

Original Research Article

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Anti-biofilm Activity of Selected Plant Essential Oils against *Pseudomonas aeruginosa* and *Staphylococcus aureus*

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ABSTRACT

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Now-a-days, many antibiotics are being used to control infectious diseases. Inappropriate use of antibiotics leads to emergence of highly resistant bacteria. The increasing resistance of microorganism to conventional chemicals and drugs is a serious global problem that has opened up the promotion research into the identification of new biocides with broad activity. Derivatives of the plants such as essential oils contain such biocides. In order to observe the anti-biofilm effect of four essential oils namely *Melaleuca alternifolia* (Tea tree oil), *Syzygium aromatic* (Clove oil), *Cinnamomum zeylanicum* (cinnamomum oil) and *Eucalyptus globulus* oil we examined these oils on *Pseudomonas aeruginosa* and *Staphylococcus aureus*. The result of this study indicates that plant extract at low concentrations may provide a complementary medication for biofilm associated infections.

Introduction

Antibiotic resistance is a crucial grievous menace to public health on a planetary scale as it plummet the effectiveness of treatment and increase morbidity and mortality (Cassandra *et al.*, 2008). Highly resistant bacterial evolution resulted in the compromised use of new generation of antibiotics (Levy, 2002). Antibiotic resistance is due to an inherent ability of microorganism to form surface-attached communities of cell with in the extracellular Polymeric matrix called biofilm (Davery *et al.*, 2000). The biofilm act as nidus and shields the bacteria. The challenge exhibited by biofilm infection

is the remarkable resistance to both host immune response and available chemotherapies (Patel, 2005; Leid *et al.*, 2002) Chronic nosocomial infections by Gram positive and Gram negative have become more predominant in recent years with the exaggerated use of prosthetic biomedical implants. Very often bacterial associated infections are consequence of direct biomaterial contamination during implantation but they can also be caused by hematogenous sprawl of bacteria from an infectious site anywhere within the human body. A similar medical problem is posed by

wound colligated chronic infections which are also often marked as having a biofilm nature. As a consequence of biofilm mode of growth there is an increased bacterial resistance against classical antimicrobial treatment and host immune factor (Bryers, 2008; James *et al.*, 2008). Several mechanism can account for the increased antibiotic resistance in biofilms including the physical barrier formed by exopolymeric substances, a proportion of dormant bacteria that are inert towards antibiotics (Mah *et al.*, 2001) and resistance genes that are uniquely expressed in biofilms (Mulcahy *et al.*, 2008). Thus driving the need for an alternative therapy that will effectively kill bacterial biofilms.

The ancient science of Ayurveda is considered as a vital system of medicine and governed the world wide acknowledgement and having non-toxic substances such as certain essential oils. Plants essential oil have been used for hundreds of years as a natural medicines to combat plurality of pathogens subsuming bacteria, fungi and viruses (Sonboli *et al.*, 2006). Several essential oils confer antimicrobial activity by damaging the cell wall and membrane leading to cell destruction, leakage of the contents of the cell. Essential oils belong to phytoalexins group having strong bacterio-static and bactericidal activity. Also consist of monoterpenes, sesquiterpenes, diterpenes and other aromatic or aliphatic compounds.

The heed of our study was converge on the possibility of using essential oils in the scuffle against biofilms of two members of the “alert” Pathogen group. These were Gram positive *Staphylococcus aureus* and Gram negative *Pseudomonas aeruginosa*.

Umpteen essential oils are cushy to obtain, have low mammalian toxicity, because of these reasons research is ongoing for new antimicrobial agents, either by the design and synthesis of new agents or through the search

of natural plant oils for as yet undiscovered antimicrobial agents (Forbes *et al.*, 2002).

Materials and Methods

The present study was a prospective study carried out over a period of 4 months (August 2016 to Nov 2016) at Jaipur national university Institute for Medical Sciences and Research Centre, Jaipur. The clinical isolates recovered from both outdoor and indoor patients and were identified by using standard microbiological protocols (Cowan *et al.*, 2002). A total of 142 *Pseudomonas aeruginosa* and 40 *Staphylococcus aureus* were included in the study.

Collection of essential oils

Four essential oils namely *Melaleuca alternifolia* (Tea tree oil), *Syzygium aromaticum* (Clove oil), *Cinnamomum zeylanicum* (cinnamomum oil), *Eucalyptus globulus* (Eucalyptus oil) were obtained from local herbal store. These oils were picked over on the literature survey and their use in traditional medicine system.

Biofilm production assay

Congo Red agar method (CRA):-Congo red agar was prepared as concentrated aqueous solution separately from other constituent's of media, autoclaved at 121°C for 15 minutes, and then added to the autoclaved brain heart infusion agar with sucrose which is cooled at 55°C.

This media is composed of BHI (37 g/L), Sucrose (50g/L), agar no.1 (10g/L) and Congo red stain (0.8 g/L). Plates were inoculated and incubated aerobically for 24 – 48 hours at 37°C. Black colonies with a dry crystalline consistency indicated biofilm production. Weak slime usually remained pink. A darkening of colonies with absence of a dry crystalline colonial morphology

indicates an intermediate result. The experiment was performed in triplicate (Rachid *et al.*, 2000).

Antimicrobial screening

The antimicrobial activities of the essential oils are determined by employing disc diffusion method, following the method described by Bauer *et al.*, (1966).

Disc diffusion assay

Bacterial inoculums were prepared from overnight culture (24h) on tryptone soya blood agar. Colonies were directly suspended in saline to obtain turbidity comparable to that

of the 0.5 McFarland standards (approximately 1.5x10⁸ CFU/ml). Aliquots (100µl) of inoculums were spread over the surface of pre-dried Mueller-Hinton agar plates with a sterile glass spreader. Sterile 6 mm filter paper discs were placed on the plates and immediately 10 µl portions of the essential oils were added. Sterile PS was used as control. The plates were left for 30 min at room temperature to allow the diffusion of oil and then they were incubated at 35°C for 24h. The inhibition zone was measured in millimetre and the assay was carried out in triplicate. Biofilm formation is one of the cogent factors for pathogenicity of both Gram positive bacteria and Gram negative bacteria.

Table.1 The antimicrobial activity of four selected essential

S. No.	Bacterial isolate	No. of isolates	Biofilm (+)	Biofilm (+)%	Biofilm (-)	Biofilm (-)%
1	<i>Pseudomonas aeruginosa</i>	142	81	57.04%	61	42.9%
2	<i>S. aureus</i>	40	24	60%	16	40%

Table.2 Effect of *Melaleuca alternifolia* oil on *Pseudomonas aeruginosa* and *Staphylococcus aureus*

Test organisms	Biofilm/Non biofilm producers	Sensitive to <i>Melaleuca alternifolia</i>	Sensitive	Resistant to <i>Melaleuca alternifolia</i>	Resistant
<i>Pseudomonas aeruginosa</i>	Biofilm producers (n=81)	21	25.92%	60	74.07%
	Non Biofilm producers (n=61)	33	54.09%	28	45.90%
<i>Staphylococcus aureus</i>	Biofilm producers (n=24)	08	33.33%	16	66.66%
	Non Biofilm producers(n=16)	10	62.5%	06	37.5%

Table.3 Effect of *Syzygium aromaticum* oil on *Pseudomonas aeruginosa* and *Staphylococcus aureus*

Test organisms	Biofilm/Non biofilm producers	Sensitive to <i>Syzygium aromaticum</i> Oil	Sensitive	Resistant to <i>Syzygium aromaticum</i> Oil	Resistant
<i>Pseudomonas aeruginosa</i>	Biofilm producers (n=81)	35	43.20%	46	56.79%
	Non Biofilm producers (n=61)	43	70.49%	18	29.50%
<i>S.aureus</i>	Biofilm producers (n=24)	07	29.16%	17	70.8%
	Non Biofilm producers (n=16)	09	56.25%	07	43.75%

Table.4 Effect of *Cinnamomum zeylanicum* oil on *pseudomonas aeruginosa* and *Staphylococcus aureus*

Test organisms	Biofilm/Non biofilm producers	Sensitive to <i>Cinnamomum zeylanicum</i> oil	Sensitive	Resistant to <i>Cinnamomum zeylanicum</i> oil	Resistant
<i>Pseudomonas aeruginosa</i>	Biofilm producers (n=81)	34	41.97%	47	58.02%
	Non Biofilm producers (n=61)	22	36.06%	39	63.93%
<i>Staphylococcus aureus</i>	Biofilm producers (n=24)	08	33.33%	16	66.66%
	Non Biofilm producers (n=16)	11	68.75%	05	31.25%

Table.5 Effect of *Eucalyptus globulus* oil on *Pseudomonas aeruginosa* and *Staphylococcus aureus*

Test organisms	Biofilm/Non biofilm producers	Sensitive to <i>Eucalyptus globulus</i> oil	Sensitive	Resistant to <i>Eucalyptus globulus</i> oil	Resistant
<i>Pseudomonas aeruginosa</i>	Biofilm producers (n=81)	53	65.43%	28	34.56%
	Non Biofilm producers (n=61)	49	80.32%	12	19.67%
<i>Staphylococcus aureus</i>	Biofilm producers (n=24)	13	54.16%	11	45.83%
	Non Biofilm producers (n=16)	11	68.75%	5	31.25%

Formation of biofilm by bacteria secures the organisms from many environmental stresses like antibiotics and human immune system. The biofilm formed by *Pseudomonas aeruginosa* and *Staphylococcus aureus* restricts the diffusion of antimicrobials agents and thereby develops resistance to these substances, which is of major clinical importance.

Novel strategies are therefore required to deal with these biofilm-mediated infections. Natural substance in plants rich in bioactive compounds has been focused for their antimicrobial properties. Enormous array of secondary metabolites produced by plants are commonly accepted for their role in protection against microbial pathogens. Several traditional plants have been studied for their antibacterial activities against an array of pathogens. Similarly, a number of plant extracts have been screened for their antibacterial activity against *Pseudomonas aeruginosa* and *Staphylococcus aureus* using different antibacterial assays. Thai medicinal plants have been studied and reported for their anti-bacterial properties. The present research

work was conducted with aim to study the Anti-biofilm activity of selected plant essential oils against *Pseudomonas aeruginosa* and *Staphylococcus aureus*. The present study was a prospective study carried out at Jaipur National University Institute for Medical Sciences and Research Centre, Jaipur over a period of 4 months *i.e.* August 2016 to Nov 2016. The clinical isolates recovered from both outdoor and indoor patients and were identified by using standard microbiological protocols. A total of 142 *Pseudomonas aeruginosa* and 40 *Staphylococcus aureus* were included in the present study. All isolates were subjected to inoculate on Congo red agar; formation of black colonies on the agar with a dry crystalline consistency indicates biofilm production. Out of 142 *Pseudomonas aeruginosa* isolates, 81 were biofilm producers *i.e.* 57.04% whereas 61 isolates of *Pseudomonas aeruginosa* were non biofilm producers *i.e.* 42.9%. A total of 40 *Staphylococcus aureus* were isolated, out of which 24 isolates were biofilm producers *i.e.* 60% whereas 16 were non biofilm producers *i.e.* 40%. Four essential oils namely

Melaleuca alternifolia (Tea tree oil), *Syzygium aromaticum* (Clove oil), *Cinnamomum zeylanicum* (cinnamomum oil), *Eucalyptus globulus* (Eucalyptus oil) were obtained from local herbal store were used in the study. These oils were picked over on the literature survey and their use in traditional medicine system. In our study *Pseudomonas aeruginosa*, producing biofilm showed 65.43% sensitivity to eucalyptus oil whereas non biofilm producing *Pseudomonas aeruginosa* showed 80.32% sensitivity respectively which was highest among all the plant oils used and our result is in concordant with the study conducted by Boles *et al.*, (2005) Our findings suggested that *Staphylococcus aureus* isolates showed high level of sensitivity to eucalyptus oil *i.e.* 54.16% by biofilm producers and 68.75% by biofilm non producers *S. aureus* which is in concordant with the study conducted by Fux *et al.*, (2004) Furthermore, the findings of have pointed out that the plants with well known antibiotic properties could also potentially possess anti-pathogenic activities, which may not be related to growth inhibition of the microorganism, which is well matched with our results.

In conclusion, this study emphasizes antimicrobial properties of plant essential oil against human pathogenic bacteria. It is known that essential oils are composed of numerous different chemical compounds and their antimicrobial activity might be attributed to several different mechanisms. In the present study, essential oils have shown nearly equal bacterio-static and bactericidal activity effects on both gram-positive and gram-negative bacteria when tested in vitro. These essential oils may be effective on other gram negative and gram positive bacteria. More importantly, these can be included in the list of herbal medicines due to their high antimicrobial potential and lesser side effects. Hence, essential oils and their components

can be recommended for therapeutic purposes and be used as an alternative medicine.

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Conflict of interest statement: We declare that we have no conflict of interest.

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