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Antibacterial and antibiofilm activity of some essential oils and compounds against clinical strains of *Staphylococcus aureus*

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ABSTRACT

Increased emergence and spread of multidrug resistant bacteria including methicillin resistant *S. aureus*(MRSA) and their biofilms have created immense clinical problem in treatment of bacterial diseases. Biofilms are difficult to eradicate with standard antimicrobial treatments due to high antibiotic resistance level compared to free living cells. Biofilm formation by isolated *S. aureus* was first assayed by macro tube and microtitre plate assays. We demonstrate here that the selected essential oils and some of their compounds (eugenol, cinnamaldehyde,citral and geraniol) showed varying levels of antibacterial activity and sub-MICs of these agents effectively inhibit biofilm formation of *Staphylococcus aureus* strains. The order of antibacterial activity was found as *T. vulgaris*> *S. aromaticum*> eugenol> cinnamaldehyde> citral> *C. citratus*> *C. martini*> geraniol> *C. nardus*. The antibofilm activity of tested oil (*Thymus vulgaris*) and compounds (eugenol) at 2xMIC values indicated their potential therapeutic application alone or in combination with antibiotics for treating biofilm assocated clinical problems caused by *Staphylococcus aureus.*

Keywords: Antibacterial, antibiofilm activity, biofilm, essential oils, S.aureus, MRSA

INTRODUCTION

An increase in antibiotic resistant bacteria is a major threat to world population with recurrence of infectious diseases. Among problematic multidrug resistant bacterial pathogens, *Staphylococcus aureus* has occupied prominent place. In the recent decades, an increase in the number of Staphylococcal infection has been observed both in hospital and community. *S aureus* has caused 278,000 hospitalization and 19000 deaths annually in the United States alone.[1,2] Methicillin resistant *S. aureus* (MRSA) has been recognized as a major nosocomial pathogens worldwide. MRSA pose a special problem due to its intrinsic resistant to β-lactam antibiotics, they have tendency to develop resistance to other unrelated antibiotics. Production of β-lactamases and *mecA* gene products are two widely known important resistance mechanisms to β-lactam drugs.[3] Presently, the only effective agents for treatment of MRSA infections are Vancomycin, daptomycin and linezolid.[4,5] Therefore, there is greater need to develop new drugs with novel mode of action or new strategy for combating biofilm associated infections caused by drug resistant bacteria.

Biofilms are surface associated multicellular communities in which cell are held together by means of a self-produced extracellular matrix. *S. aureus* frequently forms biofilm in clinical setting most often catheters and other implanted devices but also in chronic wounds.[6,7] Poor antimicrobial penetration, nutrient limitation, adaptive stress responses, phenotypic variability and persister cell formation all contribute towards making biofilm eradication difficult.[6,8] Thus new anti-MRSA therapeutic strategies is needed to be developed.

Plant essential oils have been used for hundreds of years a natural medicine to combat a variety of bacterial infection and other ailments. Several essential oils have been documented for their antibacterial, antifungal, antimutagenic and anti-quorum sensing activities.[9-14] However little efforts have been made to assess their antibiofilm activity and therapeutic potential against bacterial infection.[8,9,12] Antimicrobial action of essential oils has been attributed due to the damage to cell wall and cell membrane leading to cell lysis leakage of cell contents and inhibition of proton motive force.[15] Many essential oils have relatively low mammalian toxicity and degrade quickly making them safe and ecofriendly.

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In this study we investigated the effect of certain oils and compounds on antibacterial action against *S. aureus* strains. Most active essential oils were further tested for their effect on inhibition of biofilm formation at sub MICs *in vitro*.

MATERIALS AND METHODS

Antibiotic sensitivity of *S.aureus* (disk diffusion method)

The *S. aureus* strains were obtained from JN Medical College, AMU, Aligarh from clinical sources. The antimicrobial sensitivity testing for *S. aureus* was conducted by disc diffusion assay described by Bauer et al.,[15] modified according to as described by the Clinical and Laboratory Standards Institute (CLSI) disk diffusion standard M7-A8.[16] After 24 h at 37°C, the zone of inhibition was measured.

MIC determination of antibiotics

The MIC of drugs was determined against the bacteria by CLSI broth macrodilution method M7-A8.[16] Briefly, bacterial suspension was prepared in NB broth. 0.1 ml of two-fold serial dilutions of test drugs (10X concentrations) were made in the test tubes and 0.9 ml of diluted inoculum medium was added to each tubes and incubated at 37 ºC for 28 h . Drug free control was included. MIC was defined as the lowest concentration that inhibited visible growth.

Essential oils used in the study

A total of 5 essential oils and 4 major active compounds were used in the study. Essential oils/phytocompounds of *Cymbopogon nardus*, *Cymbopogon citratus*, *Cymbopogon martini*, *Thymus vulagaris*, eugenol, cinnamaldehyde, citral and greniol were purchased from Fragrance and Flavour Development Centre, Kannauj, India while *Syzygium aromaticum* oil was purchased from Dabur India Ltd. Delhi, India. All the essential oils or active compounds were diluted ten times in 1% DMSO before use in assays.

Antibacterial activity of essential oil

The antimicrobial activities of oils/phytocompounds were determined by agar well diffusion method.[19] Briefly, One hundred microlitres (100 µl) of the standardized inoculum (0.5 Mac-Farland) of each test bacterium was spread with the help of sterile spreader on to a sterile Muller-Hinton Agar plate (HiMedia) so as to achieve a confluent growth. The plates were allowed to dry and a sterile cork borer of diameter 6.0 mm was used to bore wells in the agar plates. Subsequently, a 100 µl volume of the oil was introduced in triplicate wells into Muller-Hinton Agar plate. Sterile DMSO served as negative control.

Visual detection of biofilm formation

The strains of *Staphylococcus aureus* were screened for their ability to form biofilm under static condition in polystyrene tubes as described by Gokce et al.[17] Briefly, 10 ml of Luria Bertani (LB) medium supplemented with glucose (final concentration 8% w/v) was inoculated with loopful of organism and incubated at 37 °C for 48 h. After incubation, the broth in the tube was aspirated gently and decanted. Tubes were washed thrice with sterile PBS and then stained with 0.1% (w/v) crystal violet for 10 min and examined for the adherent biofilm layer. Biofilm formation ability was tentatively scored as strong (+++), moderate (++), weak (+) and negative (-) by visually comparing the thickness of adherent layer.

Quantitative determination of biofilm formation in microtiter plate

The strains of *Staphylococcus aureus* were also evaluated by 96 well microtiter plates based XTT reduction assay for biofilm formation using the method as described by Sun et al.[18] with little modifications. Briefly, *Staphylococcus* cells were grown in LB medium (glucose 0.25% w/v) at 37 °C for 24 h. Biofilms were formed by adding 100 µl of this standardized cell suspension to wells of microtiter plates that contained 100 µl of BHI media (0.25% glucose) and incubating at 37°C for 48 h. Thereafter, the medium was aspirated gently, and non-adherent cells were removed by washing the biofilms thrice in sterile PBS. Further biofilm formation was analysed by XTT reduction assay. Briefly, 0.091 ml of XTT (Sigma, N. Delhi) (1 mg/l in PBS) and 0.009 ml of menadione (Sigma, N. Delhi) (1 m mol/l in acetone) was added to each well and incubated in the dark for 4 h. The colorimetric change was measured at 620 nm using a Lab system Multiskan Ex MTP Reader. Each assay was performed three times with three replicates per experiment and the mean absorbance values were used to measure the biofilm formation.

Determination of effect of essential oils and antibiotics on sessile *Staphylococcus aureus* cells

For susceptibility of sessile cells to oils/compounds and drugs a modified CLSI broth micro dilution method M07-A8 was used with some modifications.[16] Biofilms were allowed to form in microtiter plates as described earlier. Further, 0.1 ml of two-fold serial dilutions of test agents made in BHI medium was added to each biofilm wells of microtiter plates and incubated at 37 ºC for next 72 h. A series of antibiotics agent-free wells and biofilms-free wells were also included to serve as positive and negative controls, respectively. Sessile MIC (SMIC) was determined by XTT reduction assay.

Determination of effect of antimicrobial agent on biofilm formation in *S. aureus*

The effect of different compounds and drugs on the ability of *Staphylococcus aureus* cells to form biofilm was determined by the modified method of Nostro et al.[8] with some modifications. 0.1 ml of test agents (2 X final concentrations) in LB medium was added to each well of microtiter plates. Subsequently, 0.1 ml of standardized bacterial cell suspension was added and plates were incubated at 37 ºC for 48 h. Antimicrobial agent-free wells served as positive controls for biofilm growth. After incubation, the medium and non-adherent cells were removed from wells and washed three times with sterile PBS. Each assay was performed three times in triplicates and mean absorbance values were used to measure the inhibition of biofilm formation as follows: (mean OD_{405} of treated well / mean OD_{405} of untreated control well)×100.

Light microscopy of biofilm formed in the presence and absence of essential oils

Polyvinyl chloride catheter discs (18 mm diameter) were placed in 6 well tissue culture plates. Wells of the plates were dispensed with 1 ml of BHI media containing 2X of sub-MICs of test agents. Further, 1 ml of standardized cell suspension was

Table 1. Susceptibility of *S.aureus* strains to antibiotics.

Strains	Diameter of the zone of inhibition (mm)							
	AMP	CEF	CEP	CLOX	MET	NOV	PEN	VAN
JSA01	28.33±0.58	-	15.00±0.81	20.00±0.81	20.25±2.23	21.33±1.24	12.33±0.47	10.00±0.81
JSA02	10.00±0.81	-	22.33±1.24	-	-	15.00±0.81	15.66±0.47	10.00±0.81
JSA03	10.33±0.47	-	22.00±0.81	-	10.00±0.81	22.00±0.81	-	10.00±0.81
JSA04	10.33±0.47	-	20.25±2.23	14.33±0.47	-	22.33±1.24	17.00±0.81	10.00±0.81
JSA05	10.66±0.66	-	28.33±0.58	12.33±0.47	10.66±0.66	20.25±2.23	18.00±0.81	10.66±0.66
JSA06	10.00±0.81	10.33±0.47	-	10.00±0.81	-	19.22±1.69	14.33±1.24	16.00±0.81
JSA07	10.00±0.81	-	-	-	10.66±0.66	10.33±0.47	-	9.33±0.47
JSA08	12.33±0.47	-	13.66±1.24	13.66±1.24	-	21.33±1.24	15.00±0.81	10.33±0.47
JSA09	15.66±0.47	10.33±0.47	17.00±0.81	13.66±1.24	10.00±0.81	10.00±0.81	10.33±0.47	10.33±0.47
JSA10	11.33±1.24	-	27.33±0.58	17.00±0.81	-	25.66±2.05	18.00±0.81	11.33±1.24
JSA11	10.66±0.66	-	17.33±0.58	12.00±0.81	18.33±1.24	11.33±1.24	16.00±0.81	11.00±0.81

AMP; ampicillin (10µg/disc), CEF; cefuroxime(30µg/disc), CEP; cephalothin (30µg/disc), CLOX; cloxacillin (µg/disc), MET; methicillin (5µg/disc), NOV; novobiocin(30µg/disc),PEN; penicillin (10 units),VAN; vancomycin (30µg/disc)
-; indicates resistant (no inhibition of growth)

added to each well and incubated at 37 ºC for 48 h. Well without test agents served as positive control. After the formation of biofilm, medium was discarded and discs were washed three times with sterile PBS and stained with 0.1% crystal violet and incubated at 37ºC for 10 min. The discs were visualized under bright field light microscope (Olympus, Japan) at 40 X.

RESULTS

A total of 11 strains of *S. aureus* were previously isolated from various sources. Susceptibility of *S. aureus* strains against the seven antibiotics namely penicillin, ampicillin, cloxacillin, cephalathion, methicillin, novobiocin and vancomycin showed that zone of inhibition of cloxacillin, penicillin and vancomycin varied from nil to ≤ 17 mm whereas zone of inhibition of ampicillin, methicillin, cephalothion, and novobiocin ranged from ≥17 to 28 mm against JSA01, JSA02, JSA06 and JSA07 strains respectively (Table 1). Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of ampicillin, ceftriaxone and vancomycinis summarized in table 2. The strains considered as resistant at MIC value ≥ 64 µg/ml, 1 and 4 µg/ml for vancomycin, ampicillin and ceftrriaxone respectively. MIC and MBC of vancomycin and ampicillin was found to be in the range of 64 to 1024 µg/ml and 128 to 2048 µg/ml respectively. For ceftriaxone

Table 2. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of antimicrobial drugs against *S. aureus* strains.

Strains	AMP		CEFT		VAN	
	MIC	MBC	MIC	MBC	MIC	MBC
JSA-01	2	4	1024	2048	1024	2048
JSA-02	1024	2048	256	512	1024	2048
JSA-03	1024	2048	512	1024	1024	2048
JSA-04	1024	2048	512	1024	1024	2048
JSA05	1024	2048	512	1024	1024	2048
JSA-06	1024	2048	256	512	64	128
JSA-07	1024	2048	512	1024	1024	2048
JSA-08	256	2048	128	512	1024	2048
JSA-09	64	128	1024	2048	1024	2048
JSA-10	512	2048	1024	2048	1024	2048
JSA-11	1024	2048	1024	2048	1024	2048

MIC and MBC values are presented in µg/ml.

MIC and MBC lie in the range of 256 to 1024 µg/ml and 512 to 2048 µg/ml respectively. All strains were resistant to ceftriaxone and vancomycin. Only JSA01 was sensitive to ampicillin.

Table 3. Susceptibility of *S. aureus* strains to essential oils by using disc diffusion method.

Strains	Diameter of the zone of inhibition (mm) Test oils								
	C. citrates	*C. martini*	*C. nardus*	*S. aromaticum*	*T. vulgaris*	Cinnamaldehyde	Citral	Eugenol	Geraniol
JSA01		-	-		11.66±0.94	12.33±0.47	-	10.33±0.47	-
JSA02	10.00±0.81	-	10.33±0.47	10.66±0.66	14.66±0.94	15.00±0.81	10.00±0.81	11.66±0.94	-
JSA03	-	-	-		11.33±1.24	10.66±0.66	-	-	-
JSA04	10.66±0.66	10.00±0.81	11.33±1.24	13.66±1.24	19.00±1.81	24.33 ±0.58	11.33±1.24	17.00±0.81	10.00±0.81
JSA05	-	-	10.00±0.81	12.00±0.81	14.33±24	16.66±1.47	10.00±0.81	11.00± 0.81	-
JSA06	-	10.00±0.81	0.33±0.47	18.00±0.81	13.66±1.24	10.66±0.66	12.33±0.47	-	
JSA07	10.66±0.66	10.00±0.81	11.33±1.24	14.66±0.94	11.00±0.81	22.00±0.81	11.66±0.94	18.00±0.81	12.33±0.47
JSA08	10.00±0.81	10.66±0.66	-	10.66±0.66	13.66±1.24	15.00±0.81	-	11.33±1.24	-
JSA09	-	-	-	-	10.00±0.81	15.66±0.47	-	-	-
JSA10	-	-	-	10.00±0.81	10.00±0.81	15.66±0.47	-	11.33±1.24	-
JSA11	-	-	-	10.00±0.81	11.33±1.24	17.00±0.81	-	11.66±0.94	-

Antibacterial activity of the essential oil and phytocompounds

Antibacterial activity of essential oils against the *S. aureus* strains were determined by disc diffusion method. Nine essential oils/phytocompounds namely, *Thymus vulgaris, Cymbopogon nardus, Cymbopogon citratus, Cymbopogon martini, Thymus vulgaris, Syzygium aromaticum,* Cinnamaldehyde, geraniol, eugenol and citral were screened for antibacterial activity. Essential oils of *S. aromaticum*, *T. vulgaris*, Cinnamaldehyde and eugenol showed good antimicrobial activity against most of the test strains. All these strains showed least proclivity to *Cymbopogon nardus, Cymbopogon citratus, Cymbopogon martini,* geraniol, citral. *S. aureus* strains were highly susceptible to eugenol and thyme oil and zone of inhibition ranged from 10 to 19 mm (Table 3).

Biofilm forming ability of *Staphylococcus aureus*

Test strains of *S. aureus* were investigated for their biofilm forming ability. Nine strains formed moderate to strong biofilm and one strain formed weak and one strain could not formed biofilm as determined by crystal violet staining assay using polystyrene tubes (Table 4 and figure 1). These strains were divided as strong (OD_{620} >0.892), moderate (OD_{620}> 0.4 to ≤ 0.8) and weak (OD_{620} > 0.1 to ≤ 0.4) biofilm former based on their absorbance in crystal violet assay (Table 4). Strains visually exhibiting strong biofilm showed absorbance values >1.0 when assessed by crystal violet assay in 96 well microtitre plate. Further, XTT reduction assay revealed that JSA02, JSA06, JSA10 displayed strong ability to form biofilm showing OD_{620} of 1.38 ±0.027, 1.33 ± 0.045, 1.40 ± 0.039, 1.11 ± 0.021, 1.01 ± 0.084, 0.79 ± 0.021 respectively. Therefore the above isolates were selected for biofilm inhibition assays.

Effect of essential oils on planktonic and sessile cells of *S. aureus*

MIC of planktonic cells (PMIC) ranged from 0.2 to 3.3 % v/v

Table 4. Biofilm formation by the strains of *S. aureus*

Strains	Visual detection on polystyrene tubes	Visual detection microtitre plate	Absorbance at 620 nm
JSA-01	+	++	0.30 ± 0.064
JSA-02	+++	+++	1.11 ± 0.021
JSA-03	+	-	0.19 ± 0.011
JSA-04	++	++	0.46 ± 0.026
JSA05	++	++	0.29 ± 0.015
JSA-06	+++	+++	1.01 ± 0.084
JSA-07	+++	+++	0.88 ± 0.025
JSA-08	++	+++	1.12 ± 0.220
JSA-09	-	++	0.32 ± 0.023
JSA-10	-	++	0.79 ± 0.021
JSA-11	-	+	0.25 ± 0.045

+++; Strong biofilm ;OD_{620} > 0.892
++; Moderate biofilm ; OD_{620} > 0.4 to ≤ 0.8
+; weak biofilm ; OD_{620} > 0.1 to ≤ 0.4
-; No biofilm ; OD_{620} < 0.1

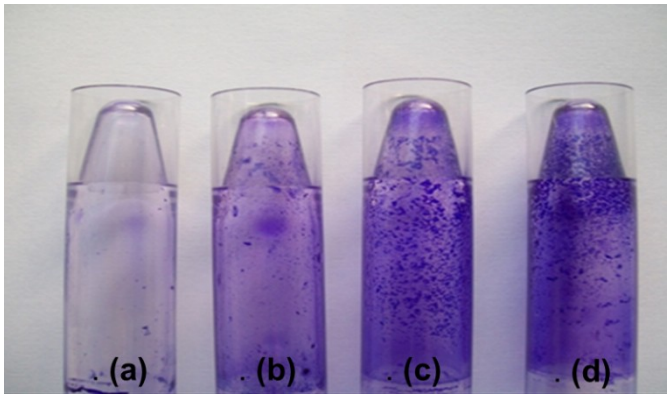

Figure 1. Visual detection of strains of *S.aureus* for their biofilm forming ability in polystyrene tubes . **(a)** JSA-11;- **(b)** JSA-03;+ **(c)** JSA-08;++ **(d)** JSA-10;+++

Table 5. Susceptibility of planktonic and sessile *S. aureus* cells to essential oils

Test agents	JSA-02		JSA-06		JSA-10	
Essential oils	**PMIC**	**SMIC**	**PMIC**	**SMIC**	**PMIC**	**SMIC**
Cymbopogon citrates	0.8	0.8	0.8	1.6	0.8	1.6
Cymbopogon nardus	1.6	3.2	0.4	1.6	1.6	3.2
Syzygium aromaticum	1.6	3.2	O.8	1.6	1.6	1.6
Thymus vulgaris	0.8	0.8	0.8	1.6	0.2	0.8
Cinnamaldehyde	0.8	0.8	0.8	0.8	0.8	1.6
Citral	3.2	6.4	1.6	3.2	1.6	3.2
Eugenol	0.8	0.8	1.6	1.6	1.6	3.2
Vancomycin	512	>1024	512	>1024	512	>1024

Values of sessile MIC (SMIC) are given in % V/V for oils and µg/ml for drug.

whereas MIC of sessile cells (SMIC) ranged from 0.8 to 6.4 % v/v as depicted in table 5. Cinnamaldehyde and eugenol showed no increase in SMIC compared to PMIC in JSA-02and JSA-06. Similar pattern was shown by oils of *Cymbopogon citratus* and *Thymus vulgaris* against JSA-02. SMIC of all the oils tested increased upto 2-3 fold in comparison to PMIC against one or other test strains. Vancomycin showed a PMIC of 512 µg/ml against each of the test strains whereas SMIC was found to be ≥ 1024 µg/ml.

Inhibition of biofilm formation by essential oils/phytocompounds in microtitre plate

Varying level of attenuation in the biofilm formation by planktonic *S.aureus* cells was observed in the presence of eugenol and thyme oil. Concentration dependent biofilm inhibition was demonstrated by eugenol and thyme oil against *S.aureus* strains. At 0.2 % v/v concentration of eugenol, biofilm forming ability of *S. aureus* JSA10 was found to be 80.7 % which was reduced to 8.4 % at 12.8% v/v concentration of eugenol (figure 2c). Similar dose dependent decrease in biofilm

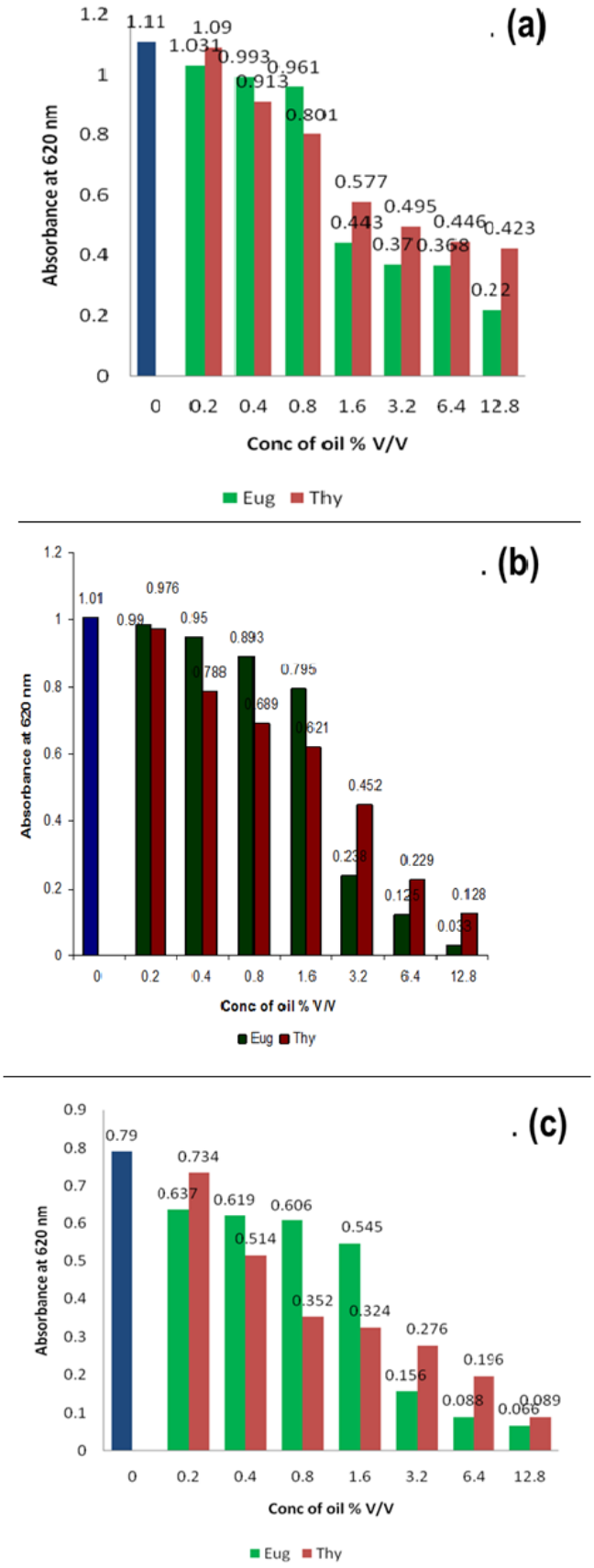

Figure 2. Concentration dependent effect of eugenol and thyme oil on *S. aureus* biofilm. a) JSA02; b) JSA06; c). JSA10

formation of strains of JSA02 and JSA06 was also observed and maximum inhibition of 80.1 and 96.7% was observed at the highest tested concentration, respectively. Thyme oilalso demonstrated a similar trend as 61.8%, 87.3% 88.7% biofilm inhibition was recorded in the presence of the test oils at 12.8 % v/v against JSA02, JSA06 and JSA10 respectively (figure 2a, 2b, 2c).

Light microscopic analysis of biofilm formation in the presence of essential oils

Inhibition of biofilm formation by thyme oil and eugenol in JSA10 strain was observed under light microscope. Figure 3a depicts, controlled untreated biofilm 0f 48 h exhibiting dense network of cells with extracellular polymeric matrix. In the presence of eugenol and thyme oil efficiently reduced the number of microcolonies and scattered aggregation of cells was observed indicating that the biofilm formation was inhibited to varying levels (Figure 3b, 3c).

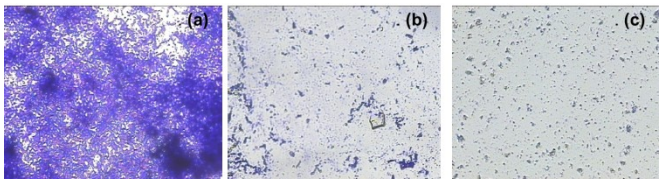

Figure 3. Inhibition of biofilm formation *in S. aureus* by eugenol and *T. vulgaris* on catheter discs in 48 h under light microscope (40 X). a). Untreated control; b). 12.8% v/v eugenol; c). 12.8% v/v thyme oil

DISCUSSION

Pathogenic microorganisms have become a leading cause of human mortality due to the increasing frequency of bacterial infections in immunocompromised populations and the limited armamentarium of clinically useful antibiotics. A significant proportion of human microbial infections are biofilm associated, creating a protected environment that allows for survival to external assaults and facilitates different microbial interactions that it is related with the enhanced resistance of biofilm cells to antimicrobial drugs.[20]

In the present study *S. aureus* strains have shown resistance to vancomycin and ceftriaxone (128- ≥1024µg/ml). Our results find support from the reports of several other workers.[21,22]

Antimicrobial activity of essential oils against drug resistant organisms

Plant essential oils have been used for hundreds of years as natural medicines to combat a multitude of pathogens, including bacteria, fungi and viruses.[23] Therefore, considering the problem of drug-resistance to conventional antimicrobials in pathogenic strains of fungi and bacteria, certain essential oils and some of their active compounds were screened for their efficacy against the drug-resistant strains of *S. aureus.*

Our study revealed broad spectrum inhibitory activity from essential oils of *T. vulgaris*, *Cymbopogon citratus*, *Cymbopogon nardus*, *Cymbopogon martin*, *Syzygium aromaticum* and major active ingredients citral, eugenol, geraniol and cinnamaldehyde against multi-drug resistant strains of *S. aureus*. Eugenol and thyme oil showed antimicrobial activity against all the test strains. Antimicrobial activity of essential oils has been reported by many other workers.[24-26]

Biofilm forming ability of *S.aureus*

Biofilm forming ability was demonstrated by 81.80 % of *S. aureus* strains from different clinical sites as revealed by both visual and spectroscopic method. Biofilm formation among these strains has been reported by several other workers.[6,27]

Anti-biofilm activity of essential oils against drug-resistant strains of *S. aureus*

Treatment with antibiotics may kill planktonic bacteria shed from the biofilm surface; however, they fail to eradicate those embedded within the biofilm, which can then subsequently act as a source for recurrent infection.[28] Following standard antibiotic treatment, a minority of drug-resistant bacteria exist that repopulate the biofilm. Subsequent retreatment of the repopulated biofilm results only in a modest reduction in bacterial numbers, indicating that the repopulated biofilm is much more resistant to treatment.[29] At present, conventional systemic therapies, using standard antimicrobial agents, represent the main strategy for the treatment and prevention of biofilm infection. Therefore, essential oils namely *Thymus vulgaris*, *Cymbopogon citratus*, *Cymbopogon nardus*, *Cymbopogon martini* and major active ingredients citral, eugenol and cinnamaldehyde were tested for their antibiofilm activity against the test strains.

In our study, antibacterial susceptibility studies of the test strains demonstrated a rise from the planktonic MICs to corresponding sessile MICs. The drug susceptibility of these strains under biofilm condition exhibited several fold increase in MICs. Sessile MIC of vancomycin was raised up to 1000 folds in the strains of *S. aureus*. Similar trend was observed by several other workers.[22,30,31] Sessile MICs of essential oils was raised only 2-3 folds against the test strains. However, *Cymbopogon nardus, Thymus vulgaris* and eugenol showed no increase in SMICs compared to PMICs against the test strains.

Thyme oil and eugenol were further tested for their biofilm inhibtory activity against JSA02, JSA06 and JSA10. A dose dependent decrease in the biofilm formimg ability was recorded at all tested concnetrations of the thyme oil and eugenol. Our resuts find support from the observations with clove oil, [13, 32] Quercetin,[31] Tannic acid[33] and honey.[34]

CONCLUSION

In conclusion, the study reveals the antimicrobial and biofilm inhibitory activity of essential oils and phytocompounds against *S. aureus*. Based onour findings, thyme oil and eugenol have potential application as as alternative antibiofilm agent against *S. aureus*. The above property could be exploited in developing them as an antipathogenic agent alone or in combination with antibiotics against drug resistant pathogenic bacteria.

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