Antimicrobial Activity of Some Essential Oils Against *Pseudomonas aeruginosa*

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Abstract. The emergence of multidrug resistance in bacteria due to overuse of antibiotics is becoming an important health concern in recent years, which requires development of novel alternatives to fight against these bacteria. Essential oils (EOs) are secondary metabolites that have different components and chemical compositions which may provide promising solution to the problem of rising number of drug resistant bacteria, as they can effectively kill bacteria. Here, in this study our aim is to determine the efficacy of lemongrass, rosemary, clary sage, geranium and tea tree essential oil against *Pseudomonas aeruginosa*, using agar well diffusion method. The minimum inhibitory concentration (MIC) of these EOs were also determined. The chemical composition of these essential oils were known by gas chromatography-mass spectrometry (GC-MS) analysis. It was revealed in this study that most of the essential oils show antimicrobial property against the test bacterium. The MIC of lemongrass is 0.25% (v/v), rosemary is 1% (v/v), clary sage is 2% (v/v), geranium is 0.5% (v/v) and for tea tree oil is 1% (v/v). We can infer from this data that lemongrass, rosemary, clary sage, geranium and tree oil can be utilized to treat infections caused by *Pseudomonas aeruginosa*, which is a gram-negative bacterium.

Keywords: Multidrug resistance · Essential oil · Antimicrobial activity · MIC · Pseudomonas aeruginosa

1 Introduction

Essential oils are volatile, hydrophobic, complex aromatic compounds having a specific odor and existing in liquid, solid, or resinous forms at room temperature. They have an oily consistency and are soluble in lipids and organic solvents. They are produced by different parts of the plant, like buds, flowers, leaves, stems, twigs, seeds, fruits, roots, wood, or bark, and are accumulated in canals, epidermic cells, glandular trichomes, secretory cells, and cavities. They have a high concentration of terpenoids, phenolic content, along with other aromatic compounds, which imparts them properties like antimicrobial, antioxidant, antiviral, antifungal, antidepressant, and insecticidal [1, 2]. They have different colors, ranging from dark green to pale yellow and from blue to dark reddish brown [3]. Essential oils have been used as therapeutics and for health

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reasons for thousands of years. They could offer a natural, safe, and cost-effective alternative for treating different diseases [4]. Different methods have been adopted to extract essential oils from different parts of the plant, like expression and fermentation, but the most common method involved is steam or hydro distillation, solvent extraction [5].

The excessive use of antibiotics by humans has led to the development of resistance in bacteria against them as a result of selection pressure and due to which several multi drug resistant bacteria are emerging. It poses a threat to public health, so we need novel alternatives like essential oils having antimicrobial properties to combat multi drug resistant bacteria [6]. The hydrophobic nature of essential oils enables them to cross the lipid membrane of bacteria easily and disturb their cell walls, making them more permeable to essential oils. As a result, the proton pumps and the ion channels are affected, membrane potential is reduced, and there is coagulation of cellular components, which ultimately results in the death of bacteria [7, 8].

Currently, the food industry is looking for food preservation alternatives that are mild and do not include chemicals, so in that regard, essential oils can turn out to be a potential substitute [9]. Around 3000 essential oils are known, out of which approximately 300 are currently being explored for their use in the food or fragrance industries [10]. They can also be used in conjunction with aromatherapy to treat cardiovascular diseases, diabetes, and cancer [11].

In this study, we used lemongrass (*Cymbopogon citratus*), rosemary (*Rosmarinus officinalis),* clary sage (*Salvia sclarea*), geranium (*Pelargonium graveolens*) and tea tree oil (*Melaleuca alternifolia*) against *Pseudomonas aeruginosa.* It is a gram-negative bacterium that is responsible for severe complications in patients suffering from cystic fibrosis due to the formation of biofilm [12]. The main components in lemongrass oil are citral, (z)-citral and geraniol [13], in rosemary it is pinene, camphor and eucalyptol [14], in clary sage it is linalyl acetate, linalool and geranyl acetate [15], in geranium it is citronellol, geraniol and linalool [16], and in tea tree oil it is terpineol, terpinene and cymene [17].

Our aim in this study is to determine the antimicrobial activities of these essential oils against *P. aeruginosa.*

2 Materials and Methods

2.1 Essential Oils

Lemongrass, rosemary, clary sage, geranium and tea tree essential oil were procured from Moksha Lifestyle Products (New Delhi, India), DMSO (Dimethyl sulfoxide) was used as a solvent for these essential oils and it was purchased from SRL (India). These essential oils were kept at room temperature and their GC-MS analysis (Table 1), chemical composition list, and certificate of analysis were provided by the supplier itself.

Table 1. GC-MS analysis data of essential oil contents and their composition provided by supplier

Sr no.	Botanical name and common name	Specific gravity (at 20 ºC)	Composition (%)
1.)	*Cymbopogon citratus* (Lemongrass)	0.865 to 0.904	Citral (33.78), (Z)-citral (28.91), Geraniol (7.448), Geranyl acetate (3.605), Caryophyllene (2.25), Phthalate (2.63), (-) -cis-Isopiperitenol (2.01), Isogeranial (1.76), Camphene (1.43), (-)-trans-Isopiperitenol (1.25)
2.)	*Rosmarinus officinalis* (Rosemary)	0.894 to 0.912	α-pinene (16.58), Camphor (13.76), Eucalyptol (11.30), D-Limonene (6.94), Terpineol (5.95), Cymene (5.76), β-pinene (5.21), Tricyclene (2.45), γ- terpinene (2.19), α-terpinene (1.76)
3.)	*Salvia sclarea* (Clary sage)	0.886 to 0.929	Linalyl acetate (43.58), Linalool (21.94), Geranyl acetate (5.77), Caryophyllene (5.66), Terpineol (4.73), Geranyl isobutyrate (3.41), Myrcene (2.05), Limonene (1.23), Camphor (1.04), γ-terpineol (0.77)
4.)	*Pelargonium graveolens* (Geranium)	0.880 to 0.899	Citronellol (33.44), Geraniol (22.57), Linalool (10.02), Citronellyl formate (5.20), Geranyl formate (2.95), Caryophyllene (2.31), Phenethyl alcohol (2.14), Isomenthone (1.69), Citronellyl propionate (1.66), Menthol (1.25)
5.)	*Melaleuca alternifolia* (Tea tree)	0.885 to 0.906	Terpineol (28.95), γ-terpinene (23.77), Cymene (6.64), Terpinolene (5.61), α-pinene (4.93), Eucalyptol (4.71), γ-terpineol (4.67), Limonene (3.92), Terpinen-4-ol (2.70), Borneol (2.10)

2.2 Bacterial Strains and Culture Conditions

This study used the bacterial strain of *Pseudomonas aeruginosa* PA01, which was obtained from the Microbial Type Culture Collection and Gene Bank (MTCC), IMTECH, Chandigarh. The bacteria were grown in Luria Bertani (LB) broth at 37 ºC. LB and Luria Bertani Agar (LBA) were purchased from HiMedia. Before using the bacterial culture for experiments, it was cultured in LB broth and streaked on agar medium to observe the colonies and hence check for its viability and purity. The culture of *Pseudomonas aeruginosa* had a characteristic blue-green color, which is mainly due to the formation of water soluble pigments like pyoverdine and pyocyanin.

2.3 Antimicrobial Activity of Essential Oils

Briefly, the bacterial strain was grown at 37 ºC for 24 h in LB broth and its turbidity was adjusted to that of 0.5 McFarland standards [18]. Then, 100 µl of this culture was spread uniformly on LB Agar plates with the help of a sterile spreader. Then, 6 mm wells were cut on agar plates using an autoclaved well borer, and 100 µl of test samples of EOs were poured into these wells, and the plates were kept in an incubator at 37 ºC for 24 h. The diameter of the zone of inhibition (ZOI) was measured for all the EOs, depicting the antimicrobial efficacy of different EOs against *P. aeruginosa.*

2.4 Determination of Minimum Inhibitory Concentration (MIC) of EOs

The MIC of EOs against *Pseudomonas aeruginosa* PAO1 was determined in accordance with the guidelines of the NCCLS, USA (2006) [19, 20] using the micro-broth dilution method. In brief, an overnight grown culture of *Pseudomonas aeruginosa* PAO1 having an O.D of 1 (at 600 nm) was grown for 24 h at 37 ºC in LB broth mixed with different concentrations of essential oils in a 96-well microtiter plate. The lowest concentration of essential oil that completely inhibited growth was taken as the MIC of that EO. Wells, having no essential oil but only the culture of the test organism, and DMSO was taken as a positive control to monitor growth.

3 Results and Discussion

3.1 Specification of EOs

Physical parameters linked with different essential oils are mentioned in Table 2, all these essential oils are soluble in alcohol and oils but not in water.

3.2 Antimicrobial Activity of EOs

Agar well diffusion assay was used to study the antimicrobial activity of essential oils against *Pseudomonas aeruginosa*, which is majorly responsible for nosocomial infections [21], and the World Health Organization (WHO) has mentioned it in its “critical” category priority list of bacterial pathogens for which novel medications are needed [22]. It was done by measuring the zone of inhibition (ZOI) around the wells (Table 3),

Table 2. Physical characteristics of different EOs used in this.

a.) Lemongrass EO

Physical parameter	Specification
Appearance	Yellow to yellow brown liquid
Odor	Very strong characteristic lemon odor
Optical Rotation	-3.0 to +1.0 at 20°C
Refractive Index	1.478 to 1.488 at 20°C

b.) Rosemary EO

Physical parameter	Specification
Appearance	Colorless to pale yellow liquid
Odor	Characteristic aromatic herbaceous odor
Optical Rotation	-10.0 to +10.0 at 20°C
Refractive Index	1.464 to 1.476 at 20°C

c.) Clary sage EO

Physical parameter	Specification
Appearance	Colorless to pale yellow clear liquid
Odor	Characteristic herbaceous odor
Optical Rotation	-0.0 to -20.0 at 20°C
Refractive Index	1.450 to 1.473 at 20°C

d.) Geranium EO

Physical parameter	Specification
Appearance	Pale yellow to greenish yellow liquid
Odor	Characteristic minty, rose-like odor
Optical Rotation	-30.0 to 0.0@ 20°C
Refractive Index	1.461 to 1.469@ 20°C

e.) Tea Tree EO

Physical parameter	Specification
Appearance	Colorless to pale yellow clear liquid
Odor	Characteristic warm, spicy odor
Optical Rotation	-5.0 to +15.0 at 20°C
Refractive Index	1.475 to 1.482 at 20°C

which was clearly visible in the case of rosemary, geranium, and tea tree essential oils. However, it was not that significant in the case of clary sage oil. The ZOI of lemongrass EO was found to be 6.5 mm and 7.1 mm at 10% and 25%, respectively, for rosemary, it was 7 mm and 8.2 mm, for geranium, it was 6.4 mm and 7.4 mm, and for tea tree oil, it was 6.7 mm and 7.5 mm. DMSO, along with LB medium, was used as a control and it showed no antimicrobial activity. Any essential oil that produces a visible clearance zone is used in experiments as the rate at which each EO diffuses in agar differs [23] and also EOs being volatile in nature may evaporate when exposed to air.

3.3 MIC of Essential Oils

Minimum inhibitory concentration (MIC) reveals the antimicrobial efficiency of that EO against the test organism. In this study, the micro-broth dilution method was used

Table 3. Antimicrobial activity of EOs against *Pseudomonas aeruginosa*

Sample	Zone of inhibition (mm)	
	10% (v/v)	25% (v/v)
Lemon grass	6.5	7.1
Rosemary	7	8.2
Clary sage	-	6.2
Geranium	6.4	7.4
Tea Tree	6.7	7.5
DMSO (Control)	-	-

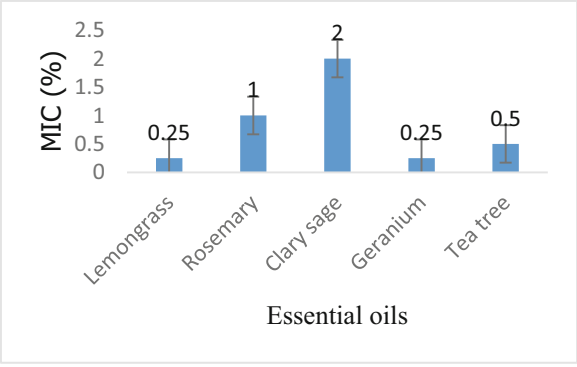

Fig. 1. MIC of different EOs against *Pseudomonas aeruginosa*

to calculate the MICs of different essential oils (Fig. 1). A concentration ranging from 10% to 0.0018% was taken for the same. Among the tested essential oils, clary sage showed the maximum MIC of 2% (v/v), while lemongrass, rosemary, geranium, and tea tree had MICs of 0.25% (v/v), 1% (v/v), 0.25% (v/v) and 0.5% (v/v) respectively. From these MIC results, we can infer that *Pseudomonas aeruginosa* is susceptible to these 5 EOs, which have different MIC values.

4 Conclusion

On the basis of these results, it can be concluded that lemongrass, rosemary, clary sage, geranium, and tea tree essential oils have the potential to be used as antimicrobials against *Pseudomonas aeruginosa*, which is an ambient microbe and a leading cause of infection in patients who have undergone surgery and are in the vicinity of healthcare equipment. The development of drug resistance in bacteria requires novel therapeutics, and essential oils from plants can address this issue. However, further studies relating to cytotoxicity, mechanism of action, and *in vivo* analysis should be conducted.

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