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Comparative Antibacterial Activity of *Azadirachta indica* (Neem) and *Curcuma longa* (Turmeric) Hydroalcoholic Extracts Against Common Clinical Isolates: An In Vitro Study

Dr. Pratap Sahu[1], Dr. A. Sowmya[2], Komal Parashar[3], Li Minmin[4], Balasaheb Shiraskar[5*], Dhuruv Kumar Rawal[6], Mavluda Khakimova[7], Elyor Akhmedov[8]

[1]Professor, Department of Pharmaceutical Sciences, School of Pharmaceutical Sciences, Siksha 'O' Anusandhan (Deemed to be University), Bhubaneswar, Odisha, India, pratapsahu@soa.ac.in, 0000-0002-8805-8682
[2]Associate Professor, Department of Microbiology, Vinayaka Mission's Kirupananda Variyar Medical College & Hospitals, Vinayaka Mission's Research Foundation (DU), Salem, Tamil Nadu, India. sowmyahari91@gmail.com, 0009-0001-4548-4382
[3]Centre for Research Impact & Outcome, Chitkara University Institute of Engineering and Technology, Chitkara University, Rajpura, 140401, Punjab, India. komal.parashar.orp@chitkara.edu.in https://orcid.org/0009-0007-8120-2083
[4]Faculty of Education, Shinawatra University, Pathum Thani, Thailand. 1401079264@qq.com
[5]Krishna Institute of Pharmacy, Krishna Vishwa Vidyapeeth "Deemed to be University", Taluka-Karad, Dist-Satara, Pin-415 539, Maharashtra, India. balasahe.siraskar@kvv.edu.in
[6]Department of Orthopedics, Noida international University, Greater Noida, Uttar Pradesh 203201, India., drdhruvrawal23@gmail.com
[7] Samarkand State Medical University, Samarkand, Uzbekistan, mia.khakimova@gmail.com
[8] PhD, Associate Professor, Department of Medical Radiology No. 2, Tashkent State Medical University axmedov.e.a@tashmeduni.uz

ABSTRACT:

Background: The relentless global rise of antimicrobial resistance (AMR) has renewed scientific interest in medicinal plants as reservoirs of novel antibacterial compounds. In South India, *Azadirachta indica* (neem) and *Curcuma longa* (turmeric) are deeply embedded in traditional medicine, yet head-to-head comparisons of their antibacterial potency against contemporary clinical isolates are scarce.

Objective: To compare the in vitro antibacterial activity of hydroalcoholic extracts of neem leaf and turmeric rhizome against five common clinical bacterial isolates, and to relate activity to phenolic and flavonoid content.

Methods: In this in vitro experimental study, hydroalcoholic (ethanol–water) extracts were prepared from neem leaf and turmeric rhizome. Antibacterial activity was assessed by agar well diffusion at 25, 50 and 100 mg/mL in triplicate against *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Enterococcus faecalis*, with a standard antibiotic as positive control and solvent as negative control. Minimum inhibitory (MIC) and minimum bactericidal (MBC) concentrations were determined by broth

microdilution. Total phenolic (TPC) and total flavonoid (TFC) contents were quantified. Data were analysed using mean ± SD, independent t-test and one-way ANOVA ($p < 0.05$).

Results: Both extracts showed concentration-dependent activity. The overall mean zone of inhibition was significantly larger for neem (15.44 ± 4.65 mm) than turmeric (13.13 ± 4.32 mm) (Welch $t = 2.44$, $p = 0.017$), while both were far exceeded by the positive control (24.03 mm) and negligible for solvent (0.42 mm). At 100 mg/mL, zones ranged 20.4–21.8 mm (neem) and 17.4–18.9 mm (turmeric), with no significant inter-species difference (neem $F = 1.28$, $p = 0.34$; turmeric $F = 1.47$, $p = 0.28$). MIC/MBC patterns were organism-dependent: turmeric was more potent against *E. coli*, whereas neem was more potent against *P. aeruginosa* and *E. faecalis*. Neem contained higher TPC (165.91 vs 148.54 mg GAE/g) and TFC (74.84 vs 57.79 mg QE/g).

Conclusion: Both hydroalcoholic extracts exhibited meaningful, dose-dependent antibacterial activity against Gram-positive and Gram-negative clinical isolates, with neem generally superior—plausibly linked to its higher phenolic/flavonoid content. These low-cost screening findings support the traditional South Indian use of neem and turmeric and their potential relevance amid escalating AMR.

1. INTRODUCTION

Antimicrobial resistance (AMR) has emerged as one of the most pressing threats to global public health in the twenty-first century. The steady erosion of the therapeutic value of conventional antibiotics, driven by their overuse and misuse in human medicine, veterinary practice and agriculture, has created a widening gap between the pace of resistance emergence and the discovery of new antibacterial agents. Common nosocomial and community-acquired pathogens such as methicillin-resistant *Staphylococcus aureus*, extended-spectrum β-lactamase–producing *Escherichia coli* and *Klebsiella pneumoniae*, and multidrug-resistant *Pseudomonas aeruginosa* now routinely complicate the management of infections that were once readily treatable. In resource-constrained settings, including many parts of South India, the burden of drug-resistant infection is compounded by high infection prevalence, variable stewardship and limited access to newer antibiotics. Against this backdrop, the search for affordable, accessible and effective antibacterial agents from natural sources has acquired renewed urgency [1,2].

Medicinal plants have historically served as a rich source of therapeutic molecules, and their secondary metabolites—phenolic, flavonoids, alkaloids, terpenoids and essential oils—continue to attract attention as templates for antimicrobial discovery. This multi-target activity is thought to make the development of resistance comparatively more difficult than with single-target thetic antibiotics, positioning botanicals as attractive candidates for adjuvant or stand-alone antibacterial strategies [3,4].

Two plants occupy a place of particular prominence in the traditional medical systems of the Indian subcontinent: *Azadirachta indica* A. Juss (neem) and *Curcuma longa* L. (turmeric). Neem, a member of the Meliaceae family, is often described as the "village pharmacy" of India; virtually every part of the tree—leaf, bark, seed and oil—has been employed in Ayurveda and Siddha for skin disorders, oral hygiene, diarrhoeal disease, fever and wound care. Its leaf extracts have documented antibacterial, antisecretory, antiplaque, anti-biofilm and anticarcinogenic properties, attributed to constituents such as nimbidin, nimbin, azadirachtin, quercetin and a range of phenolic compounds [5]. Turmeric, the rhizome of *Curcuma longa* (Zingiberaceae), is equally ubiquitous in South Indian households and rituals, valued as both a culinary spice and a medicinal agent. Its principal polyphenol, curcumin, together with related curcuminoids and turmeric oil, exhibits a broad spectrum of biological activities including antioxidant, anti-inflammatory, antimicrobial and antimutagenic effects [6]. Curcumin and its bioconjugates have demonstrated activity against β-lactamase–producing organisms and multidrug-resistant mycobacteria, underscoring the antibacterial promise of this "golden spice" [7-9].

Despite the substantial individual literature on each plant, direct, standardised comparisons of neem and turmeric under identical laboratory conditions against a shared panel of clinically relevant isolates remain limited. Most published studies employ

heterogeneous solvents, extraction methods, concentrations and test organisms, which frustrates meaningful comparison. Hydroalcoholic (ethanol–water) extraction is particularly attractive for screening work because it efficiently recovers both moderately polar and polar phytoconstituents—including phenolics and flavonoids—while remaining inexpensive and reproducible. A head-to-head evaluation of hydroalcoholic neem and turmeric extracts, coupled with quantification of their phenolic and flavonoid content, therefore offers an opportunity to link chemical composition to antibacterial performance and to contextualise the traditional South Indian use of these plants.

The present study was designed to address this gap. Its objectives were: (i) to prepare hydroalcoholic extracts of neem leaf and turmeric rhizome and determine their extraction yield, total phenolic content and total flavonoid content; (ii) to compare their antibacterial activity by agar well diffusion at graded concentrations against five common clinical isolates spanning Gram-positive and Gram-negative organisms; (iii) to determine MIC and MBC for each extract–organism pair; and (iv) to interpret the comparative activity in relation to phytochemical content and to the wider literature on neem and turmeric antibacterial effects. We hypothesised that both extracts would display concentration-dependent activity and that differences in phenolic/flavonoid content would be reflected in differences in inhibitory potency.

2. MATERIALS AND METHODS

2.1 Study design and setting

This was an in vitro experimental antibacterial study conducted in the Department of Microbiology, India, during six months. The work involved no human participants and no animals; all testing was performed on stored bacterial isolates.

2.2 Plant collection and authentication

Fresh, healthy leaves of *Azadirachta indica* and rhizomes of *Curcuma longa* were collected locally in South India, and taxonomically authenticated in the Department of Pharmacognosy, a voucher specimen [vno.2113] was deposited for future reference. Plant material was washed, shade-dried, and pulverised to a coarse powder using a mechanical grinder, then stored in airtight containers protected from light and moisture until extraction.

2.3 Preparation of hydroalcoholic extracts

Hydroalcoholic (ethanol–water) extraction was performed on the dried powders of neem leaf and turmeric rhizome. A weighed quantity of each powder was macerated/percolated in an ethanol–water solvent system, filtered, and the filtrate concentrated under reduced pressure to yield a semi-solid extract. The percentage extraction yield was calculated on a dry-weight basis. The neem leaf extract gave a yield of 15.91% and the turmeric rhizome extract 16.17%. Stock solutions were reconstituted in the extraction solvent to prepare working concentrations of 25, 50 and 100 mg/mL for antibacterial testing.

2.4 Bacterial clinical isolates

Five clinically important bacterial isolates were tested: *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Enterococcus faecalis*. These represent a mix of Gram-positive (*S. aureus*, *E. faecalis*) and Gram-negative (*E. coli*, *P. aeruginosa*, *K. pneumoniae*) organisms commonly implicated in clinical infection. Isolates were obtained from the Department of Microbiology and identified to species level by standard microbiological and biochemical methods; reference strains [ATCC/reference strain numbers] were used for quality control. Inocula were standardised to 0.5 McFarland turbidity prior to testing.

2.5 Agar well diffusion assay

Antibacterial activity was evaluated by the agar well diffusion method. Standardized bacterial suspensions were uniformly spread on Mueller–Hinton agar plates, and wells were bored into the medium. Each well was charged with the respective extract at 25, 50 or 100 mg/mL. A standard antibiotic ([standard antibiotic used as positive control]) served as the positive control, and the extraction solvent alone served as the negative control. Plates were incubated under appropriate conditions, and the diameter of the zone of inhibition (including the well) was measured in millimeters. All assays were performed in triplicate, and results are expressed as mean ± standard deviation [6,10].

2.6 Determination of MIC and MBC

Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined by the broth micro dilution method. Serial two-fold dilutions of each extract were prepared in broth and inoculated with standardized bacterial suspensions. The MIC was defined as the lowest concentration showing no visible growth after incubation. To determine the MBC, aliquots from wells showing no visible growth were subculture onto agar; the MBC was defined as the lowest concentration yielding no bacterial growth on subculture [9,11].

2.7 Phytochemical quantification

Total phenolic content (TPC) was measured by the Folin–Ciocalteu method and expressed as milligrams of Gallic acid equivalents per gram of extract (mg GAE/g). Total flavonoid content (TFC) was determined by the aluminum chloride colorimetric method and expressed as milligrams of quercetin equivalents per gram of extract (mg QE/g).

2.8 Statistical analysis

Quantitative data are presented as mean ± standard deviation. Comparison of overall mean zones of inhibition between neem and turmeric extracts was performed using an independent (Welch) t-test. Differences in zone of inhibition across the five clinical isolates at 100 mg/mL were assessed for each extract using one-way analysis of variance (ANOVA). A two-sided p value < 0.05 was considered statistically significant. Analyses were performed using standard statistical software.

3. RESULTS

3.1 Extraction yield and phytochemical content

The two hydro alcoholic extracts gave broadly comparable extraction yields (neem 15.91%, turmeric 16.17%), but differed appreciably in phytochemical content (Table 1). The neem leaf extract contained higher total phenolic content (165.91 vs 148.54 mg GAE/g) and substantially higher total flavonoid content (74.84 vs 57.79 mg QE/g) than the turmeric rhizome extract. This richer polyphenolic profile of neem provides a plausible chemical basis for the antibacterial differences observed below.

Table 1. Extraction yield, total phenolic content and total flavonoid content of hydro alcoholic neem and turmeric extracts.

Plant (part)	Solvent	Yield (%)	TPC (mg GAE/g)	TFC (mg QE/g)
Azadirachta indica (leaf)	Hydroalcoholic	15.91	165.91	74.84
Curcuma longa (rhizome)	Hydroalcoholic	16.17	148.54	57.79

3.2 Overall antibacterial activity and dose–response

Both extracts produced clear, concentration-dependent zones of inhibition against all five isolates (Table 2, Figure 1). Pooled across all organisms and concentrations, the mean zone of inhibition was significantly larger for neem (15.44 ± 4.65 mm) than for turmeric (13.13 ± 4.32 mm) (Welch $t = 2.44$, $p = 0.017$), indicating that neem was, overall, the more potent extract. As expected, the positive-control antibiotic produced the largest zones (mean 24.03 mm), while the negative solvent control was essentially inactive (mean 0.42 mm), confirming assay validity and that the observed activity was attributable to the extracts rather than the solvent.

The dose–response relationship was consistent for both extracts (Figure 1). Mean zones rose from 10.05 mm at 25 mg/mL to 15.19 mm at 50 mg/mL and 21.07 mm at 100 mg/mL for neem, and from 8.01 mm to 13.11 mm to 18.27 mm across the same concentrations for turmeric. At every concentration tested, neem produced a larger mean zone than turmeric, and the margin was maintained across the dose range.

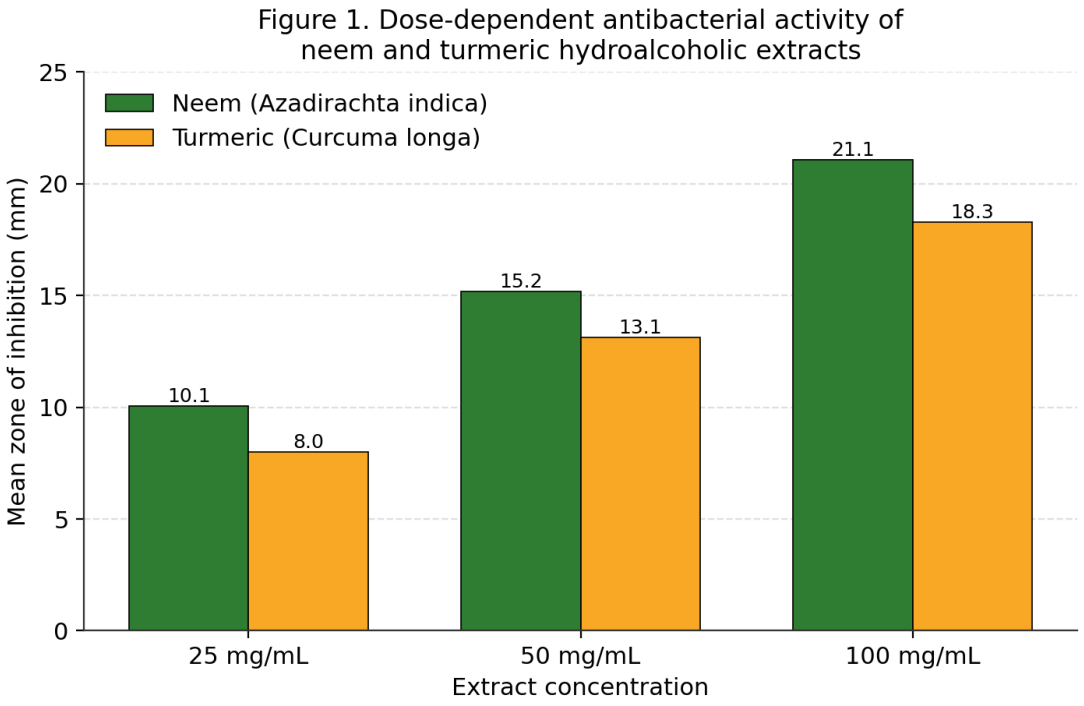

Figure 1. Mean zone of inhibition (mm) of neem and turmeric hydroalcoholic extracts at 25, 50 and 100 mg/mL, demonstrating a clear concentration-dependent (dose–response) increase in antibacterial activity for both extracts, with neem exceeding turmeric at each concentration.

Table 2. Mean zone of inhibition (mm) by extract and concentration, including positive and negative controls (values pooled across the five clinical isolates).

Concentration (mg/mL)	Neem (mm)	Turmeric (mm)	Positive control (mm)	Negative control (mm)
25	10.05	8.01	—	—
50	15.19	13.11	—	—
100	21.07	18.27	—	—
Overall mean	15.44	13.13	24.03	0.42

3.3 Activity by clinical isolate at 100 mg/mL

At the highest concentration (100 mg/mL), both extracts were active against every isolate, with neem consistently producing larger zones (Table 3, Figure 2). Neem zones ranged narrowly from 20.37 mm (*S. aureus*) to 21.80 mm (*P. aeruginosa*), while turmeric zones ranged from 17.43 mm (*P. aeruginosa*) to 18.93 mm (*E. coli*). One-way ANOVA showed no statistically significant difference in zone size across the five organisms for either extract (neem $F = 1.28$, $p = 0.34$; turmeric $F = 1.47$, $p = 0.28$), indicating broadly similar susceptibility of Gram-positive and Gram-negative isolates to each extract at this concentration.

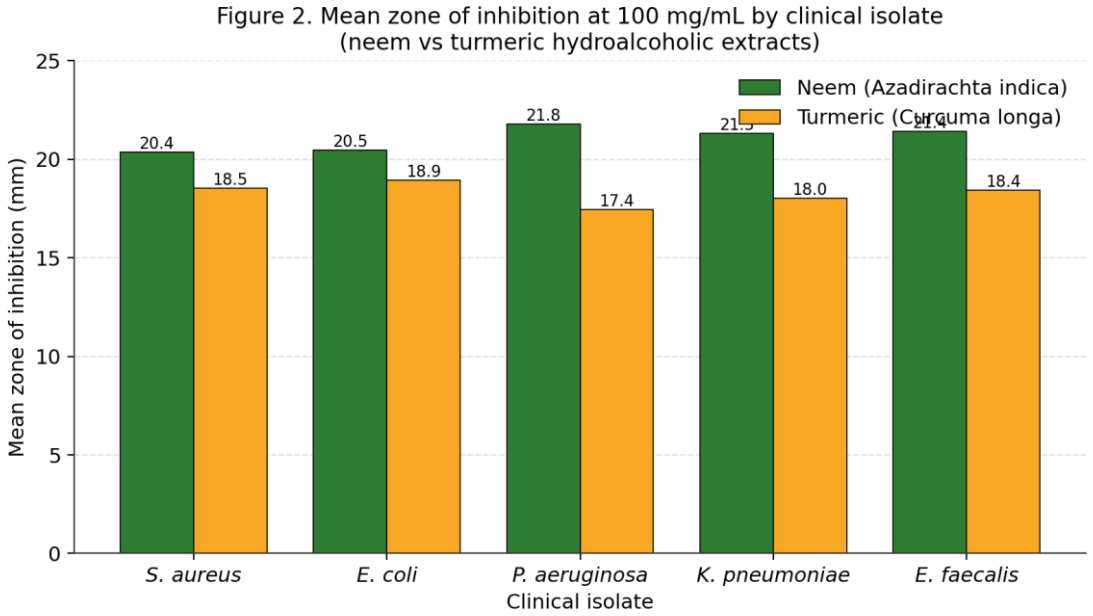

Figure 2. Mean zone of inhibition (mm) at 100 mg/mL for each of the five clinical isolates, comparing neem and turmeric hydro alcoholic extracts. Neem produced larger zones against every organism; inter-species differences were not statistically significant for either extract.

Table 3. Mean zone of inhibition (mm) at 100 mg/mL by clinical isolate for neem and turmeric extracts.

Clinical isolate	Neem (mm)	Turmeric (mm)
Staphylococcus aureus	20.37	18.53
Escherichia coli	20.47	18.93
Pseudomonas aeruginosa	21.80	17.43
Klebsiella pneumoniae	21.30	18.03
Enterococcus faecalis	21.43	18.43

3.4 MIC and MBC

Broth micro dilution revealed that inhibitory and bactericidal potency was organism-dependent rather than uniformly favoring one extract (Table 4). Both extracts shared identical MIC/MBC (0.5/2.0 mg/mL) against *S. aureus*. Turmeric was notably more potent than neem against *E. coli* (MIC 0.125 vs 0.5 mg/mL; MBC 0.5 vs 1.0 mg/mL). Conversely, neem was more potent against *P. aeruginosa* (MIC 0.125 vs 0.5 mg/mL; MBC 0.5 vs 2.0 mg/mL) and *E. faecalis* (MIC 0.125 vs 0.5 mg/mL; MBC 0.5 vs 1.0 mg/mL), and modestly more potent against *K. pneumoniae* (MIC 0.5 vs 1.0 mg/mL). For most organisms the MBC was within two- to four-fold of the MIC, consistent with a bactericidal rather than merely bacteriostatic effect. These findings complement the zone-of-inhibition data by showing that, although neem was superior in overall diffusion assays, turmeric held a specific potency advantage against *E. coli*.

Table 4. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) in mg/mL for neem and turmeric extracts by clinical isolate.

Clinical isolate	Neem MIC	Neem MBC	Turmeric MIC	Turmeric MBC
Staphylococcus aureus	0.5	2.0	0.5	2.0
Escherichia coli	0.5	1.0	0.125	0.5

Pseudomonas aeruginosa	0.125	0.5	0.5	2.0
Klebsiella pneumoniae	0.5	1.0	1.0	2.0
Enterococcus faecalis	0.125	0.5	0.5	1.0

4. DISCUSSION

This in vitro study provides a standardised, head-to-head comparison of hydroalcoholic extracts of neem leaf and turmeric rhizome against a panel of five clinically important bacterial isolates. Three principal findings emerge. First, both extracts exhibited robust, concentration-dependent antibacterial activity against Gram-positive and Gram-negative organisms. Second, neem was, overall, the more potent extract in the agar well diffusion assay, producing significantly larger zones of inhibition than turmeric (15.44 vs 13.13 mm; $p = 0.017$). Third, potency at the level of MIC/MBC was organism-dependent, with turmeric outperforming neem against *E. coli* while neem was superior against *P. aeruginosa* and *E. faecalis*. Together these observations paint a nuanced picture in which the two botanicals are complementary rather than simply ranked [11-14].

The superior overall diffusion activity of neem is biologically coherent in light of its phytochemical profile. The neem extract contained both higher total phenolic content (165.91 vs 148.54 mg GAE/g) and markedly higher total flavonoid content (74.84 vs 57.79 mg QE/g) than turmeric. The larger neem zones are consistent with a greater dose of active polyphenols delivered per well, and the parallel dose–response curves for both extracts (Figure 1) reinforce a concentration-driven mechanism. This phenolic–activity link aligns with earlier reports that neem leaf preparations possess broad antibacterial and ant secretory activity relevant to enteric and other infections [15], inhibit resistant *S. aureus* biofilm formation [2], serve as effective endodontic and antiplaque agents [3,16], and more generally underpin the extensive ethnomedical reputation of the plant [17,6].

Turmeric's activity, though somewhat lower in the diffusion assay, is equally consistent with the literature on curcumin and curcuminoids. Curcumin has been comprehensively documented as a multifunctional polyphenol with antioxidant, anti-inflammatory and antimicrobial properties [8,9], and both curcumin bio conjugates and structural analogues have shown activity against β-lactamase–producing bacteria and multidrug-resistant mycobacteria [10,18]. Turmeric oil, a byproduct of curcumin production, also carries antioxidant and ant mutagenic activity [19]. Notably, turmeric's lower diffusion-assay performance may partly reflect the physicochemical behavior of curcumin—its poor aqueous solubility and limited diffusion through agar can understate its intrinsic potency. This interpretation is supported by the MIC data, where turmeric matched or exceeded neem against *S. aureus* and clearly outperformed it against *E. coli*, indicating that agar diffusion and broth micro dilution capture partly different facets of activity.

The organism-dependent MIC/MBC pattern is one of the more clinically interesting outputs of this work. Turmeric's enhanced potency against *E. coli* and neem's advantage against *P. aeruginosa*, *E. faecalis* and *K. pneumoniae* suggest that the two extracts engage bacterial targets that differ in their susceptibility across species. The absence of a significant inter-species difference in diffusion zones for either extract (neem $p = 0.34$; turmeric $p = 0.28$) indicates that, at high concentration, both preparations are broadly active regardless of Gram status. This is encouraging from a screening perspective, since Gram-negative organisms—with their additional outer membrane barrier—are frequently less susceptible to plant extracts; the retained activity against *P. aeruginosa* and *K. pneumoniae* here is therefore noteworthy. It also echoes prior work on Indian medicinal plants demonstrating activity against multidrug-resistant enteropathogens [20].

Crude botanical extracts are unlikely to replace conventional antibiotics for the treatment of established, severe infection. Their value lies rather in low-cost preliminary screening, in the identification of lead molecules for subsequent purification, and potentially in adjuvant roles—for example, as resistance-modifying agents, topical antiseptics, or components of oral and dermatological preparations, applications for which neem in particular already has clinical precedent [3,21,22]. In the context of the traditional South Indian use of neem and turmeric, these results lend laboratory support to long-standing ethnomedical practice, situating that practice within a plausible pharmacological framework rather than treating it as merely anecdotal.

The relevance to AMR deserves emphasis. As resistance narrows the therapeutic options available in South India and elsewhere, inexpensive, locally sourced botanicals with reproducible antibacterial activity are attractive candidates for further development, particularly where the photochemistry can be standardized. The complementary organism-specific profiles of neem and turmeric also raise the possibility of combination approaches, in which the two extracts—or their purified constituents—might together cover a broader spectrum than either alone.

Several limitations should be acknowledged. The study evaluated single representative isolates of each species rather than multiple strains, and did not include characterized multidrug-resistant clinical strains, time–kill kinetics, cytotoxicity testing, or chemical fingerprinting (e.g., HPLC) of the active constituents. Agar well diffusion, while standard, is influenced by extract solubility and diffusion characteristics that may disadvantage lipophilic components such as curcumin. Finally, the extracts were crude hydro alcoholic preparations; bioassay-guided fractionation would be required to identify and quantify the specific molecules responsible for activity. Future work should address these gaps with panels of well-characterized clinical strains, mechanistic and synergy studies, and in vivo validation [23].

5. CONCLUSION

Under identical in vitro conditions, hydro alcoholic extracts of both neem leaf and turmeric rhizome demonstrated clear, concentration-dependent antibacterial activity against a panel of five common Gram-positive and Gram-negative clinical isolates. Neem was overall the more potent extract in agar well diffusion (mean zone 15.44 vs 13.13 mm; $p = 0.017$), a difference plausibly grounded in its higher phenolic and flavonoid content, whereas MIC/MBC testing revealed an organism-dependent picture in which turmeric was more potent against *E. coli* and neem against *P. aeruginosa* and *E. faecalis*. Both extracts were less potent than a standard antibiotic but vastly more active than the solvent control. These low-cost screening findings provide laboratory support for the traditional South Indian medicinal use of neem and turmeric and highlight their potential relevance as sources of antibacterial leads in an era of escalating antimicrobial resistance.

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