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Research Paper

Study of the Combined Effect of *Azadirachta indica*, *Ocimum sanctum*, and *Curcuma longa* Extracts on Common Drug-Resistant Microbial Species in India: Evaluation of Antibacterial and Antifungal Activities

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ABSTRACT

The need for alternative sources of antimicrobial agents, such as medicinal plants and polyherbal combinations, has grown due to antimicrobial resistance. The current study assessed the antibacterial efficacy of ethanolic extracts of *Curcuma longa* (turmeric), *Ocimum sanctum* (tulsi), and *Azadirachta indica* (neem) against specific bacterial and fungal infections, both separately and in combination. Soxhlet extraction was used to authenticate and extract plant materials using 95% ethanol. Neem, Tulsi, and Turmeric extract yield percentages were 18.5%, 15.8%, and 21.4%, respectively. Alkaloids, flavonoids, tannins, phenolic chemicals, and glycosides were found in all three extracts, according to preliminary phytochemical screening. Antifungal activity was measured using the agar well diffusion method against *Candida albicans* and *Aspergillus niger*, whereas antibacterial activity was examined against *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*. The combined extract generated 19.5 and 18 mm inhibition zones against *Candida albicans* and *Candida niger*, respectively. For Neem and Tulsi, the reported MIC values were 1250 µg/mL; for Turmeric, they were 625 µg/mL; and for the combined extract, they were ≤156.25 µg/mL. Neem, Tulsi, and Turmeric have FIC values of 0.125, 0.125, and 0.25, respectively, when the reported FIC values were recalculated, yielding a FICI of 0.50. According to the used FICI criterion, the results show increased antibacterial activity of the combined extract and offer preliminary evidence consistent with a synergistic interaction. To validate the relationship, more organism-specific MIC and checkerboard research is needed.

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INTRODUCTION

Due to antimicrobial resistance (AMR), which decreases the efficacy of traditional antimicrobial agents and necessitates the use of other sources of antimicrobial compounds, AMR has emerged as a significant global health concern. Flavonoids, tannins, phenolics, and alkaloids are examples of physiologically active secondary metabolites found in medicinal plants that may have antibacterial properties. [1,2] Neem, or *Azadirachta indica*, has been extensively studied for its therapeutic qualities and includes a variety of bioactive components that have been shown to have antibacterial activity. [3,8] Another traditional medicinal plant with a number of pharmacologically active components and documented antibacterial qualities is *Ocimum sanctum*, or Tulsi. [4,9] Similar biological activity, including antibacterial properties, have been shown by *Curcuma longa* (turmeric) and its primary bioactive component, curcumin. [5, 10, 15]

The use of combinations of natural products may provide enhanced antimicrobial effects through complementary or synergistic interactions among their phytoconstituents. Previous studies have highlighted the potential of combining plant-

derived products to improve antimicrobial efficacy. [6,7] Several medicinal plants and spices have also demonstrated activity against pathogenic and multidrug-resistant microorganisms, supporting their investigation as potential sources of antimicrobial agents. [11–14,16,17]

2. MATERIALS AND METHODS

2.1 Collection and Authentication of Plant Materials

Fresh healthy leaves of *Azadirachta indica* (Neem), fresh leaves of *Ocimum sanctum* (Tulsi), and mature rhizomes of *Curcuma longa* (Turmeric) were collected during May–September from authenticated herbal gardens and local medicinal-plant suppliers in Maharashtra, India. Diseased, damaged, and insect-infested materials were excluded. The plant materials were washed with tap water followed by distilled water and air-dried before processing.

The collected plant materials were authenticated by Dr. Arun K. Zingare, Ph.D. Supervisor (Botany), M. B. Patel College of Arts, Commerce and Science, Deori. Voucher/herbarium specimens were preserved for future reference. The assigned herbarium numbers were 01 for Neem, 02 for Tulsi, and 03 for Turmeric.

Table 1. Authentication details of plant materials

Plant	Botanical name	Family	Part used	Herbarium No.
Neem	<i>Azadirachta indica</i>	Meliaceae	Leaves	01
Tulsi	<i>Ocimum sanctum</i>	Lamiaceae	Leaves	02
Turmeric	<i>Curcuma longa</i>	Zingiberaceae	Rhizomes	03

2.2 Preparation of Ethanolic Extracts

The dried plant materials were powdered and extracted separately by continuous Soxhlet extraction using 95% ethanol. For each extraction, 100 g of powdered plant material was extracted with 500 mL ethanol using a Soxhlet apparatus. The solvent was subsequently recovered and the

extracts were concentrated and dried. The percentage yield was calculated as:

$$\text{Percentage yield} = \frac{\text{Weight of dried extract}}{\text{Weight of plant powder}} \times 100$$

The extraction procedure and quantities were based on the experimental procedure documented in the thesis.



2.3 Preliminary Phytochemical Screening

Approximately 1 g of each dried ethanolic extract was dissolved in 10 mL ethanol and filtered. The filtrates were subjected to standard qualitative phytochemical tests for alkaloids, flavonoids, tannins, phenolic compounds, and glycosides.

The reported screening showed positive reactions for alkaloids, flavonoids, tannins, phenolic compounds, and glycosides in all three extracts.

2.4 Antibacterial Activity

Antibacterial activity was evaluated by the agar well diffusion method against *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*. Nutrient agar containing peptone (5 g/L), beef extract (3 g/L), sodium chloride (5 g/L), and agar (15 g/L) was prepared and adjusted to pH 7.2. The medium was sterilized at 121°C and 15 lb pressure for 15 min.

Fresh bacterial cultures were grown in nutrient broth at 37°C for 24 h and adjusted to 0.5 McFarland turbidity, corresponding to approximately 1.5×10^8 CFU/mL.

Wells of approximately 6 mm diameter were prepared in inoculated nutrient agar plates. Approximately 100 µL of each individual extract and the combined extract was introduced into the respective wells. After diffusion, plates were incubated at 37°C for 24 h. Zones of inhibition were measured in millimetres. Experiments were performed in triplicate and results were expressed as mean values with standard deviation.

2.5 Antifungal Activity

Antifungal activity was evaluated against *Candida albicans* and *Aspergillus niger* using Sabouraud Dextrose Agar (SDA). The medium consisted of peptone (10 g/L), dextrose (40 g/L), agar (15 g/L), and distilled water, with the pH adjusted to approximately 5.6. The medium was sterilized at 121°C and 15 lb pressure for 15 min.

Fungal cultures were subcultured on SDA slants and suspended in sterile saline. The suspensions were uniformly inoculated onto SDA plates. Wells approximately 6 mm in diameter were prepared, and 100 µL of each extract or combined extract was added. Plates were allowed to stand for approximately 30 min for diffusion and were then incubated at 28°C for 48 h. Zones of inhibition were measured in millimetres.

2.6 Preparation of Extract Stock Solutions

Stock solutions were prepared by dissolving 100 mg of dried extract in 10 mL solvent to obtain a concentration of 10 mg/mL, equivalent to 10,000 µg/mL. The thesis records sterile distilled water as the solvent for the stock solution.

The prepared stocks were used for antimicrobial and MIC studies.

2.7 Determination of Minimum Inhibitory Concentration

MIC was determined by the tube dilution method using serial two-fold dilutions of the extract. The tested concentrations were 5000, 2500, 1250, 625, 312.5, and 156.25 µg/mL. The MIC was defined as the lowest concentration showing no visible turbidity.

The MIC experiment in the thesis was conducted using a mixed bacterial inoculum containing *S. aureus*, *E. coli*, and *P. aeruginosa*.

The individual MIC values reported were:

- Neem: 1250 µg/mL
- Tulsi: 1250 µg/mL
- Turmeric: 625 µg/mL
- Combined extract: ≤ 156.25 µg/mL

For the combined extract, all tested concentrations down to 156.25 µg/mL were reported as clear. Because 156.25 µg/mL was the lowest concentration tested, the scientifically conservative expression is ≤ 156.25 µg/mL, rather than an exact MIC of 156.25 µg/mL.



2.8 FIC Index Calculation

The fractional inhibitory concentration was calculated using:

$$FIC_A = \frac{MIC_A \text{ in combination}}{MIC_A \text{ alone}}$$

and:

$$FICI = FIC_{Neem} + FIC_{Tulsi} + FIC_{Turmeric}$$

Thus:

$$FICI = 0.125 + 0.125 + 0.25$$

$$FICI = 0.50$$

The thesis used ≤ 0.5 as the criterion for synergistic interaction.

Important methodological qualification: because the thesis reports a single combined MIC against a mixed bacterial inoculum and does not provide organism-specific combination concentrations, the FICI should be considered **preliminary evidence consistent with synergism**, rather than definitive confirmation.

2.9 Statistical Analysis

Antimicrobial experiments were performed in triplicate. Results were expressed as mean $\pm$ SD. One-way analysis of variance (ANOVA) was used for comparison, with $p < 0.05$ considered statistically significant according to the thesis methodology.

Because replicate-level raw measurements were not available in the manuscript source, the reported SD values are retained as thesis-reported values and should ideally be verified against the original laboratory observations before journal submission.

3. RESULTS

3.1 Extraction Yield

The extraction yields differed among the three medicinal plants. Turmeric showed the highest yield (21.4%), followed by Neem (18.5%) and Tulsi (15.8%).

Table 2. Percentage yield of ethanolic extracts

Plant	Powder taken (g)	Extract obtained (g)	Yield (%)
<i>A. indica</i>	100	18.5	18.5
<i>O. sanctum</i>	100	15.8	15.8
<i>C. longa</i>	100	21.4	21.4

The relatively higher extractive yield of Turmeric indicates greater recovery of ethanol-soluble constituents under the selected extraction conditions.

All three ethanolic extracts showed positive reactions for the major phytochemical groups evaluated.

3.2 Preliminary Phytochemical Screening

Table 3. Preliminary phytochemical screening

Phytoconstituent	Neem	Tulsi	Turmeric
Alkaloids	+	+	+
Flavonoids	+	+	+
Tannins	+	+	+
Phenolic compounds	+	+	+
Glycosides	+	+	+



The presence of these classes of secondary metabolites provides a possible chemical basis for the observed antimicrobial activity, although qualitative screening does not establish the identity, concentration, or contribution of individual compounds.

3.3 Antibacterial Activity

The individual extracts demonstrated antibacterial activity against all three tested organisms. Turmeric showed the greatest individual antibacterial activity, whereas the combined extract produced the largest zones of inhibition.

Table 4. Antibacterial activity of individual and combined extracts

Test organism	Neem (mm)	Tulsi (mm)	Turmeric (mm)	Combined (mm)
<i>S. aureus</i>	10.0	9.5	14.0	21.0
<i>E. coli</i>	9.5	8.0	13.0	20.0
<i>P. aeruginosa</i>	8.0	7.5	13.0	20.5

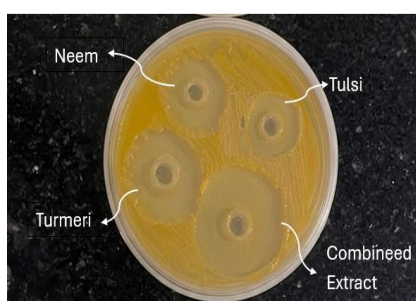


Figure 01 - Cup Plate Method of *S. Aureus*

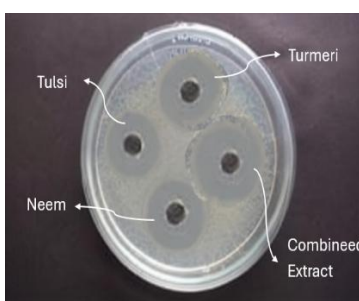


Figure 02 - Cup Plate Method of *E. Coli*

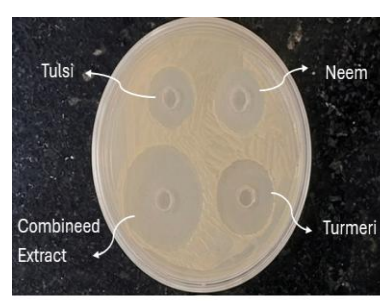
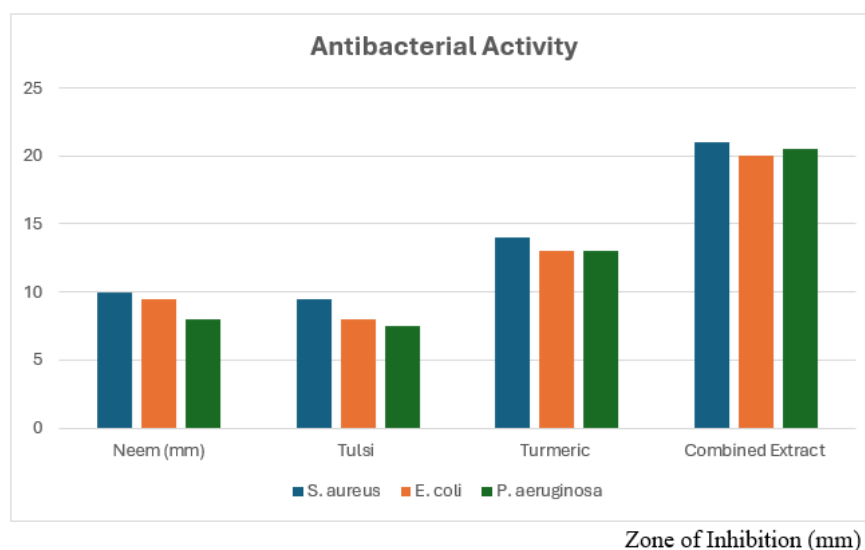


Figure 03 - Cup Plate Method of *P. Aeruginosa*

The combined extract produced the highest inhibition against all three bacterial organisms. The reported overall mean inhibition zones were

9.16 ± 0.5 mm for Neem, 8.3 ± 0.4 mm for Tulsi, 13.33 ± 0.3 mm for Turmeric, and 20.5 ± 0.6 mm for the combined extract, with reported statistical significance at $p < 0.05$.



Graph 01: Antibacterial Activity

These findings demonstrate **enhanced antibacterial activity** of the combined preparation compared with the individual extracts. However, inhibition-zone enhancement alone

should not be interpreted as definitive proof of synergism.

3.4 Antifungal Activity

All tested extracts showed antifungal activity against *C. albicans* and *A. niger*. Among the

individual extracts, Neem produced the greatest inhibition zones.

Table 5. Antifungal activity of individual and combined extracts

Test organism	Neem (mm)	Tulsi (mm)	Turmeric (mm)	Combined (mm)
<i>C. albicans</i>	12.5	10.0	10.5	19.5
<i>A. niger</i>	11.0	10.0	9.0	18.0

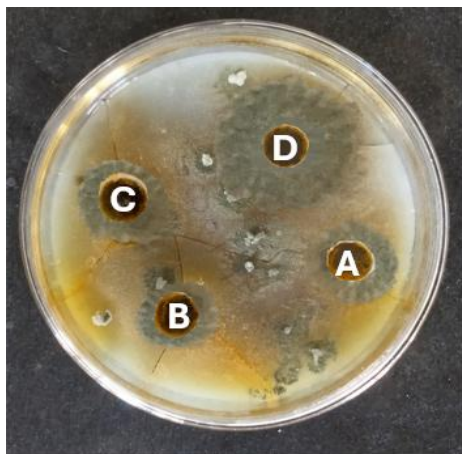


Figure 04 - Cup Plate of *Candida albicans*

The combined extract produced 19.5 mm inhibition against *C. albicans* and 18 mm against *A. niger*, compared with 12.5 and 11 mm, respectively, for Neem, the most active individual extract.

Relative to Neem, the combined extract increased the inhibition zone by approximately **56.0% against *C. albicans*** and **63.6% against *A. niger***.

The thesis reported overall mean zones of 11.75 ± 0.4 mm for Neem, 10.0 ± 0.3 mm for Tulsi, $9.75 \pm$

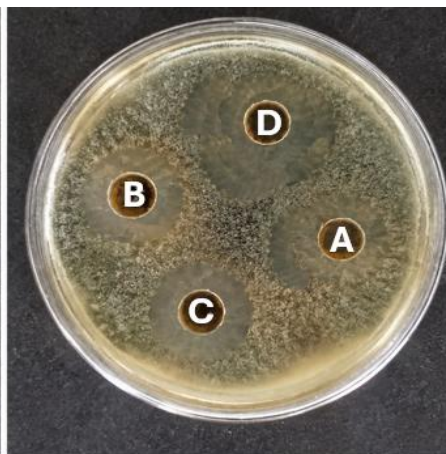


Figure 05 - Cup Plate of *Aspergillus niger*

0.2 mm for Turmeric, and 18.75 ± 0.5 mm for the combined extract, with $p < 0.05$.

3.5 Minimum Inhibitory Concentration

The MIC results showed greater inhibitory potency of the combined preparation than the individual extracts.

Table 6. Comparative MIC values

Extract	MIC reported ($\mu\text{g/mL}$)
Neem	1250
Tulsi	1250
Turmeric	625
Combined extract	≤ 156.25

Neem and Tulsi showed growth inhibition up to $1250 \mu\text{g/mL}$, while turbidity appeared at $625 \mu\text{g/mL}$ and lower concentrations.

Turmeric showed a lower MIC of $625 \mu\text{g/mL}$. The combined extract remained clear at all tested concentrations, including $156.25 \mu\text{g/mL}$.

The combined preparation therefore required substantially less extract to inhibit the tested mixed bacterial inoculum than the individual extracts.

3.6 Corrected FIC Index

Table 7. Corrected FIC calculation

Extract	MIC alone (µg/mL)	MIC in combination used for calculation (µg/mL)	Corrected FIC
Neem	1250	156.25	0.125
Tulsi	1250	156.25	0.125
Turmeric	625	156.25	0.250
FICI	—	—	0.500

The original thesis reported Neem FIC as 0.25, but $156.25/1250$ equals 0.125. Correcting this arithmetic produces a total FICI of 0.50.

According to the criterion used in the thesis, a FICI ≤ 0.5 was interpreted as synergistic.

Therefore, the corrected FICI of **0.50 is consistent with synergistic interaction under the predefined criterion.**

Nevertheless, because the MIC experiment was performed against a mixed bacterial inoculum, this result should be regarded as **preliminary evidence** rather than definitive confirmation of organism-specific synergism.

DISCUSSION

The present study investigated the antimicrobial potential of ethanolic extracts of Neem, Tulsi, and Turmeric, both individually and in combination. The study was motivated by the increasing need for alternative antimicrobial sources in the context of growing antimicrobial resistance. Plant-derived products contain diverse secondary metabolites that can exhibit antimicrobial effects through multiple mechanisms.

Soxhlet extraction with 95% ethanol produced yields of 18.5%, 15.8%, and 21.4% for Neem, Tulsi, and Turmeric, respectively. Turmeric gave the highest yield under the selected extraction conditions. Differences in extractive yield may reflect variation in plant part, chemical composition, polarity of constituents, moisture

content, and extraction efficiency. The present yield results are therefore useful for comparing recovery under the specific experimental conditions but should not be interpreted as direct measures of antimicrobial potency.

Preliminary phytochemical screening demonstrated alkaloids, flavonoids, tannins, phenolic compounds, and glycosides in all three extracts. These findings provide a broad phytochemical basis for the observed antimicrobial effects. However, qualitative phytochemical screening cannot establish which individual compounds are responsible for activity. Quantitative chromatographic or spectroscopic characterization would therefore be required to correlate specific constituents with antimicrobial effects.

The antibacterial results demonstrated clear differences among the extracts. Turmeric was the most active individual extract, producing inhibition zones of 14 mm against *S. aureus* and 13 mm against both *E. coli* and *P. aeruginosa*. Neem and Tulsi showed lower inhibition zones. The stronger activity of Turmeric may reflect its characteristic bioactive constituents, although the present study did not chemically quantify these compounds.

The combined extract produced considerably larger inhibition zones than the individual preparations. Zones of 21, 20, and 20.5 mm were observed against *S. aureus*, *E. coli*, and *P. aeruginosa*, respectively. This consistent increase



across both Gram-positive and Gram-negative bacteria suggests that combining the extracts may improve overall antimicrobial activity. Plant combinations can theoretically provide complementary mechanisms of action because their constituents may interact with multiple microbial targets. Such multi-component interactions have been discussed in the phytopharmaceutical literature.

The antifungal results followed a similar pattern. Neem demonstrated the strongest individual antifungal activity, whereas the combined preparation produced the greatest inhibition against both *C. albicans* and *A. niger*. The increase from 12.5 to 19.5 mm against *C. albicans* and from 11 to 18 mm against *A. niger* indicates substantially enhanced activity of the combined extract.

The MIC findings further support the enhanced antimicrobial activity of the combination. Neem and Tulsi showed MIC values of 1250 µg/mL, while Turmeric showed 625 µg/mL. The combined extract remained inhibitory at the lowest tested concentration of 156.25 µg/mL. Because this was the lowest concentration tested, its MIC should conservatively be reported as **≤156.25 µg/mL** rather than exactly 156.25 µg/mL.

The FIC calculation required correction. The thesis stated:

$$FIC_{Neem} = \frac{156.25}{1250} = 0.25$$

but the correct arithmetic is:

$$\frac{156.25}{1250} = 0.125$$

Thus, the corrected FIC values are 0.125 for Neem, 0.125 for Tulsi, and 0.25 for Turmeric, giving:

$$FICI = 0.125 + 0.125 + 0.25 = 0.50$$

The corrected value is therefore consistent with the thesis's predefined criterion for synergistic interaction.

However, an important methodological limitation must be acknowledged. The MIC assay was conducted using a mixed bacterial inoculum containing *S. aureus*, *E. coli*, and *P. aeruginosa*. Consequently, the reported MIC represents inhibition of the mixed inoculum rather than a species-specific MIC. Similarly, the FICI calculation is based on the reported overall combination value rather than separate organism-specific checkerboard matrices. FICI methodology is useful for investigating antimicrobial interactions, but interpretation depends strongly on experimental design and reproducibility. ([PubMed](#))

Therefore, the present findings should be described as **enhanced antimicrobial activity with preliminary evidence consistent with synergistic interaction**, rather than definitive confirmation of synergism.

A further limitation is that the study used preliminary phytochemical screening rather than quantitative chemical profiling. Future work should identify and quantify the principal active constituents using chromatographic techniques such as HPLC, LC-MS, or GC-MS where appropriate. Species-specific MIC testing and checkerboard assays should also be conducted separately against each microorganism. Time-kill experiments could provide additional evidence regarding the dynamics of the interaction.

CONCLUSION

The present study demonstrated that ethanolic extracts of *Azadirachta indica*, *Ocimum sanctum*, and *Curcuma longa* possess antimicrobial activity against selected bacterial and fungal pathogens. Turmeric exhibited the highest antibacterial activity among the individual extracts, whereas Neem showed the greatest individual antifungal activity.

The combined extract demonstrated markedly enhanced antimicrobial activity, producing larger



inhibition zones against all tested microorganisms than the individual extracts. The combined preparation also showed a substantially lower reported MIC ($\leq 156.25 \mu\text{g/mL}$) than Neem, Tulsi, or Turmeric individually.

Correction of the FIC arithmetic yielded FIC values of 0.125, 0.125, and 0.25 for Neem, Tulsi, and Turmeric, respectively, resulting in a FICI of 0.50. According to the criterion used in the study, this value is consistent with a synergistic interaction.

However, because the MIC/FIC experiment was performed using a mixed bacterial inoculum and did not include organism-specific checkerboard testing, the result should be regarded as **preliminary evidence of synergism** rather than definitive confirmation. Further species-specific MIC, checkerboard FICI, time-kill, quantitative phytochemical, toxicity, and in-vivo studies are recommended to establish the reproducibility, mechanism, safety, and therapeutic relevance of the polyherbal combination.

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