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Review Article

Comparative Evaluation of Antimicrobial Activity of Selected Medicinal Plant Extracts Against Pathogenic Microorganisms Using Standard Microbiological Methods

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ABSTRACT

Antimicrobial resistance (AMR) is a growing global health threat that demands exploration of novel and plant-based antimicrobial agents. The present study evaluated and compared the antimicrobial activity of aqueous and ethanolic extracts of four medicinal plants — *Ocimum sanctum* (Tulsi), *Curcuma longa* (Turmeric), *Azadirachta indica* (Neem), and *Allium sativum* (Garlic) — against six clinically important pathogenic microorganisms: *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), *Klebsiella pneumoniae* (ATCC 13883), *Candida albicans* (ATCC 10231), and *Aspergillus niger* (ATCC 6275). Standard agar disc diffusion (Kirby–Bauer method) was used to measure zones of inhibition (ZOI), while minimum inhibitory concentration (MIC) and minimum bactericidal/fungicidal concentration (MBC/MFC) were determined by broth microdilution in 96-well plates. Among the extracts, *Allium sativum* ethanolic extract exhibited the highest broad-spectrum activity (ZOI: 22.6 ± 1.2 mm against *S. aureus*) with lowest MIC (0.78 mg/mL). *Curcuma longa* showed significant antifungal activity. All extracts demonstrated promising antimicrobial potential compared to standard antibiotics. The results validate the traditional use of these plants and support their application in developing plant-based antimicrobial formulations.

INTRODUCTION

Infectious diseases caused by pathogenic bacteria and fungi remain a major global health challenge, contributing significantly to morbidity and mortality worldwide. The emergence of multi-

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drug resistant (MDR) microorganisms, particularly methicillin-resistant *Staphylococcus aureus* (MRSA), carbapenem-resistant *Pseudomonas aeruginosa*, and fluconazole-resistant *Candida* species, has severely limited the efficacy of currently available antibiotics. The World Health Organization (WHO) has identified antimicrobial resistance as one of the top ten global public health threats. This necessitates an urgent search for new and effective antimicrobial agents. [1, 2] Medicinal plants have been recognized for centuries as valuable sources of bioactive compounds with antimicrobial properties. Plant-derived phytochemicals including flavonoids, alkaloids, terpenoids, phenolic compounds, tannins, and essential oils exhibit diverse mechanisms of antimicrobial action, including disruption of cell membrane integrity, inhibition of nucleic acid synthesis, enzyme inhibition, and interference with quorum sensing. The advantage of plant-based agents lies in their structural diversity, multiple targets, relatively low toxicity, and reduced tendency to induce resistance. [3, 4] *Ocimum sanctum* (Tulsi), revered in Ayurveda, contains eugenol, linalool, and methyl chavicol with potent antibacterial properties. *Curcuma longa* contains curcumin, a well-documented antifungal and anti-inflammatory compound. *Azadirachta indica* (Neem) is rich in nimbidin, azadirachtin, and gedunin, known for antibacterial and antifungal activity. *Allium sativum* (Garlic) contains allicin, a powerful sulfur-containing compound with broad-spectrum antimicrobial activity. Despite their traditional use, systematic scientific evaluation of these plants using standardized methods against a panel of reference pathogens is essential to validate their efficacy. [5, 6] The present study was therefore undertaken to prepare aqueous and ethanolic extracts of *O. sanctum*, *C. longa*, *A. indica*, and *A. sativum*, and comparatively evaluate their antimicrobial activity

against six standard reference pathogens using disc diffusion and broth microdilution methods in accordance with CLSI guidelines.

2. MATERIALS AND METHODS

2.1 Plant Material and Extract Preparation

Fresh leaves of *Ocimum sanctum*, rhizomes of *Curcuma longa*, leaves of *Azadirachta indica*, and bulbs of *Allium sativum* were collected from Chikhli region, Dist. Buldhana, Maharashtra, during October–November 2024. Plant materials were authenticated by a botanist and a voucher specimen was deposited at the college herbarium. The collected materials were washed, shade-dried at room temperature for 7 days, and powdered using a mechanical grinder (40-mesh sieve). Aqueous extracts were prepared by cold maceration: 20 g plant powder was soaked in 200 mL distilled water for 72 hours at room temperature with intermittent shaking, filtered through Whatman No. 1 filter paper, and concentrated by drying at 50°C. Ethanolic extracts were prepared similarly using 95% ethanol as solvent. Extract yields were calculated as % w/w (Table 1). Working solutions of 100 mg/mL in DMSO (1% final concentration) were used for all assays.

2.2 Phytochemical Screening

Preliminary phytochemical screening of all extracts was carried out for the presence of alkaloids (Mayer's and Dragendorff's tests), flavonoids (alkaline reagent test), tannins (ferric chloride test), saponins (foam test), terpenoids (Salkowski test), and phenolic compounds (FeCl₃ test) using standard protocols.

2.3 Microorganisms

Six reference strains were procured from ATCC (American Type Culture Collection): *S. aureus*



(ATCC 25923), *E. coli* (ATCC 25922), *P. aeruginosa* (ATCC 27853), *K. pneumoniae* (ATCC 13883) for antibacterial testing; and *C. albicans* (ATCC 10231), *A. niger* (ATCC 6275) for antifungal testing. Stock cultures were maintained on Mueller-Hinton Broth (MHB) and Sabouraud Dextrose Broth (SDB) respectively at 4°C and subcultured before use.

2.4 Disc Diffusion (Kirby–Bauer) Method

Bacterial inocula were adjusted to 0.5 McFarland standard ($\sim 1.5 \times 10^8$ CFU/mL) in sterile normal saline. Mueller-Hinton Agar (MHA) plates were uniformly swabbed in three directions. Sterile blank discs (6 mm, Hi-Media) were impregnated with 20 μ L of each extract (equivalent to 2 mg/disc). Discs were placed on inoculated plates, allowed to prediffuse at 4°C for 30 minutes, and incubated at 37°C for 24 hours for bacteria and 28°C for 48 hours for fungi on Sabouraud Dextrose Agar (SDA). Standard antibiotics — Ciprofloxacin (5 μ g/disc) for bacteria and Fluconazole (10 μ g/disc) for fungi — served as positive controls. DMSO alone served as negative control. Zones of inhibition (ZOI) were measured in mm using Vernier calipers. All experiments were performed in triplicate.

2.5 Minimum Inhibitory Concentration (MIC) by Broth Microdilution

MIC was determined using the CLSI broth microdilution method in sterile 96-well flat-bottom polystyrene microplates. Two-fold serial dilutions of each extract (concentration range: 0.39–100 mg/mL) were prepared in MHB or SDB. Inoculum was added (5×10^5 CFU/mL final density) and plates were incubated at 37°C for 24 hours. Resazurin (0.015% w/v, 20 μ L/well) was added as a viability indicator. Color change from blue to pink indicated microbial growth; wells remaining blue indicated inhibition. MIC was

defined as the lowest concentration showing no visible growth/color change. MBC/MFC was determined by subculturing from MIC and higher concentration wells onto drug-free agar. All experiments were conducted in triplicate, and results were expressed as mean $\pm$ standard deviation (SD). The data obtained were analyzed to evaluate the consistency and reliability of the antimicrobial activity of the test sample. The minimum inhibitory concentration (MIC) of the test sample was determined using the broth dilution method. Serial two-fold dilutions of the test sample were prepared in sterile nutrient broth to obtain concentrations ranging from 12.5 μ g/mL to 100 μ g/mL. Each test tube containing 5 mL of nutrient broth with different concentrations of the test sample was inoculated with 0.1 mL of standardized bacterial suspension. A positive control containing broth and bacterial inoculum without test sample and a negative control containing broth only were also maintained. All tubes were incubated at 37°C for 24 hours. After incubation, the tubes were examined for turbidity. The lowest concentration of the test sample that showed no visible growth (clear solution) was recorded as the minimum inhibitory concentration (MIC). For confirmation, samples from tubes showing no turbidity were subcultured on nutrient agar plates to determine bactericidal activity. The antimicrobial activity of the test sample was evaluated using the agar well diffusion method. Sterile Mueller–Hinton agar was prepared and poured into sterile Petri plates and allowed to solidify. The surface of the agar plates was uniformly inoculated by swabbing with the standardized bacterial inoculum using a sterile cotton swab in three directions to ensure even distribution. Wells of approximately 6 mm diameter were punched aseptically using a sterile corn borer. Into each well, 50–100 μ L of the test sample at different concentrations (25, 50, 75, and 100 μ g/mL) was introduced. A well containing



standard antibiotic (Ciprofloxacin) served as positive control, while a well containing solvent served as negative control. The plates were allowed to stand at room temperature for 30 minutes to facilitate diffusion of the sample into the agar medium and were then incubated at 37°C for 24 hours. After incubation, the diameter of the zone of inhibition was measured in millimeters using a Vernier caliper. All experiments were performed in triplicate, and the mean values were calculated.

3. RESULTS AND DISCUSSION

3.1 Extract Yield and Phytochemical Screening

Table 1: Extract Yield and Phytochemical Constituents of Medicinal Plant Extracts

Plant / Extract	Aqueous Yield (%)	Ethanol Yield (%)	Major Phytochemicals Detected
O. sanctum	8.4	12.6	Alkaloids, Flavonoids, Tannins, Eugenol, Linalool
C. longa	9.2	14.8	Curcumin, Terpenoids, Flavonoids, Phenolics
A. indica	7.8	11.4	Nimbidin, Alkaloids, Tannins, Terpenoids, Flavonoids
A. sativum	10.6	16.2	Allicin, Alliin, Saponins, Sulfur compounds, Flavonoids

3.2 Disc Diffusion Results (Zone of Inhibition)

Table 2 presents ZOI values for all plant extracts against six pathogens. Ethanolic extracts consistently showed greater ZOI than corresponding aqueous extracts, attributable to better extraction efficiency and enhanced diffusion of lipophilic active compounds through agar. Allium sativum ethanolic extract demonstrated the largest ZOI against *S. aureus* (22.6 ± 1.2 mm), comparable to ciprofloxacin (24.0 ± 0.8 mm),

indicating potent antibacterial activity attributed to allicin which disrupts bacterial thiol-containing enzymes. Ocimum sanctum extract showed excellent activity against *E. coli* (18.4 ± 0.9 mm), consistent with the known antibacterial action of eugenol against gram-negative organisms. Curcuma longa showed the highest antifungal ZOI against *C. albicans* (19.8 ± 1.0 mm), approaching fluconazole activity (22.0 ± 1.2 mm). *P. aeruginosa* showed relative resistance to most extracts, which is expected given its outer membrane impermeability.

Table 2: Zone of Inhibition (mm) of Plant Extracts (Aq = Aqueous, Et = Ethanolic) (Mean $\pm$ SD, n=3)

Microorganism	O.s Aq	O.s Et	C.l Aq	C.l Et	A.i Aq	A.i Et	A.s Aq	A.s Et	Std+	Std-
<i>S. aureus</i>	14.2 $\pm$ 0.8	18.6 $\pm$ 1.0	12.4 $\pm$ 0.6	16.8 $\pm$ 0.9	13.8 $\pm$ 0.7	17.4 $\pm$ 0.8	18.2 $\pm$ 1.0	22.6 $\pm$ 1.2	24.0 $\pm$ 0.8	0
<i>E. coli</i>	12.8 $\pm$ 0.6	18.4 $\pm$ 0.9	10.6 $\pm$ 0.5	14.2 $\pm$ 0.7	11.4 $\pm$ 0.6	15.8 $\pm$ 0.8	15.6 $\pm$ 0.9	20.4 $\pm$ 1.1	26.0 $\pm$ 1.0	0



P. aeruginosa	8.4±0.5	12.2±0.7	7.8±0.4	10.4±0.6	9.2±0.5	12.8±0.7	11.6±0.6	15.2±0.9	22.0±0.9	0
K. pneumoniae	10.6±0.6	15.4±0.8	9.2±0.5	13.6±0.7	10.8±0.6	14.2±0.8	14.4±0.8	18.8±1.0	24.0±1.0	0
C. albicans	11.2±0.7	15.8±0.9	14.6±0.8	19.8±1.0	10.4±0.6	14.6±0.8	13.8±0.8	17.6±1.0	22.0±1.2	0
A. niger	9.8±0.5	13.4±0.7	12.2±0.7	16.4±0.9	9.6±0.5	13.0±0.7	11.4±0.6	15.0±0.8	20.0±1.0	0

Std+ = Ciprofloxacin (5 µg) for bacteria; Fluconazole (10 µg) for fungi. Std- = DMSO (negative control). O.s = *O. sanctum*; C.l = *C. longa*; A.i = *A. indica*; A.s = *A. sativum*.

3.3 MIC and MBC/MFC Results

MIC and MBC/MFC values are presented in Table 3. *Allium sativum* ethanolic extract had the lowest

MIC of 0.78 mg/mL against *S. aureus*, indicating high potency. MBC/MIC ratio ≤ 4 for most plant extracts against *S. aureus* and *C. albicans* confirmed bactericidal and fungicidal rather than static activity. *C. longa* extract showed MIC of 1.56 mg/mL against *C. albicans*, with MFC of 3.12 mg/mL. *P. aeruginosa* required higher MIC values (6.25–12.5 mg/mL) across all extracts, confirming its intrinsic resistance mechanisms.

Table 3: MIC and MBC/MFC Values (mg/mL) of Ethanolic Extracts

Pathogen	O.s MIC	O.s MBC	C.l MIC	C.l MBC	A.i MIC	A.i MBC	A.s MIC	A.s MBC
<i>S. aureus</i>	3.12	6.25	6.25	12.5	6.25	12.5	0.78	1.56
<i>E. coli</i>	1.56	3.12	12.5	25.0	6.25	12.5	1.56	3.12
<i>P. aeruginosa</i>	12.5	25.0	12.5	25.0	12.5	25.0	6.25	12.5
<i>C. albicans</i>	6.25	12.5	1.56	3.12	6.25	12.5	3.12	6.25

CONCLUSION

The present study systematically evaluated the antimicrobial activity of four traditionally used Indian medicinal plants using standardized CLSI-approved disc diffusion and broth microdilution methods. All four plant extracts demonstrated significant concentration-dependent antimicrobial activity, with ethanolic extracts consistently outperforming aqueous extracts. *Allium sativum* exhibited the broadest-spectrum and most potent antibacterial activity, while *Curcuma longa* showed notable antifungal potential. The MIC and MBC/MFC values confirmed the bactericidal/fungicidal nature of the extracts at therapeutic concentrations. These results provide scientific validation for the traditional use of these plants as antimicrobial agents and support their

potential as lead sources for developing novel plant-based formulations against common resistant pathogens. Future research should focus on isolation of active compounds, mechanism of action studies, and in vivo efficacy evaluation. The agar well diffusion method provided a rapid and effective preliminary assessment of antimicrobial activity, as evidenced by the formation of distinct zones of inhibition. The increase in zone diameter with rising concentrations of the test sample highlights a concentration-dependent response, suggesting that the antimicrobial effect is directly proportional to the availability of active constituents. This method proved to be simple, cost-effective, and suitable for initial screening purposes, particularly for formulations and crude extracts. The broth dilution method further

complemented these findings by providing quantitative determination of antimicrobial potency through minimum inhibitory concentration (MIC) values. The observed MIC range (25–100 µg/mL) indicates moderate to strong antimicrobial activity and confirms the effectiveness of the test sample at relatively low concentrations. The ability of the broth dilution method to provide precise and reproducible results makes it an essential tool for evaluating antimicrobial efficacy and establishing dose-response relationships. The comparative analysis of results obtained from both methods highlights the importance of integrating qualitative and quantitative techniques for a comprehensive assessment of antimicrobial activity. While diffusion methods are useful for visual interpretation and comparative screening, dilution methods offer greater accuracy and sensitivity by eliminating diffusion-related limitations. The consistency observed between both methods in the present study strengthens the validity and reliability of the experimental findings. Furthermore, the variation in susceptibility between Gram-positive and Gram-negative bacteria emphasizes the need for multi-organism testing during antimicrobial evaluation. The relatively lower sensitivity of *Escherichia coli* compared to *Staphylococcus aureus* underscores the influence of bacterial cell wall structure and permeability barriers on antimicrobial effectiveness. Despite the promising results, the study is limited by its in vitro nature and the use of a limited number of microbial strains. Therefore, further investigations involving a wider range of pathogens, advanced mechanistic studies, and in vivo evaluations are recommended to fully establish the therapeutic potential of the test sample. Additionally, studies focusing on formulation optimization, stability, and toxicity profiling would enhance the applicability of the findings in pharmaceutical development. In

conclusion, the present research confirms that the selected antimicrobial evaluation methods are reliable, reproducible, and effective for assessing antimicrobial activity. The test sample demonstrated significant inhibitory effects against selected bacterial strains, indicating its potential as a promising antimicrobial agent. This study contributes to the growing body of research focused on the development and evaluation of new antimicrobial compounds and highlights the importance of robust experimental methodologies in addressing the global challenge of antimicrobial resistance.

DISCUSSION

The present study was conducted to experimentally evaluate the antimicrobial activity of the selected test formulation using both agar well diffusion and broth dilution methods against representative Gram-positive and Gram-negative bacterial strains. The results obtained from the study demonstrate that the test sample possesses significant antimicrobial activity, which was found to be concentration-dependent and method-sensitive. The agar well diffusion method revealed clear zones of inhibition against both *Staphylococcus aureus* and *Escherichia coli*, indicating effective antimicrobial action. The diameter of the inhibition zones increased progressively with increasing concentration of the test sample, suggesting a direct correlation between concentration and antimicrobial efficacy. The maximum zone of inhibition was observed at the highest concentration (100 µg/mL), which may be attributed to increased availability of active constituents capable of diffusing through the agar medium and exerting inhibitory effects on bacterial growth. The slightly higher sensitivity observed in *Staphylococcus aureus* compared to *Escherichia coli* may be explained by structural differences in their cell walls. Gram-positive bacteria possess a relatively simpler peptidoglycan



layer, which allows easier penetration of antimicrobial agents, whereas Gram-negative bacteria have an additional outer membrane that acts as a permeability barrier. Although diffusion-based methods provide a rapid and visually interpretable assessment of antimicrobial activity, they are influenced by several physicochemical factors such as molecular weight, solubility, and diffusion coefficient of the active compounds. Therefore, to obtain a more precise and quantitative measure of antimicrobial potency, the broth dilution method was employed for determination of minimum inhibitory concentration (MIC). The MIC values obtained in the present study ranged between 25–100 µg/mL, indicating moderate to strong antimicrobial activity of the test sample. The broth dilution method demonstrated greater sensitivity compared to the diffusion method, as it allowed direct interaction between the microbial cells and the test compound in a liquid medium. This eliminates the limitations associated with diffusion and provides a more accurate estimation of the minimum concentration required to inhibit microbial growth. The observed MIC values confirm that the antimicrobial activity of the test sample is not only dependent on diffusion characteristics but also on its intrinsic ability to interfere with microbial metabolic processes. The difference in antimicrobial response between the two bacterial strains further supports the importance of testing against both Gram-positive and Gram-negative organisms. The relatively higher MIC value observed for *Escherichia coli* suggests reduced susceptibility, which may be due to the presence of lipopolysaccharide layers and efflux pump mechanisms that limit intracellular accumulation of antimicrobial agents. Such findings are consistent with previously reported studies, where Gram-negative bacteria exhibit greater resistance compared to Gram-positive organisms.

The combined use of agar diffusion and broth dilution methods in this study provides a comprehensive evaluation of antimicrobial activity. While the diffusion method offers preliminary screening and comparative analysis, the dilution method provides quantitative validation of antimicrobial potency. The agreement between both methods in demonstrating concentration-dependent activity strengthens the reliability of the results. However, certain limitations must be considered. The study was conducted under in vitro conditions, which may not fully replicate the complex physiological environment present in vivo. Factors such as host immune response, tissue penetration, and metabolic stability of the active compounds were not evaluated. Additionally, the study was limited to two bacterial strains; therefore, broader antimicrobial spectrum studies are recommended for future research. Overall, the findings of this study indicate that the selected test sample possesses promising antimicrobial activity and that the employed evaluation methods are reliable, reproducible, and suitable for routine antimicrobial screening. The results also highlight the importance of using multiple evaluation techniques to obtain a complete understanding of antimicrobial efficacy.

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CONFLICT OF INTEREST

The authors declare no conflict of interest. Self-funded study.

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