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**ORIGINAL RESEARCH ARTICLE**

# Study of Antimicrobial Activity of Natural Plant Oils against Bacterial Species Isolated From Hospital Sample

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## ABSTRACT

The aim of this work was to investigate the antimicrobial activity of six natural plant oils (lemon oil, Neem oil, Orange oil, Jasmine oil, Eucalyptus oil and lime oil) against Gram positive and Gram negative bacteria, isolated from hospital sample. The antimicrobial activity of plant oils was determined by an Agar Diffusion method against the isolated bacteria. Further bacteriostatic concentration were determined for each bacteria that evidenced sensitivity to the plants oil .All the plants oil showed bacteriostatic activity against *E. coli* and *Staphylococcus* was resistant to lemon oil .The test organism showed resistance to Eucalyptus oil. Hence the plant oil considered in this research showed a satisfactory antimicrobial activity and in conclusion, plants oil can be used as antimicrobial agents.

**Key words:** Plants oil, *E.coli*, *Staphylococcus*, *Pseudomonas* and agar diffusion method, MIC assay.

## INTRODUCTION

Plants oil (also called volatile oils) are aromatic oily liquids obtained from plant materials (flowers, buds, seeds, leaves, twigs, bark and roots).Essential oils are complex mixers comprising many single compounds obtained by fermentation or method of steam distillation used commercially in fragrance market . These oils are gaining interest in ayurveda worldwide in medicinal field [1, 4]. Gram negative bacteria (*E. coli*, *Pseudomonas*) and Gram positive bacteria (*Staphylococcus*) were used.

Neem oil (Azadirachta indica) is an herbal plant which is effective against pathogenic bacteria of fish. It is also effective against diarrhea and cholera. Anticarcinogenic and hepatocarcinogenic activity is seen in Neem oil extract [2]. Lemon oil (*Citrus aurifolia*) contains about 90% monoterpine hydrocarbons, composed mainly of limonene and 2 to 6 % aldehydes [3] .Lemon oil is widely used for its antioxidant properties [2], and in perfume industry for fragnance and aroma [4]. Orange oil (Citrus sinensis) has a fresh tangy smell, yellow to orange in color; with watery in viscocity.It has shelf life of 6 months. It is used in flavoring of food and to polish the furniture to protect from damage against insects. The main chemical components of orange oil are a-pinene, sabinene, myrcene, limonene, linalool, citronellal, neral and geranial [5]. Jasmine oil is cheap synthetic oil widely used as room freshner, soap and aroma therapy [6]. It is also used to treat ulcer and in expelling worms. Eucalyptus and lime oil also have specific chemical composition which act as antimicrobial property. Some oils have also been used in cancer treatment [9].plant oil is also effective in food preservation [7].

## MATERIALS AND METHODS

### Collection of plant material:

The plant sample was collected from a healthy disease free plant. The Leaves of the healthy tree was used.

### Isolation of essential oil:

The leaves sample was washed carefully and air dried and then ground to semi-powdered state.50gms of the sample was subjected to hydro distillation in a Clevenger apparatus for 5 hrs. The sample oil was stored in air tight brown bottle at low temperature under aseptic condition.

### Antimicrobial activity:

The microbial strains isolated from hospital sample were maintained in pure culture and refrigerated and stored under aseptic condition.

### Antibacterial screening:

Screening of essential oils for antibacterial activity was done by the disk diffusion method, which is normally used as a preliminary check and

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to select between efficient essential oils [7]. The disc diffusion method was employed for the determination of antibacterial activities of plant oil. The details of procedure are described below;

1.Mueller-Hinton agar was prepared and sterilized .It was poured into sterile petriplates and allowed to solidify under aseptic condition. It was surface spread with 0.1ml of bacterial suspension adjusted to $10^7$CFU/ml with sterile saline solution (18hr culture at 37$^o$C in 10ml Mueller-Hinton Broth was used).

2.The plant oil were dissolved in 10%v/v aqueous Dimethyl sulfoxide (DMSO)with Tween 80(0.5%v/v for easy diffusion of oil in agar)and sterilized by filtration through 0.45µm membrane filter.Sterilised paper disc(6mm diameter) were impregnated with 15µl of the plant oil(At concentration of 1:1,1:5,1:10 and 1:20).The disc were then transferred onto the agar surface.(The inoculated plate were allowed to dry at room temperature for 30min prior to transfer of the disc) [6] .

3.Paper disc moistened with aqueous DMSO solvent was used as negative control.

4.The standard antibiotic Cephalosporin (30µg/disc), and Penicillin (30µg/disc) were used as positive controls.

5.The petriplate were sealed with parafilm to avoid contamination and evaporation of test sample. The plate was left at room temperature for 30min to allow diffusion of oil.

6. After incubation at 37$^0$C for 18hrs, the diameter of zone of inhibition was measured in mm. (18 h was fixed as the optimum since there was no change in the inhibition up to 24 h).The test was carried out in triplicate and the mean value was considered.

**MIC (Minimum Inhibitory Concentration) assay:**

Based on the previous screening five essential oils (lemon, lime, Orange, Neem and Jasmine) were identified to have potent antibacterial activity and their Minimum inhibitory Concentrations (MIC) were determined. The agar dilution method recommended by the National Committee for Clinical Laboratory Standards [6] was used with the following modification; a final concentration of 0.5% (v/v) Tween-80 was incorporated into the agar medium to enhance oil solubility. A series of two fold dilution of each oil, ranging from 0.2 to 25.6 mg/ml, was prepared in Mueller Hinton agar at 48°C.The media was poured into sterile petriplates and the plates were dried at room temperature for 30 min prior to spot inoculation with 3 µl aliquots of culture containing approximately $10^7$ CFU/ml of each organism. Inoculated plates were incubated at 37°C for 18 h and the MIC was determined. Experiments were carried out in triplicate. Inhibition of bacterial growth in the plates containing test oil was judged by comparison with growth in blank control plates. The MICs were determined as the lowest concentration of oil inhibiting visible growth of each organism on the agar plate.

**RESULTS AND DISCUSSION**

The antimicrobial activity of the plant oil obtained by process of Distillation depends on their chemical composition. The antimicrobial activity of the plant oil was evaluated (against Gram positive *Staphylococcus* and Gram negative *E.coli*. and *Pseudomonas* organism isolated from hospital sample), by disc diffusion and MIC assay methods. The Disc diffusion (Zone of inhibition in mm is listed in **Table 1**) and MIC (mg/ml) of the sensitive plant oil is listed in (Table 2).It was seen that the plant oil inhibited the growth of bacterial strains and produced a Zone of inhibition ranging from 8.5 to 26mm, depending on the susceptibility of the test bacteria .However the inhibition zone was lower than those of antibiotics, which showed wide inhibition zone at very low concentration. As shown in the table 1 *E.coli*. was the most sensitive organism with highest zone of inhibition (26mm) and lowest MIC value (>1.6mg/ml) to the oil of Neem and jasmine, whereas orange oil was not effective against this bacteria. On the other hand it was seen that Staphylococcus was resistant to lemon and lime oil and showed high MIC value (12.8mg/ml).Eucalyptus oil was not effective against the test organism. The MIC value for selected plant oil ranged from 1.6 to 12.8mg/ml. Hence the study revealed that Neem oil showed maximum activity with MIC value ranging from 1.6 to 3.2mg/ml followed by Jasmine oil with MIC value ranging from 1.6 to 6.4mg/ml against all the test organism whereas the remaining plant oil showed moderate MIC values.

**Table 1: List of Zone of Inhibition in mm for each dilution of plant oil and the test organism**

| Test organism | *Staphylococcus* | | | | *Pseudomonas* | | | | *E. coli.* | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Dilution of plant oil** | **1:1** | **1:5** | **1:10** | **1:20** | **1:1** | **1:5** | **1:10** | **1:20** | **1:1** | **1:5** | **1:10** | **1:20** |
| Lemon oil | 16mm | 15.4mm | 11mm | _ | 21mm | 20mm | 19.5mm | _ | 21mm | 19mm | 17.5mm | _ |
| Neem oil | 25mm | 22.7mm | 21mm | _ | 25mm | 24.6mm | 22mm | _ | 26mm | 25mm | 23.5mm | _ |
| Orange oil | 17.8mm | 16.5mm | 15.6mm | _ | 21.6mm | 19mm | 17.5mm | _ | 16mm | 15mm | 8.5mm | _ |
| Lime oil | 15.8mm | 14mm | 12.5mm | _ | 25mm | 23.8mm | 21mm | _ | 21mm | 19mm | 12mm | _ |
| Jasmine oil | 21.5mm | 20mm | 18.5mm | _ | 24.5mm | 23mm | 20mm | _ | 26mm | 24.5mm | 21.5mm | _ |
| Eucalyptus oil | _ | _ | _ | _ | 10.5mm | _ | _ | _ | 13.5mm | _ | _ | _ |
| DMSO solvent. | - | - | - | - | - | - | - | - | - | - | - | - |

**Table 2: Minimum Inhibitory Concentration of the plant oil (mg/ml)**

| Test organism | *Staphylococcus* | *Pseudomonas* | *E.coli.* |
|---|---|---|---|
| Lemon oil | 12.8 | >6.4 | >6.4 |
| Neem oil | 3.2 | >1.6 | >1.6 |
| Orange oil | 12.8 | >6.4 | 12.8 |
| Lime oil | 12.8 | >3.2 | >6.4 |
| Jasmine oil | 6.4 | >3.2 | >1.6 |

## CONCLUSION

The result of this work showed that the Neem oil and Jasmine oil possess high antimicrobial properties which can be used as natural antimicrobial agents for human infections and in food preservation. Furthermore the development of natural antimicrobial agents will help to decrease the use and negative effects of environmental pollution by synthetic chemicals and drugs.

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