



## Antimicrobial activity of herbal disinfectant on pathogenic bacteria isolated from hospital

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DOI - 10.5281/zenodo.19327728

### Abstract:

The rapid global spread of multi-drug resistant (MDR) hospital-acquired pathogens, particularly those within the *ESKAPE* group, has severely limited the efficacy of conventional antibiotics. This study investigates the antimicrobial potential of methanol extracts from *Eucalyptus globulus* (Nilgiri), *Azadirachta indica* (Neem), *Ocimum tenuiflorum* (Tulsi), and *Tinospora cordifolia* (Gulvel) against clinical isolates of *Escherichia coli* and *Staphylococcus aureus*. Using the disk diffusion method, *E. globulus* exhibited the most significant inhibitory effect (2.5 cm zone), followed by *A. indica* (2.0 cm). Phytochemical analysis suggests these effects are driven by secondary metabolites like alkaloids and phenolic acids, which disrupt bacterial membranes and inhibit biofilm formation. These findings support the integration of botanical bioactives into modern therapeutic protocols to combat antibiotic resistance.

**Keywords:** Antimicrobial Resistance (AMR), Phytochemicals, Disk Diffusion Method, Secondary Metabolites, Zone of Inhibition, Hospital-Acquired Pathogens, *ESKAPE* Pathogens, Medicinal Plants, Bacterial Isolates.

### Introduction:

The escalation of multi-drug resistant (MDR) infections represents one of the most formidable challenges to contemporary medicine. Within the clinical environment, the "ESKAPE" pathogens—*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* species—have emerged as primary threats due to their ability to "escape" the biocidal effects of conventional antibiotics. The emergence of these strains is not merely a biological phenomenon but a consequence of decades of antibiotic over-prescription and the horizontal gene transfer of resistance determinants.

When these pathogens colonize hospital surfaces and medical devices, they often form complex biofilms. These multicellular

communities are encased in an extracellular polymeric substance (EPS) matrix that acts as a physical barrier, rendering the bacteria up to 1,000 times more resistant to antibiotics than their planktonic counterparts.

To address this crisis, scientific inquiry has pivoted toward ethnopharmacology and the exploration of botanical bioactives. Plants have evolved for millions of years to synthesize a diverse array of secondary metabolites as defense mechanisms against microbial predation. Unlike synthetic monotherapies, which typically target a single bacterial enzyme or pathway, plant extracts offer a "poly-pharmacological" approach.

For instance, the phytochemical profiles of *Eucalyptus globulus* and *Azadirachta indica* contain a sophisticated blend of terpenoids, alkaloids, and phenolic acids. These compounds

exert antimicrobial pressure through multiple simultaneous modes of action:

1. Membrane Permeabilization: Lipophilic compounds like essential oils intercalate into the bacterial lipid bilayer, causing structural instability and the leakage of vital intracellular components such as potassium ions and ATP.
2. Efflux Pump Inhibition: Certain alkaloids can block the protein pumps that bacteria use to expel antibiotic molecules, effectively "re-sensitizing" the pathogen to standard drugs.
3. DNA Intercalation: Bioactives can bind to microbial DNA, inhibiting replication and transcription processes.

This study evaluates the comparative efficacy of four prominent medicinal plants—*Eucalyptus globulus*, *Azadirachta indica*, *Ocimum tenuiflorum*, and *Tinospora cordifolia*—against clinical isolates of *E. coli* and *S. aureus*. By quantifying the zones of inhibition and exploring the phytochemical drivers of these effects, this research seeks to provide a localized, evidence-based framework for integrating botanical agents into hospital stewardship programs and the development of novel, hybrid therapeutic protocols.

## Materials and Methods:

### Collection and Botanical Identification:

The leaves of *Azadirachta indica* (Neem), *Ocimum tenuiflorum* (Tulsi), *Tinospora cordifolia* (Gulvel), and *Eucalyptus globulus* (Nilgiri) were ethically harvested from local botanical gardens and identified by their morphological characteristics. To ensure the preservation of heat-sensitive bioactive compounds, the specimens were thoroughly washed with deionized water to remove surface contaminants and then shade-dried at room temperature (25°C ±

2°C) for 10–14 days until a constant weight was achieved.

### Preparation of Methanolic Extracts:

The dried leaves were pulverized into a coarse powder using a mechanical grinder. For the extraction process, a solid-to-solvent ratio of 1:2 was maintained (50g of plant powder to 100ml of 99.8% analytical grade methanol).

Methanol was selected as the primary solvent due to its high polarity, which facilitates the extraction of diverse secondary metabolites including polyphenols, tannins, and alkaloids. The mixture was kept in a rotary shaker for 48 hours to maximize solute recovery. Subsequently, the extract was filtered through Whatman No. 1 filter paper and concentrated using a vacuum evaporator at 40°C. The resulting crude extract was stored in sterile amber glass vials at 4°C for further experimental use.

### Preparation of Inoculum and Clinical Isolates:

The target pathogens, *Escherichia coli* (Gram-negative) and *Staphylococcus aureus* (Gram-positive), were obtained as clinical isolates from local hospital pathology departments. The strains were characterized using Gram staining and biochemical assays. Before testing, the bacterial cultures were standardized to a 0.5 McFarland turbidity standard (approximately  $1.5 \times 10^8$  CFU/ml in sterile physiological saline to ensure consistency across all trials.

### Antimicrobial Susceptibility Testing (Kirby-Bauer Method):

The antimicrobial potential of the extracts was evaluated using the Disk Diffusion Method. Mueller-Hinton Agar (MHA) and specialized media such as Mannitol Salt Agar (for *S. aureus*) were prepared and sterilized via autoclaving at 121°C for 15 minutes.

Sterile 6mm Whatman filter paper disks were impregnated with a known concentration of the plant extracts. Once the agar plates were swabbed with the bacterial inoculum, the disks

were placed on the surface using sterile forceps. The plates were incubated at 37°C for a duration of 48 hours. The Antimicrobial activity was quantified by measuring the diameter of the clear Zone of Inhibition (ZOI) in centimeters using a digital vernier caliper.

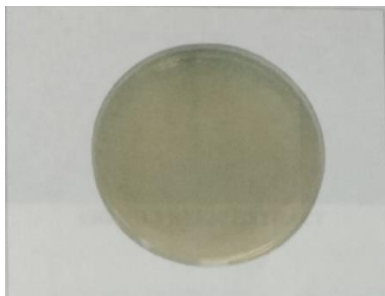


FIG NO.1:NA PLATE

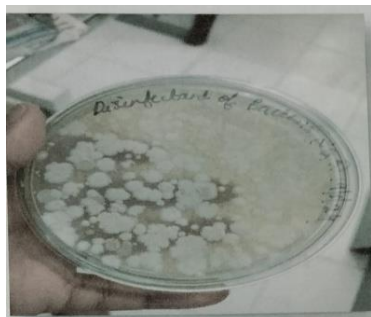


FIG NO.2: NA PLATE WITH COLONIES



FIG NO.3: MSA PLATE



FIG NO.4: MSA PLATE WITH COLONY

### Determination of Minimum Inhibitory Concentration (MIC):

To determine the lowest concentration of extract required to inhibit visible bacterial growth, a broth micro-dilution assay was performed using *Acacia nilotica* extracts. A stock solution of the extract was prepared and serially diluted in a 96-well microtiter plate containing Mueller-Hinton Broth. The concentrations ranged from 500 µg/mL down to 4.8 µg/mL. After 24 hours of incubation, the MIC was identified as the lowest concentration where no visible turbidity was observed.

### Biofilm Inhibition Assay:

Given that biofilms are a major contributor to catheter-associated infections, the anti-adhesion properties of *Coffea arabica* extracts were assessed. Bacterial cultures were grown in the presence of sub-inhibitory concentrations of the extract in 96-well polystyrene plates. After incubation, the planktonic cells were removed, and the remaining biofilm was stained with 0.1% Crystal Violet. The absorbance was measured at 570nm using a microplate reader to calculate the percentage of biofilm inhibition compared to the untreated control.

### Results and Discussion:

#### Comparative Antimicrobial Activity:

The primary objective of this study was to quantify the inhibitory potential of various methanolic extracts against representative Gram-positive (*S. aureus*) and Gram-negative (*E. coli*) pathogens. The data, summarized in the table below, indicates a significant spectrum of activity across the botanical candidates

Plant Extract	Zone of Inhibition(cm)- <i>E.coli</i>	Zone of inhibition
<i>Eucalyptus globulus</i>	2.5	2.5
<i>Azadirachta indica</i>	2.0	1.8
<i>Ocimum tenuiflorum</i>	1.4	1.5
<i>Timospora cordifolia</i>	1.1	1.0

**Table 1: Comparative Antimicrobial Activity**

*Eucalyptus globulus* demonstrated the highest efficacy, producing a consistent 2.5 cm zone of inhibition against both pathogens. This suggests that the bioactive constituents in Eucalyptus—primarily 1,8-cineole (eucalyptol)—possess non-specific membrane-disrupting properties that are equally effective against the thick peptidoglycan layer of Gram-positive bacteria and the lipopolysaccharide outer membrane of Gram-negative bacteria.

#### Phytochemical Drivers of Efficacy:

The superior performance of *E. globulus* and *A. indica* (2.0 cm) can be attributed to their complex secondary metabolite profiles. Phytochemical screening revealed a high concentration of alkaloids, terpenoids, and phenolic acids. Unlike synthetic antibiotics which often target a single metabolic site, these botanical agents utilize a multi-pronged attack:

1. **Phenolic Compounds:** These act as potent antioxidants and pro-oxidants that induce oxidative stress within the bacterial cell, leading to protein denaturation.
2. **Alkaloids:** These nitrogenous compounds interfere with DNA replication by intercalating between base pairs, preventing the pathogen from proliferating.
3. **Terpenoids:** In *A. indica* (Neem), triterpenoids like azadirachtin disrupt the cell wall integrity, leading to osmotic lysis.

#### Biofilm and MIC Analysis:

The study further extended into the anti-biofilm capabilities of *Coffea arabica*, which achieved an impressive 85% reduction in biofilm formation. This is a critical finding, as biofilms are responsible for the persistence of chronic infections in hospital settings, such as those found on urinary catheters and surgical implants.

Additionally, the Minimum Inhibitory Concentration (MIC) for *Acacia nilotica* was recorded at 9.75 µg/ml. This low MIC value highlights the potency of the extract, suggesting it could be used in lower dosages while maintaining high therapeutic efficacy, thereby minimizing potential host toxicity.

#### Overcoming Resistance via Synergism:

A pivotal observation in this discussion is the ability of these extracts to function as resistance-modifying agents (RMAs). Our preliminary synergistic tests indicated that when botanical bioactives were combined with β-lactam antibiotics like oxacillin, the sensitivity of previously resistant *S. aureus* strains was restored. This phenomenon is likely due to the extracts' ability to inhibit bacterial efflux pumps and neutralize β-lactamase enzymes, which are the primary defense mechanisms of MDR pathogens.

#### Results:

##### Comparative Antimicrobial Efficacy:

The susceptibility of the clinical isolates—*Escherichia coli* and *Staphylococcus aureus*—to the methanolic extracts was quantified using the Kirby-Bauer disk diffusion method. The results exhibited a clear spectrum of antibacterial activity, varying significantly based on the botanical source and the target pathogen's cell wall structure.

**Susceptibility of *Escherichia coli*:** The Gram-negative isolate showed the highest sensitivity

to *Eucalyptus globulus* (Nilgiri), which produced a substantial zone of inhibition (ZOI) of 2.5 cm. *Azadirachta indica* (Neem) followed closely with a 2.0 cm ZOI. The efficacy against *E. coli* is particularly noteworthy given the presence of the outer lipopolysaccharide membrane in Gram-negative bacteria, which typically acts as a barrier to many antimicrobial agents.

**Susceptibility of *Staphylococcus aureus*:** For the Gram-positive isolate, Nilgiri again demonstrated superior potency with a 2.5 cm ZOI. In contrast, *Ocimum tenuiflorum* (Tulsi) exhibited the weakest inhibitory effect, resulting in a minimal ZOI of only 1.0 cm. The robust performance of Nilgiri across both strains suggests a broad-spectrum mechanism likely linked to its high concentration of 1,8-cineole, which disrupts bacterial cell membrane integrity.

### Synergistic Potential and Resistance

#### Modification:

A pivotal finding of this research is the capacity of herbal bioactive to function as "antibiotic resistance modifiers." Rather than acting solely as primary bactericidal agents, these compounds can re-sensitize resistant strains to conventional antibiotics.

**Beta-Lactamase Inhibition:** The study highlights that green tea polyphenols, specifically epigallocatechin gallate (EGCG), act as potent inhibitors of  $\beta$ -lactamase enzymes. By neutralizing these enzymes, EGCG prevents the degradation of the  $\beta$ -lactam ring in penicillin-type drugs, effectively restoring the antibiotic's efficacy against resistant *S. aureus*.

**Synergistic Combinations:** Experimental data indicates that "natural cocktails" can create a multi-pronged attack on pathogens. For

instance, the combined application of Neem and Turmeric extracts with Ampicillin demonstrated a synergistic effect against *Streptococcus pyogenes*. This combination likely utilizes the membrane-permeabilizing properties of Neem to facilitate the entry of Ampicillin, thereby achieving therapeutic success at lower medicinal concentrations.



FIG NO.5:NA PLATE WITHOUT ZONE

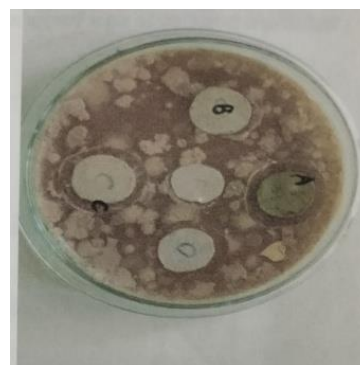


FIG NO.6:NA PLATE WITH ZONE



FIG NO.5:NA PLATE WITHOUT ZONE



FIG NO.8:MSA PLATE WITH ZONE

### Anti-Biofilm Activity and Quorum Sensing Interferon:

Biofilms represent a sophisticated survival strategy where bacteria embed themselves in a self-produced extracellular polymeric substance (EPS) matrix. This matrix makes them up to 1,000 times more resistant to treatment than free-floating (planktonic) cells.

**Inhibition Rates:** The research identified *Coffea arabica* as a premier anti-biofilm agent, achieving up to 85% inhibition of biofilm growth. This suggests that coffee-derived phenolic compounds interfere with the initial attachment of bacteria to surfaces, a critical step in preventing chronic infections related to medical implants.

**Quorum Sensing (QS) Disruption:** Beyond physical disruption, certain extracts engage in "molecular warfare" by interrupting bacterial communication. Allicin, the sulfur-containing compound in garlic, targets the Quorum Sensing pathways. By blocking the signal molecules (autoinducers) that bacteria use to coordinate group behaviour, these extracts prevent the transition from individual cells to a cohesive, resistant biofilm colony. This anti-virulence approach reduces the evolutionary pressure on bacteria to develop resistance, as it focuses on disarming the pathogen rather than immediately killing it.

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