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ANTIMICROBIAL ACTIVITY OF SOME ESSENTIAL OILS

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Antimicrobial activity of essential oils against skin infection causing pathogenic bacteria *i.e., Staphylococcus* and *Pseudomonas* sps. was carried out using four essential oils, namely *Rosemarinus officianalis* (Rosemary oil), *Syzygium aromaticum* (Clove oil), *Eucalyptus* species (Eucalyptus oil), *Cymbopogon martini* (Palmarosa oil) by 'Agar well Assay' and 'Tube Assay growth' of organisms. The results indicate *Rosemarinus officianalis* oil is more effective in inhibiting the bacterial growth while *Syzygium aromaticum* is the least effective. In the present paper, the results of antimicrobial activity of essential oils against *Staphylococcus* and *Pseudomonas* sps. are described and is discussed.

Keywords: Antimicrobial Activity; Essential oils; *Pseudomonas* species; *Staphyloccous* species.

Introduction

Plant oils and extracts have been used since time immemorial[1]. Historically, many plant oils and extracts, such as myrrh and clove etc, have been used as topical antiseptics, or have been reported to show antimicrobial properties[2]. It is therefore, important to investigate such plants which have been used in traditional medicines as potential sources of novel antimicrobial compounds[3]. Resurgence of interest in natural therapies and increasing consumer demand for effective, safe, natural products means that quantitative data on plant oils and extracts are required. Their purpose vary from the use of rosewood and cedarwood in perfumery, to flavouring drinks with lime, fennel or juniperberry oil[2], and the application of lemongrass oil for the preservation of stored food crops[4]. In particular, the antimicrobial activity of plant oils and extracts has formed the basis of many applications, including raw and processed food preservation; pharmaceuticals, alternative medicine and natural aroma therapies[5]. While the role of some of the oils used on the basis of their reputed antimicrobial properties have well documented in vitro activity[6,7]. Some studies have been exclusively dealt with one to one micro-organism. While these data are useful, the reports are not directly comparable due to different methodologies used such as choice of plant extract(s), test micro-organism(s) and antimicrobial test method[8].

Based on historical facts and the use of essential oils for various purposes, we have studied the antimicrobial activity of these oils on *Pseudomonas aeroginosa* and *Staphylococcus aureus.*

The aim of this study was to test the antimicrobial activity of the few selected essential oils against a Gram-positive *(Staphylococcus aureus)* and Gram-negative *(Pseudomonas aeroginosa)* bacteria which causes infections, and to understand the antimicrobial efficacy of these oils during their different growth phases.

Material and Method

Microorganisms used in the present work are *Pseudomonas aeroginosa* and *Staphylococcus aureus.* The cultures were obtained through the courtesy of Mahavir Hospital and Research Centre, Hyderabad.

To confirm their identification and characterization biochemical reactions *i.e.,* Sulfate reduction Test, Citrate Utilization Test, Oxidase Test, Catalase Test were carried out[9].

Oil Samples 1% concentration each- Cymbopogon martinii (Palmarosa oil), *Rosemarinus officianalis* (Rosemary oil), *Eucalyptus* species (Eucalyptus oil) and *Syzygium aromaticum* (Clove oil).

The four essential oils were selected randomly based on their availability in the local market in Hyderabad,

Media-Nutrient Broth / Agar per liter (Agar 15gms, Beef Extract 3gms, Peptone 5gms, pH 7.2)[10]

Methods used: Method 1- 2D-3D Method/Agar well assay[11]. Method 2- Tube Assay/Turbidity Method (Determination of Bacterial Growth Curve)[10].

Method 1: Agar well assay-Pre-seeded agar plates were prepared with equal volumes of Nutrient Agar. These were allowed to solidify and the wells were made with the help of sterile borer. The bacterial pure cultures were allowed to grow in Nutrient Broth and the cell density was adjusted using Mcfarland Standards[12]. Then 50µl of four oils in

Table 1. Method 1.Inhibition Zone

Oil Sample	Inhibition Zone for *Staphylococcus* sps	Inhibition zone for *Pseudomonas* sps.
Syzygium aromaticum(Clove oil)	O.2cm	0.5cm
Rosmarinus officinalis (Rosemary oil)	1cm	2.6cm
Eucalyptus species(Eucalyptus oil)	0.4cm	1.9cm
Cymbopogon martinii (Palmarosa oil)	0.3cm	0.5cm

Optical density of *Staphylococcus aureus* at Log and Stationary phases.

Oil Samples	Log Phase	Stationary Phase
Control	0.10	0.20
Cymbopogon martini (Palmarosa oil)	0.18	0.25
Syzygium aromaticum (Clove Oil)	0.19	0.38
Eucalyptus species (Eucalypsus Oil)	0.16	0.23
Rosemarinus officinalis (Rosemary oil)	0.11	0.22

Optical density of *Pseudomonas aueroginosa* at Log and Stationary phases.

Oil Samples	Log Phase	Stationary Phase
Control	0.10	0.20
Cymbopogon martini (Palmarosa oil)	0.16	0.23
Syzygium aromaticum (Clove Oil)	0.20	0.33
Eucalyptus species (Eucalypsus Oil)	0.13	0.28
Rosemarinus officinalis (Rosemary oil)	0.11	0.21

different Agar plates was added using Micro-pipetted into the wells, and kept at room temperature for 30 minutes, to enable the oils to diffuse into the agar, followed by incubation at 37° C for 24 hours. Zones of inhibition were carefully observed and measured which forms the basis of this study.

Method 2 .Tube Assay: Turbidity Method (Determination of Bacterial Growth Curve)-This experiment was designed to assess the effect of the various essential oils at different growth phases, *i.e.,* log and stationary phase. For this, 2 ml of culture from each growth phase of the culture was added to 10 ml of nutrient broth. To this 50µl of the oil sample was added and incubated at 37°C for 24 hours. After 24 hours, again the OD values at 520nm were checked and compared with the initial OD values

Results and Discussion

The inhibition zones of four essential oils were studied by Method 1, Agar well assay method and the data is given in Table 1.

Pseudomonas aeruginosa and *Staphylococcus aureus* were inhibited to varying degrees by all the oils tested. *Rosemarinus officianalis* was found to be comparatively more effective with an inhibition zone diameter of 2.6 cm and 1 cm, respectively, in comparison to results obtained for *Syzygium aromaticum* (see Table.1).

Method 2 -After 24hrs of incubation the O.D at 520 nm was checked .The results obtained are given below.

In Figs. 1-4 the data is shown for Method 2 *i.e.,* Turbidity Method/Tube Assay Method, shows a comparison of optical density measured at 520 nm for the test organisms at Log and Stationary Phases.

The optical density of the *Staphylococcus aureus* with *Rosemarinus officianalis* was found to be 0.11nm and 0.22nm and with *Syzygium aromaticum* the optical density was found to be 0.19nm and 0.38nm against a control 0.10nm and 0.20nm for the Log and Stationary phase, respectively.

Comparison of optical density of the *Pseudomonas aeruginosa* with *Rosemarinus officianalis*

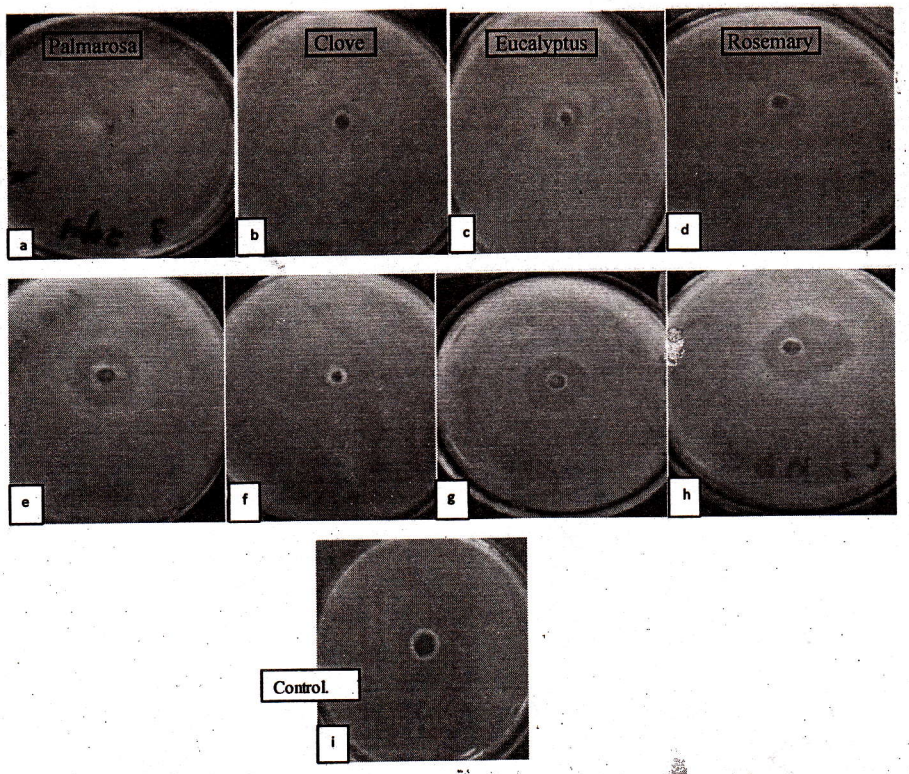

Plate 1. Comparison of Inhibitions Zones of *Staphylococcus aureus* and *Pseudomonas aeroginosa* in various oils.

was found to be 0.11nm and 0.21nm and with *Syzygium aromaticum* O.D was found to be 0.20nm and 0.33nm against a control 0.10nm and 0.20nm for the Log and Stationary phase, respectively.

From the foregoing results it is obvious that *Rosemarinus officianalis* exhibited high antimicrobial activity than other oils in both the organisms tested (Figs. 1-4, Table 1).

Recent work on *Saccharomyces cerevisiae,* has shown the cytotoxicity of some essential oils, based on colony forming ability, differed considerably depending on their chemical composition. The essential oil treated cells in stationary phase of growth showed 50% lethality[13]. In addition the state of cell growth, cell division has been shown to be critical since essential oils penetrate efficiently at the budding sites. As for, the cytotoxic effect is attributed to the presence of phenols, aldehydes and alcohols[14-15]. It is probable that these factors might be responsible for higher inhibition in *Rosemarinus officianalis* treated cultures. This property is significant in the applications of essential oils not only against human or animal pathogens / parasites but also for the preservation of agricultural or marine products. Essential oils or some of their constituents are indeed effectively used against a large variety of organisms including bacteria[16-17]. Recent work on antimicrobial activity of essential oils and plant extracts including rosemary, peppermint, basil, tea tree, celery seed[5-7], lends support to the present findings.

Based on the earlier findings about whether or not a plant oil or extract possesses activity against Gram-positive and Gram-negative bacteria and fungi[2] have shown the relative activity of plant oils and extracts by comparing results from different oils tested against the same organism(s).

Comparison of the data obtained in this study with previously published results is difficult, in view of the fact that composition of plant oils and their extracts are known to vary according to local climatic/ environmental conditions[18]. Furthermore, some oils with the same common name may be derived from different plant species[2].

Further, the method used to assess antimicrobial activity, and the choice of test organism(s), varies considerably between various workers[8]. A method frequently used to screen plant extracts for antimicrobial activity is the agar disc diffusion technique[19]. The usefulness of this method is however, limited to the

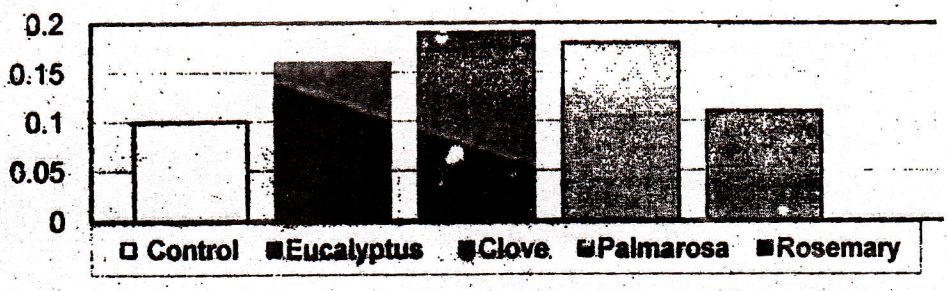

Fig.1. Comparison of O D vaues at log phase for *Staphylococcus* sps.

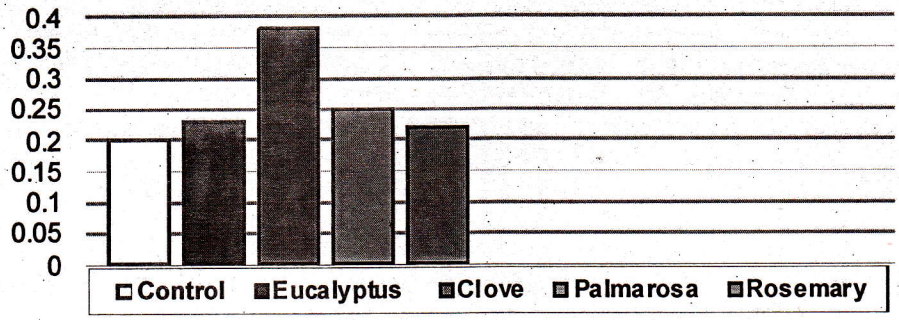

Fig.2. Comparison of O.D values at stationary phase for *Staphyloccus sps.*

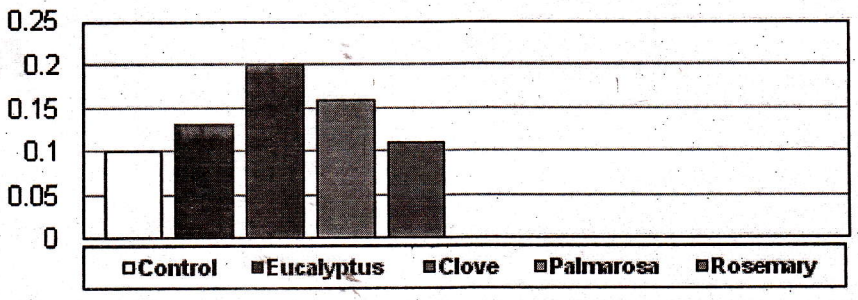

Fig.3. Comparison of O.D values at log phase for *Pseudomonas* sps.

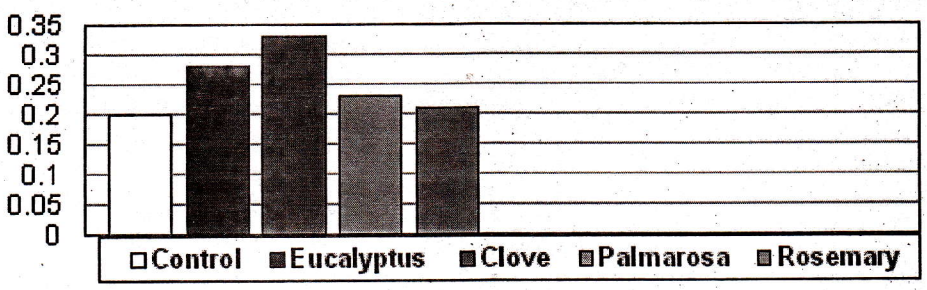

Fig.4. Comparison of O.D values at stationary phase for *Pseudomonas* sps.

Figs. 1-4. the data is shown for Method 2 *i.e.,* Turbidity Method / Tube Assay Method, shows a comparison of optical density measured at 520 nm for the test organisms at Log and Stationary Phases.

generation of preliminary, qualitative data only, as the hydrophobic nature of most essential oils and plant extracts prevents in uniform diffusion through the agar medium[8, 20]. Agar and broth dilution methods are also routinely used for the results obtained by each of these methods may differ as many factors vary between assays. These include differences in microbial growth, exposure of micro-organisms to the plant oil, the solubility of oil or oil components, and the use and quantity of an emulsifier[7].

The need for a standard, reproducible protocol method for assessing oils has been stressed[21,22], accordingly, many methods have been developed and tested specifically for determining their antimicrobial activity of essential oils[22-23]. The benefits of basing new methods on pre-existing, conventional, assays such as the NCCLS methods is that these assays tend to be more readily acceptable by regulatory bodies[21,23]. Moreover, these methods have been designed specifically for assessing the activity of antimicrobial compounds, and the factors affecting their reproducibility have been investigated. NCCLS methods[21] has developed for assessing conventional antimicrobial agents such as antibiotics, may be with minor modifications these methods can be developed for testing of essential oils and plant extracts[21].

To conclude, this study supports that essential oils presently studied posses *in vitro* antibacterial property to varying degree which is attributable to different factors. Further work on other essential oils is necessary to understand the antimicrobial activity.

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