± 0.9	30 ± 1.0	22 ± 0.8	26 ± 0.9

Figure 1. Comparative Antibacterial Activity of Herbal Extracts Against MDR Bacteria

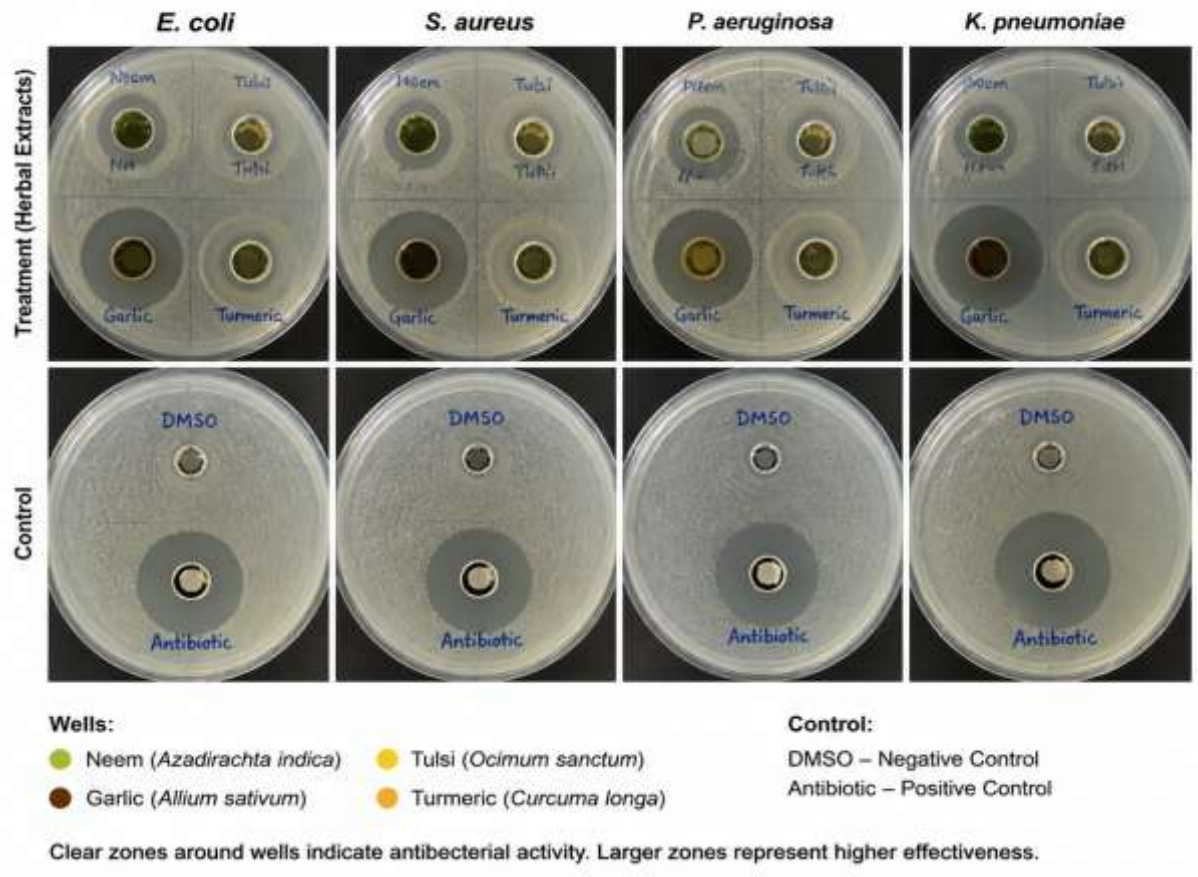


Summary of Major Findings

- Garlic extract exhibited the strongest antibacterial activity against all tested MDR bacteria.
- Neem extract showed moderate to strong antibacterial effects.
- Tulsi and turmeric extracts demonstrated comparatively moderate inhibition.
- *Staphylococcus aureus* was the most susceptible bacterial strain.
- *Pseudomonas aeruginosa* showed maximum resistance among tested organisms.
- The antibacterial activity of herbal extracts may be associated with the presence of phytochemicals such as alkaloids, flavonoids, phenolics, and essential oils.

Figure 2. Agar Well Diffusion Assay Showing Zones of Inhibition

Photographic observations of agar plates revealed distinct clear zones around wells containing herbal extracts, indicating antibacterial activity against MDR bacterial pathogens.



5. DISCUSSIONS

The present study demonstrated that the selected herbal extracts exhibited varying degrees of antibacterial activity against multidrug resistant (MDR) bacterial pathogens. Among all tested extracts, *Allium sativum* (Garlic) showed the highest antibacterial activity, followed by *Azadirachta indica* (Neem), whereas *Curcuma longa* (Turmeric) exhibited comparatively lower inhibition against the tested organisms. The results suggest that medicinal plants possess significant antimicrobial potential and may serve as alternative therapeutic agents against MDR bacterial infections.

The strong antibacterial activity of garlic extract observed in this study may be attributed to the presence of sulfur-containing bioactive compounds such as allicin, ajoene, and diallyl sulfides. These compounds are known to interfere with bacterial cell wall synthesis, protein synthesis, and enzymatic activity, ultimately inhibiting bacterial growth. Similar findings were reported by Ankri and Mirelman, who demonstrated potent antibacterial activity of garlic extract against *Staphylococcus aureus* and other pathogenic bacteria. Neem extract also showed considerable antibacterial activity, which may be due to the presence of phytochemicals such as nimbin, azadirachtin, tannins, and flavonoids possessing antimicrobial and antioxidant properties.

Tulsi extract exhibited moderate antibacterial activity against the tested MDR bacteria. The antibacterial potential of Tulsi may be associated with bioactive constituents including eugenol, ursolic acid, and essential oils. These phytochemicals can disrupt bacterial membranes and inhibit microbial metabolism. Turmeric extract showed relatively lower antibacterial activity compared to garlic and neem; however, it still demonstrated inhibitory effects against all tested bacterial strains. Curcumin, the principal active component of turmeric, is known for its antimicrobial, antioxidant, and anti-inflammatory activities.

The antibacterial efficacy of medicinal plants is mainly associated with the presence of secondary metabolites such as phenolics, flavonoids, alkaloids, tannins, terpenoids, and essential oils. Phenolic compounds can cause protein denaturation and membrane damage in bacterial cells, while flavonoids are capable of inhibiting nucleic acid synthesis and energy metabolism. Alkaloids interfere with DNA replication and enzymatic systems, whereas essential oils increase membrane permeability leading to

leakage of intracellular contents. The synergistic action of these phytochemicals may contribute to the broad-spectrum antibacterial activity observed in the present study.

Among the tested bacterial isolates, *Pseudomonas aeruginosa* exhibited the highest resistance toward herbal extracts. This increased resistance may be attributed to its intrinsic defense mechanisms including low outer membrane permeability, production of efflux pumps, enzymatic degradation of antimicrobial compounds, and strong biofilm-forming ability. In contrast, *Staphylococcus aureus* was found to be the most susceptible bacterium, showing larger zones of inhibition with most herbal extracts. The structural differences between Gram-positive and Gram-negative bacteria may also influence their susceptibility patterns. Gram-negative bacteria possess an additional outer membrane rich in lipopolysaccharides, which acts as a barrier against antimicrobial agents.

The findings of the present investigation are in agreement with previous reports on the antibacterial potential of medicinal plants against MDR pathogens. Similar studies by Batiha et al. and Cowan reported that garlic, neem, tulsi, and turmeric extracts possess significant inhibitory activity against pathogenic bacteria. Furthermore, several researchers have emphasized the importance of plant-derived antimicrobial compounds as promising alternatives to synthetic antibiotics in combating antimicrobial resistance.

Overall, the present study highlights the therapeutic importance of medicinal plants as potential sources of antibacterial agents against multidrug resistant bacteria. The observed antibacterial activity supports the traditional use of these plants in the treatment of infectious diseases. Further studies involving purification, characterization, and molecular analysis of active phytoconstituents are necessary to develop effective plant-based antimicrobial formulations for clinical applications.

CONCLUSIONS

The present study demonstrated that selected medicinal plant extracts possess considerable antibacterial activity against multidrug resistant (MDR) bacterial pathogens. Among the tested herbal extracts, *Allium sativum* (Garlic) exhibited the highest antibacterial activity, followed by *Azadirachta indica* (Neem), while *Ocimum sanctum* (Tulsi) and *Curcuma longa* (Turmeric) showed moderate inhibitory effects. The variation in antibacterial efficacy among different plants may be attributed to differences in the concentration and composition of bioactive phytochemicals such as phenolics, flavonoids, alkaloids, tannins, and essential oils. The findings of this study indicate that medicinal plants can act as valuable natural sources of antimicrobial compounds against clinically important MDR bacteria including *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae*. The observed antibacterial activity supports the traditional therapeutic use of these herbal plants in the treatment of infectious diseases. In particular, garlic and neem extracts showed strong inhibitory effects and may have potential for development into plant-based antimicrobial formulations. Although the present investigation provided encouraging results, further studies are required for purification, isolation, and characterization of the active phytoconstituents responsible for antibacterial activity. Additional research involving toxicity studies, molecular mechanisms, in vivo experiments, and clinical evaluations will be essential to establish the safety and therapeutic efficacy of these herbal extracts for pharmaceutical applications.

Medicinal plants may serve as promising alternatives for combating multidrug resistant bacterial infections.

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