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Research Article

OCIMUM SANCTUM EXTRACT COATING ON BIOMATERIAL SURFACES TO PREVENT BACTERIAL ADHESION AND BIOFILM GROWTH

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ABSTRACT

Objective: The objective of this work is to evaluate the performance of OS extract as a coating on biomaterial surfaces in preventing bacterial adhesion and biofilm growth, as an effective measure to combat Biomaterial associated infections.

Methods: Here, we have incorporated the extract from a medicinal plant as a coating to biomaterial surfaces in order to prevent bacterial adhesion and biofilm growth. To this end, *Ocimum sanctum* (OS) oil extract is coated on biomaterials (polymethyl methacrylate and polystyrene) and bacteria such as *Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa* were allowed to adhere and grow for 1 hr, 3 hrs and 24 hrs.

Results: A significant reduction ($p<0.01$) in number of adherent bacteria on OS extract coated surfaces compared to bare surfaces was observed at all-time points. The zone of inhibition of OS extract was observed for all the three bacteria and maximum inhibition was observed for *P. aeruginosa* (30 mm diameter) compared to *S. aureus* (25 mm diameter) and *E. coli* (28 mm diameter).

Conclusion: Thus, OS oil extract could be a promising coating for reduction of bacterial adhesion and biofilm formation.

Keywords: Antibacterial coating, Bacterial adhesion, Biofilm, Biomaterial, Biomaterials-associated infection, *Ocimum sanctum*.

INTRODUCTION

The emergence of biomaterials is unarguably the greatest medical achievements and at present, millions of people across the world have benefited from some kind of medical prosthesis. Biomaterials have turned out to be essential in all fields of medicine however these implants are associated with an ultimate risk of microbial infections [1-4]. Biomaterials-associated infections (BAI) is the major cause of implant failure. Biomaterial implants may become contaminated with microorganisms during the process of surgery (peri-operative contamination) or during hospitalization (post-operative contamination), causing the onset of BAI. Microorganisms involved in BAI are protected from antibiotics and host immune system due to their biofilm mode of growth. Consequently, infected implant often has to be removed. In general, *Staphylococcus aureus* is the most frequently isolated pathogen from infected implant surfaces. *S. aureus* is detected in approximately 23% of infections associated with prosthetic joints [5,6]. In addition, isolated organisms include *Escherichia coli* and *Pseudomonas aeruginosa* [7]. Biofilm formation occurs in all currently used biomaterial implants. Microbial adhesion to biomaterials is determined by the physicochemical properties of the implant surface [8]. Thus, modification of biomaterial surface may be able to prevent microbial adhesion and subsequent biofilm formation. Several biomaterial surface modifications have been developed to reduce bacterial adhesion and biofilm formation, but microbial adhesion can only be reduced by one or two log units and not fully eliminated [9-13]. Medicinal plants and its phytochemical compounds are effective against a wide array of diseases that has attracted the interest in using an extract of medicinal plants as a coating for biomaterials. *Ocimum sanctum* (OS), also known as *Ocimum tenuiflorum*, tulsi or *Holy basil*, is one of the widely used medicinal plants, an aromatic kind that belongs to the family Lamiaceae, native to the Asian continent and is cultivated throughout the tropical regions of the eastern world. Extracts of OS were identified to be extremely effective against both Gram-negative and Gram-positive bacteria [14]. OS is a plant with the most admirable properties for curing and preventing diseases. OS extract demonstrates antibacterial, antioxidant, anti-diabetic, anti-cancer, and immunomodulatory properties [15]. Therefore, in this work, an attempt has been made to evaluate the performance of OS extract as a coating on biomaterial surfaces in preventing bacterial adhesion and biofilm growth, as an effective measure to combat BAI (Fig. 1).

MATERIALS AND METHODS

OS extract

The essential oil of OS along with gas chromatography/mass spectrometry (GC/MS) report was obtained from Aromatics International, USA. The essential oil was stored at 4°C.

Biomaterial surface and characterization

Polymethyl methacrylate (PMMA), polystyrene (PS) (Industrial Insulation, Chennai, India), commonly used biomaterials, were used as substratum surfaces. Samples were rinsed thoroughly with ethanol and washed with sterile water before use. Tissue culture PS (TCPS) well plates (Nest Biotech., China) was used as a control surface. The wettability of the surfaces was determined by water contact angle measurements at room temperature using the sessile drop technique. Each value was obtained by averaging five droplets on one sample.

Bacterial growth conditions and harvesting

S. aureus, E. coli and *P. aeruginosa* were used for this study. Bacterial strains used in this study were obtained from the Culture Collection Centre for Drug Discovery and Development, Sathyabhama University, Chennai, India. Bacteria were first cultured aerobically overnight at 37°C on blood agar from a frozen stock. The plate was kept at 4°C. For each experiment, one colony was inoculated in 10 ml of tryptone soy

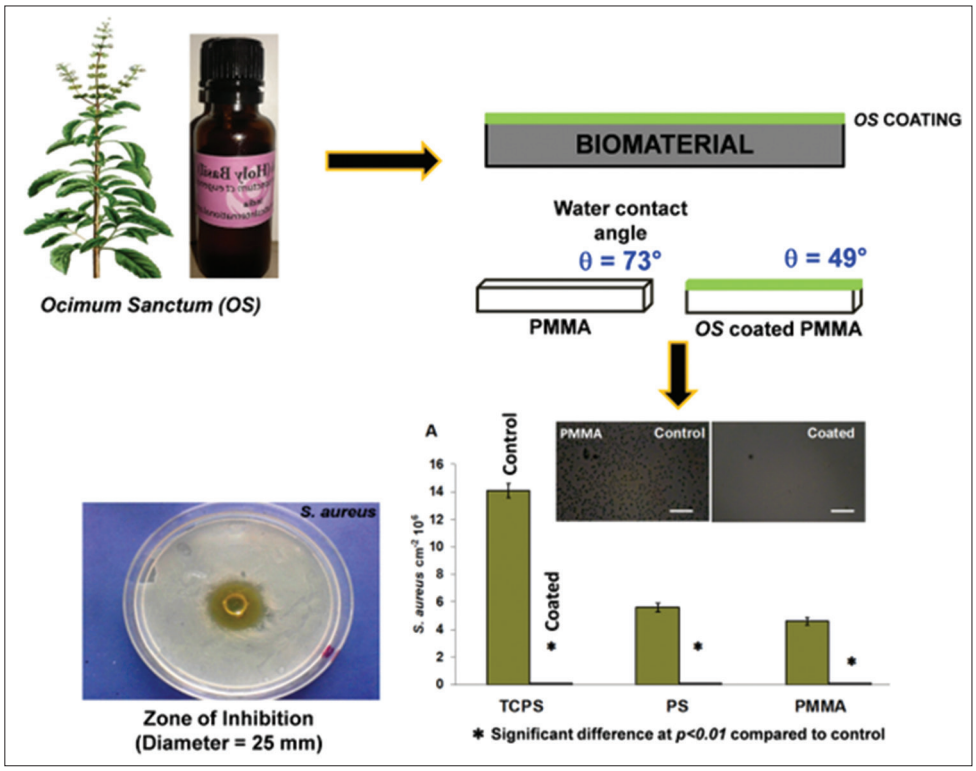


Fig. 1: Schematic diagram of the experimental methodology presenting *Ocimum sanctum* extract as a coating to implant surfaces to prevent bacterial adhesion and biofilm growth

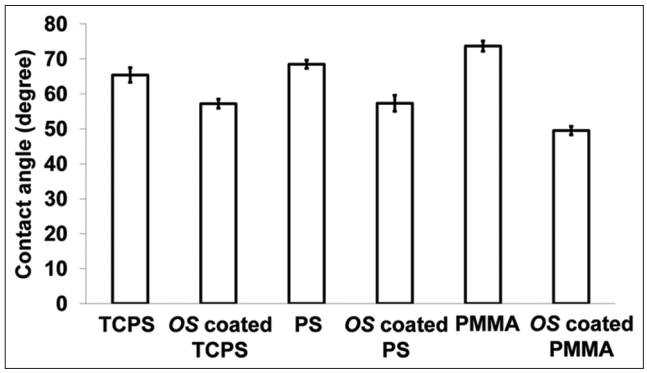


Fig. 2: Water contact angle of different biomaterial surfaces in the absence and presence of *Ocimum sanctum* extract coating

Table 1: Chemical compositions of OS extract obtained from GC-MS analytical method

Compound	Percentage
α-pinene	0.25
β-pinene	0.15
α-humulene	5.63
β-caryophyllene	29.98
a-cubebene	0.30
linalol	0.65
elemol	3.88
eudesmol	0.42
1,8-cineole	1.02
caryophyllene oxide	0.81
chavicol	0.09
Eugenol	53.06
methyl chavicol	0.55

GC-MS: Gas chromatography-mass spectrometry, OS: *Ocimum sanctum*

broth (TSB; Hi media, Mumbai) and cultured for 16 hrs. Bacteria were harvested by centrifugation at 3000 rpm for 5 minutes. Then bacteria were suspended in TSB to a concentration of 10^7 bacteria/ml.

Bacterial adhesion and biofilm growth on OS extract coated surfaces

TCPS wells containing the biomaterials (PMMA and PS) were filled with 500 µl of OS extract and allowed to adsorb to the surface at 37°C for 10 minutes. TCPS wells were used as a control surface. Subsequently, unadsorbed extracts were removed from the wells. Then, 1 ml of bacterial suspension was added to the wells and allowed to adhere and grow aerobically at 37°C for different time points 1 hr, 3 hrs, and 24 hrs. Bacterial adhesion in the absence of extract coating was considered as control. Subsequently, wells were washed with sterile phosphate buffer saline (10 mM potassium phosphate, 0.15 M NaCl, pH 7.0) to remove unbound bacteria and images were taken using phase contrast microscopy and number of adherent bacteria per cm^2 were determined using ImageJ® software. Experiments were performed in triplicate with separately cultured bacteria. Data were represented as a mean with standard deviation. For statistical analysis, analysis of variance was performed followed by Tukey's HSD *post-hoc* test and the value of $p<0.05$ was considered significant.

Antibacterial activity of OS extract

Freshly prepared nutrient agar plates were used. Bacterial cultures were inoculated to the agar plates and incubated at 37°C for 30 minutes. Holes of 6 mm diameter were punched into the nutrient agar plates. Subsequently, holes were filled with 100 µl of OS extract and incubated at 37°C for 24 hrs. The antibacterial activity was assessed by measuring the zone of inhibition.

RESULTS

The breakdown of the chemical components present in the OS extract is shown in GC/MS report as observed from Table 1. It was found that eugenol is the major component (53.06%) present in the oil extract. It

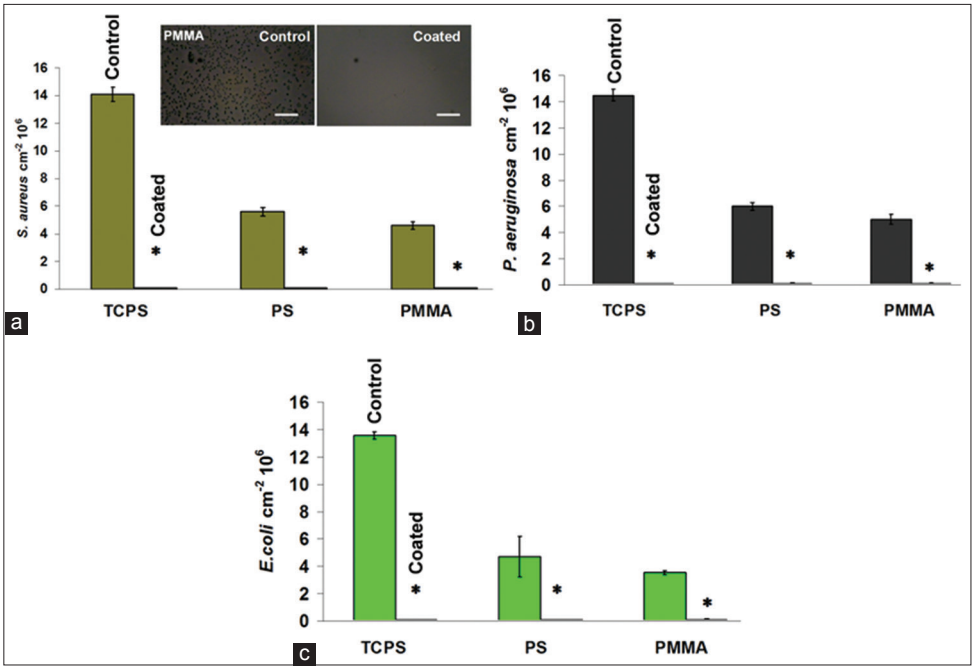


Fig. 3: Number of adherent bacteria ([a] *Staphylococcus aureus*, inset images shows the adherent *S. aureus* on control (bare polymethyl methacrylate [PMMA]) and *Ocimum sanctum* (OS) extract coated PMMA, [b] *Pseudomonas aeruginosa* and [c] *Escherichia coli*) after 1 hr of growth on different biomaterial surfaces in the absence and presence of OS extract coating. *denotes significant difference at p<0.01 compared to control

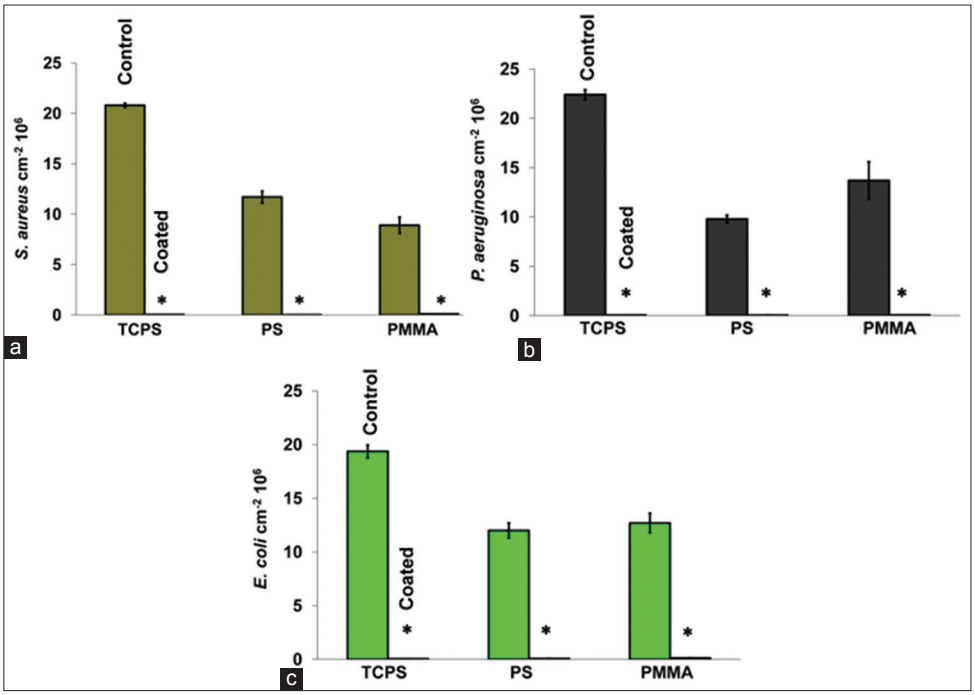
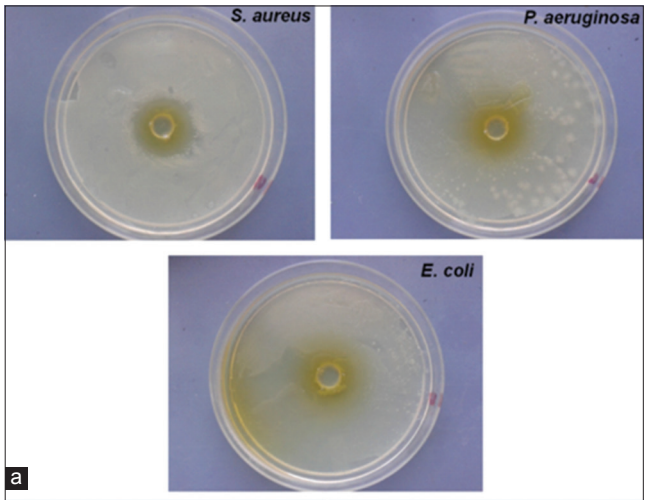


Fig. 4: Number of adherent bacteria ([a] *Staphylococcus aureus*, [b] *Pseudomonas aeruginosa* and [c] *Escherichia coli*) after 3 hr of growth on different biomaterial surfaces in the absence and presence of *Ocimum sanctum* extract coating. *denotes significant difference at p<0.01 compared to control

also includes other main components such as β-caryophyllene (29.98%), α-humulene (5.63%), sesquiterphenoids such as elemol (3.88%), oxides such as 1,8-cineole (1.02%), caryophyllene oxide (0.81%).

The water contact angles of different surfaces are shown (Fig. 2). The water contact angles of bare PMMA and PS are 73.6±1.5 and 68.5±1.2, respectively, whereas the OS extract coated surfaces were hydrophilic compared to bare surfaces. Bacterial adhesion and biofilm growth were carried out on different surfaces at different time points (1 hr, 3 hrs, and 24 hrs). Quantitative data of adherent bacteria on different surfaces at 1 hr and 3 hrs time points are shown (Figs. 3 and 4).

A significant reduction (p<0.01) in the number of adherent bacteria on OS extract coated surfaces compared to bare surfaces was observed.



Organisms	Diameter (mm)
S. aureus	25
P. aeruginosa	30
E. coli	28

b

Fig. 5: Antibacterial activity of *Ocimum sanctum* extract (a) Images represent the zone of inhibition of bacterial growth and (b) table shows the diameter of inhibition zone in mm

Even after 24 hrs of incubation, the number of adherent bacteria on OS extract coated surfaces was significantly less compared to the number of adherent bacteria on bare surfaces (data not shown). A similar trend was observed in all the three bacteria (*S. aureus, E. coli* and *P. aeruginosa*) used. The antibacterial activity of OS extract is shown in Fig. 5.

The zone of inhibition of OS extract was observed for all the three bacteria (Fig. 5a). OS extract showed maximum inhibition for *P. aeruginosa* (30 mm diameter) compared to *S. aureus* (25 mm diameter) and *E. coli* (28 mm diameter) (Fig. 5b).

DISCUSSION

Studies have demonstrated that the essential oil of OS exhibited notable antibacterial activity for all the tested pathogens (*E. coli*, *Klebsiella* spp., *Proteus mirabulus*, *P. aeruginosa* and *S. aureus*) and particularly for *S. aureus*, maximum inhibition was observed with diameter of 20 mm [16]. Researchers showed that the methanol extract of OS inhibited the growth of *E. coli*, *Salmonella typhi*, *Bacillus cereus*, *Bacillus subtilis*, *Streptococcus pyogens* and *S. aureus* with the inhibition zone lying in the range of 11.86-18.50 mm [17]. Studies showed that the antibacterial activity of OS extract could be due to eugenol component that was major component present in the OS oil extract [18-22]. Methanol extracts of OS with eugenol as major component exhibited significant antibacterial activity against *E. coli*, *Proteus mirabulus* and *S. aureus*. Similarly, OS extract exhibited high antibacterial activity against *Aspergillus niger* and *Streptococcus faecalis* [23]. The antimicrobial activity of eugenol can be ascribed to the presence of a double bond in α, β positions of the side chain, and to the methyl group located in the γ position [24].

The mechanism of action of OS extract and its components as antimicrobials has not been fully elucidated. This is complicated by the fact that there are a large number of chemical components present in OS. In general, the antibacterial mechanism of essential oil extracts may not be attributed to one specific mechanism, but may be several targets in the cell. It was shown that essential oil extracts could pass through the cell wall and cytoplasm disrupts the structure of different layers of polysaccharides, fatty acids and phospholipids [25].

CONCLUSION

This study demonstrates the effectiveness of OS extract as a coating on biomaterial surfaces in preventing bacterial adhesion and biofilm growth to combat BAI. OS coated biomaterial surfaces showed a significant reduction in bacterial adhesion and biofilm growth up to 24 hrs. The antibacterial mechanisms may be attributed to several targets in the cell. The strategy of essential oil extract coating on biomaterial surfaces could significantly reduce BAI.

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