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A Preliminary Analysis on the Bactericidal Properties of *Jasminum sambac* (L.) Aiton Essential Oil Against *Staphylococcus aureus* and *Pseudomonas aerugenosa*

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Abstract
Flowers of *Jasminum sambac* is widely used for decorative purposes as well as for the extraction of its highly aromatic essential oil. Studies had revealed biological activity potential of *Jasminum sambac* essential oil in terms of antioxidant, anticancer and antibacterial. The present study attempted to understand the bactericidal properties of *Jasminum sambac* essential oil against *Staphylococcus aureus* (ATCC 6538) and *Pseudomonas aerugenosa* (ATCC 27853) and mode of its action. Significant inhibition zones were obtained against both *Staphylococcus aureus* and *Pseudomonas aerugenosa* during disc diffusion assay. Minimum inhibitory and bactericidal concentration further supported the disc diffusion result. Estimation of leakage of reducing sugar, UV_{260} and UV_{280} absorbing materials suggested the deleterious effect of *Jasminum sambac* essential oil on bacterial membrane. The essential oil induced bacterial membrane damage was confirmed by studies on bacterial cell morphology using scanning electron microscopic method. Further studies are required to identify the active principle and to understand the underlying molecular mechanism of membrane damage caused by *Jasminum sambac* essential oil.

Keywords
Jasminum sambac, essential oil, antibacterial, disc diffusion, SEM.

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INTRODUCTION
Evolution of drug resistant strains of bacteria is a potential threat in the treatment of various infectious diseases. Drug resistance consequent in the ineffectiveness of the existing conventional antibiotics. Moreover, in spite of the favorable reaction antibiotics have been unveiled to possess concomitant side effects in the long run. Thus there is an intensified drive for finding novel compounds of therapeutic potential with no or minimalistic side effects. Plants are repository of several bioactive molecules and whilst can serve as a potential source in devising new germicidal drugs.

Genus *Jasminum* of Oleaceae consists of approximately 200 species with wide geographical distribution (Jeyrani et al., 2018) [1]. *Jasminum* plants are cultivated for its fragrant flowers and essential oil for commercial purposes. Medicinally, the leaves and flowers are used in alleviating skin problems, itching,

ulcers, toothache, pain and pus in ear and speedy wound healing (Rani et al., 2017) [2].

Essential oils from plants have enormous benefits in the field of pharmaceutical and cosmetic industries. For instance, essential oil of *Jasminum grandiflorum* is known for its tranquilizing effect, cardwood essential oil for sedative effect, lavender essential oil for analgesic activity and correcting menopausal disorder and *Aconus gramineus* for epilepsy treatment (Hamid et al., 2011) [3]. Further, essential oils also possess wide ranging germicidal properties. Studies proved thyme and oregano is effective against *E. coli*, *Salmonella typhimurium*, *Salmonella choleraesuis* and *Salmonella entritidis* infection and carvacrol against *E. coli* (Penalver *et al*. 2005; *Burt et al*. 2007) [4, 5]. Similarly, another report showed promising bactericidal and fungicidal properties in the extracted essential oil of *Croton cajucara* (Alviano *et al*. 2005) [6].

The characteristic aroma and biological activity of essential oil has been credited to the individual or synergistic action of its various constituents. The aromatic oil of jasmine contains linalool, benzyl alchohol, methyl jasmonate, linalyl acetate, benzyl benzoate, indole, jasmine, methyl anthranilate, p-cresol, geraniol, benzaldehyde, methyl benzoate, methyl salicylate, 1-epi-cubenol, cis-nerolidol, nerol, α-terpineol, cedrol, jasmolactone, farnesol, 1-pentanolide and eugenol (Mittal et al., 2011)[7].

Species *Jasminum sambac* is native to India, Philippines, Myanmar, Srilanka, Bhutan, Nepal and Pakistan (Jeyrani et al., 2018)[1]. Its various parts are used medicinally either singly or combined or in blend with others. Studies indicate the significant microbicidal potential of *Jasminum sambac* against multitudes of bacteria and fungi. Methanolic extracts of *J. sambac* is showed to be effective in suppressing spore formation as well as development of *Alternaria* species, *A. flavus, A. fumigatus* and *Curvularia* (Hussaii and Mahasneh, 2011) [8]. Further, ethanolic extracts of the callus of *J. sambac* is bactericidal against *S.typhi* and *P.mirabilis* whereas flowers and leaves extracts were active against methicillin resistant *S.aureus* and *B.subtilis*, *E.coli, S. typhimurium* and *K. pneumonia* (Joy and Raja, 2008) [9]. Also, essential oil of *J. sambac* found efficacious in inhibiting *E. coli* growth in-vitro (Rath *et al*. 2008) [10]. However, studies correlating the mechanism and bactericidal activity of *Jasminum sambac* essential oil are poorly understood. In this scenario, present study attempted a preliminary investigation on the antibacterial potential of essential oil from the flowers of *Jasminum sambac* on *Staphylococcus aureus* and *Pseudomonas aerugenosa* and its mode of action.

MATERIALS AND METHODS

Plant Material and its Collection

Freshly collected *Jasminum sambac* flowers were used for the study. The collected flowers were washed repeatedly with tap water and sliced into small pieces for the extraction of essential oil.

Extraction of Essential Oil

Hydro-distillation method was used for essential oil extraction. 1000g of flower was put in the round bottomed flask (Clevenger apparatus) containing 250 ml water and boiled for about 3 hours. The oil was recovered by using micropipette. For further purification, the collected essential oil was subjected to centrifugation at 1000rpm for 10 minutes (Akram *et al*. 2017) [11].

Microorganisms

The test organisms used in the antibacterial study include Gram positive bacteria *Staphylococcus aureus* (ATCC 6538) and Gram negative bacteria *Pseudomonas aerugenosa* (ATCC 27853). Samples purchased from Tropical Institute of Ecological Sciences, Kottayam, Kerala.

Preparation of Test Solutions

The extracted essential oil is divided into three concentrations-100µg/ml, 250µg/ml and 500µg/ml by mixing essential oil with DMSO (Dimethyl sulfoxide).

Disc Diffusion Method

Bacteria were uniformly swabbed across the MH (Mueller Hinton agar) plates. The sterile filter paper disks of 5mm, incorporated with essential oil of different concentration, was placed in the bacteria swabbed cultured nutrient medium. Pure DMSO and antibiotic streptomycin (500µg/ml) was used as negative and positive control respectively. The plates were observed after 18hrs at 37°C. All tests were performed six times and the antibacterial activity was expressed as the mean of inhibition diameters (mm) produced by essential oil at different concentrations (Patani et al., 2011) [12].

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

A series of dilutions of test sample were used for determining MIC and MBC (12). To each test tube (broth culture medium having different concentration of essential oil) 10^8 CFU/ml bacterial samples were inoculated. The samples were incubated at 37°C for 24 hours. Observed the tubes for bacterial growth. Concentrations in test tubes showing zero growth represent MIC or MKC. Innorder to distinguish MIC and MKC (MBC), bacterial samples were plated onto agar (without test sample) and looked for colony development. Only samples from the test tube having MIC concentration developed colonies where as MKC

samples showed no growth. MIC and MKC values were expressed in µg/ml (Patani *et al*. 2011) [12].

Activity Index calculation

Activity Index (AI) was determined as ratio of inhibition zone produced by test sample and standard (Rakshit and Ramalingam, 2011) [13].

Determination of Leakage of Reducing Sugar and UV_{260}/UV_{280} Solutes

To determine reducing sugar leakage 10ml of the bacterial samples (10^8 CFU/ml) were transferred to broth medium containing 100, 200, 300, 400 and 500 µg/ml essential oil. The samples were incubated at 37°C with constant shaking for about 2/4hrs. After incubation samples were centrifuged at 12,000 rpm and recovered supernatant for reducing sugar estimation by DNS (3-5 dinitrosalicylic acid) method. Samples were read at 540nm and maltose used as standard (Parveen et al., 2018) [14].

Leakage of UV_{260} (DNA) and UV_{280} (protein) absorbing material was determined by standard protocol with some modification (Rakshit and Ramalingam, 2011) [13]. Bacterial suspension (10^8 cfu/ml) in 0.9% sterile NaCl was prepared. Samples were incubated at 37°C for 30 minutes. To the samples, essential oil (MIC) was added. Aliquots of the samples were taken at 30, 60, 90 and 120 min. After centrifugation samples were read spectrophometrically at 260 and 280 nm. Bacterial sample without MIC served as control.

Scanning Electron Microscopy (SEM)

Bacterial samples for SEM analysis were prepared following standard protocol with some modification (Tang et al., 2017)[15]. 10^8 cfu/ml samples were incubated in liquid medium at 37°C for 24 hours. Added test compound (MIC) to the samples (untreated samples served as control). Treated samples were incubated for 10 hours at 37°C. Centrifuged the samples at 6000 rpm for 10 min and pellets were washed with sterile phosphate-buffered saline for three times. The samples were passed through ethanol series (10, 25, 50, 75 and 100%) for dehydration. The samples were prepared as per protocol of JEOL manufactures (JSM-6390, Mahatma Gandhi University, Kottayam, Kerala) and observed for morphological features of bacterial cells (scanning electron microscope, 5Kv, X5500).

Statistical analysis

All the experiments were repeated six times. Further, mean and standard error of the values were calculated.

RESULT AND DISCUSSION

Hydro distillation using clevenger apparatus yielded 4.3ml/Kg fresh tissue weight of essential oil from the flowers of *Jasminum sambac* (L.) Aiton. Further, antibacterial properties of the extracted essential oil were determined against *Staphylococcus aureus* (gram positive) and *Pseudomonas aerugenosa* (gram negative bacteria).

The inhibition diameter (mm) displayed during disc diffusion is calculated and shown in Table. 1. The data was compared with standard streptomycin. Among the two bacteria, *Staphylococcus aureus* found more susceptible towards *Jasminum sambac* essential oil treatment producing 1.560, 1.900 and 3.11mm inhibition zone against 100, 250 and 500 µg/ml concentration respectively. *Pseudomonas aerugenosa* produced more or less similar sensitive zone in comparison to *Staphylococcus aureus*.

Table 1. Antibacterial activity exhibited by essential oil in terms of inhibition diameter (mm).

Type	Culture	Concentration (µg/ml)			Streptomycin (500µg/ml)	DMSO
		100	250	500		
Gram +ve	*Staphylococcus aureus*	1.560±0.0265	1.900±0.0722	3.116±0.050784	4.633±0.106559	**0**
Gram -ve	*Pseudomonas aerugenosa*	1.760±0.04224	1.921±0.0532	2.000±0.07	2.340±0.03744	**0**

Mean value ± Standard error

Activity index is shown in Table. 2. AI values are higher referring high bactericidal potential of essential oil against both gram +ve and gram -ve bacteria.

Table 2. Activity index (AI) exhibited by essential oil

Type	Culture	Concentration (500µg/ml)	Streptomycin (500µg/ml)	AI
Gram +ve	*Staphylococcus aureus*	3.116±0.050784	4.633±0.106559	0.672±0.05376
Gram -ve	*Pseudomonas aerugenosa*	2.000±0.07	2.340±0.03744	0.854±0.06832

Mean value ± Standard error

MIC and MKC values further substantiate the disc diffusion result. If MBC dose is equal to or less than four times the MIC concentration, then the antibacterial agent can be regarded as bactericidal (Saida *et al*. 2016) [16]. In all the tested strains, the MBC values found to be only nearly double the concentration of MIC (Table. 3). This clearly indicates

the remarkable ability of *Jasminum sambac* essential oil as potent antimicrobial agent.

Table 3. MIC and MKC values of essential oil against tested microbes.

Type	Culture	MIC	MKC	MIC/MKC	Streptomycin	DMSO
Gram positive	*Staphylococcus aureus*	4.4±0.088	8.2±0.1558	0.536±0.4288	0.516±0.0412	0
Gram negative	*Pseudomonas aerugenosa*	3.9±0.0624	7.7±0.2926	0.506±0.0404	0.468±0.0374	0

Mean value ± Standard error

Leakage of reducing sugar and UV_{260}/ UV_{280} is an implication of bacterial cell membrane integrity loss (Lakehal *et al*. 2016) [17]. Essential oil treated bacterial samples exhibited leakage in a concentration and duration dependent manner (Table. 4; Fig. 1,2). The result proposes the bacterial membrane damage caused by *Jasminum* essential oil might be by instigating lipid peroxidation or inhibition of cell membrane protein function thereby serves as potent bactericidal agent.

Table 4. Leakage of reducing sugar (µg/mg) from essential oil treated bacterial samples

	Pseudomonas aerugenosa **(Reducing Sugar)**		***Staphylococcus aureus*** **(reducing sugar)**	
	2h	**4h**	**2h**	**4h**
Control	0.02±0.001	0.09±0.0288	0.04±00116	0.08±0.00272
100 µg/mL	1.5±0.0375	2.8±0.042	2.4±0.0912	3.7±0.1073
200 µg/mL	3.6±0.1152	4.9±0.0588	4.5±0,072	5.9±0.1003
300 µg/mL	5.3±0.1484	7.1±0.1207	6.6±0.1518	8.1±0.1782
400 µg/mL	7.5±0,1575	8.0±0.088	8.7±0.2436	11.5±0.4485
500 µg/mL	8.8±0.2029	10.3±0.2472	10.0±0.11	13.3±0.2394

Mean value ± Standard error

Various scientific studies indicate, bioactive molecules of essential oils might attach to the bacterial cell surface and gradually either adhere or penetrate into the phospholipid bilayer of the cell membrane. In the act, the structural integrity of the cell membrane is altered leading to unrestrained influx or efflux of ions. The ionic imbalance thus generated is detrimental to cellular metabolism which eventually leads into cell death (Newman and Cragg, 2012) [18]. Also, constituents in essential oil might differ in targets which pose a great difficulty in predicting susceptibility of microorganisms from strain to strain (De Martino et al., 2009) [19].

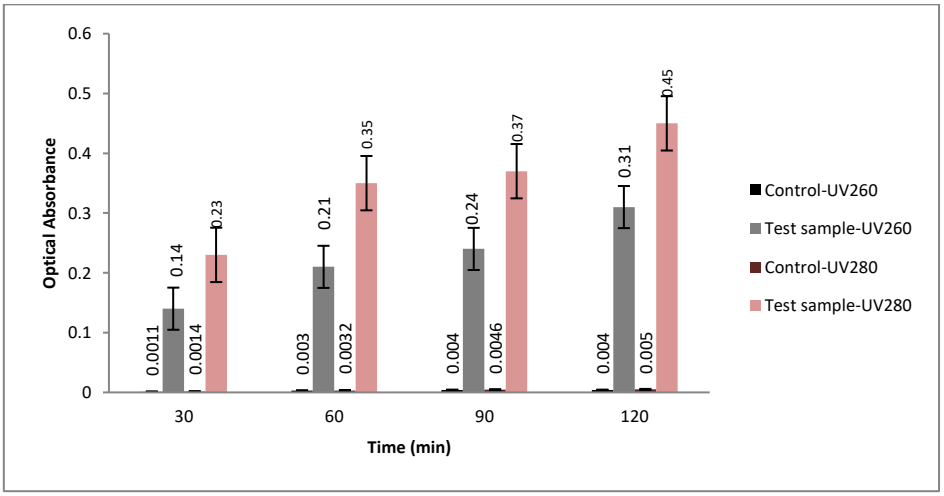

Figure 1. Leakage of UV_{260}/ UV_{280} from essential oil treated *Staphylococcus aureus* (Mean value ± Standard error)

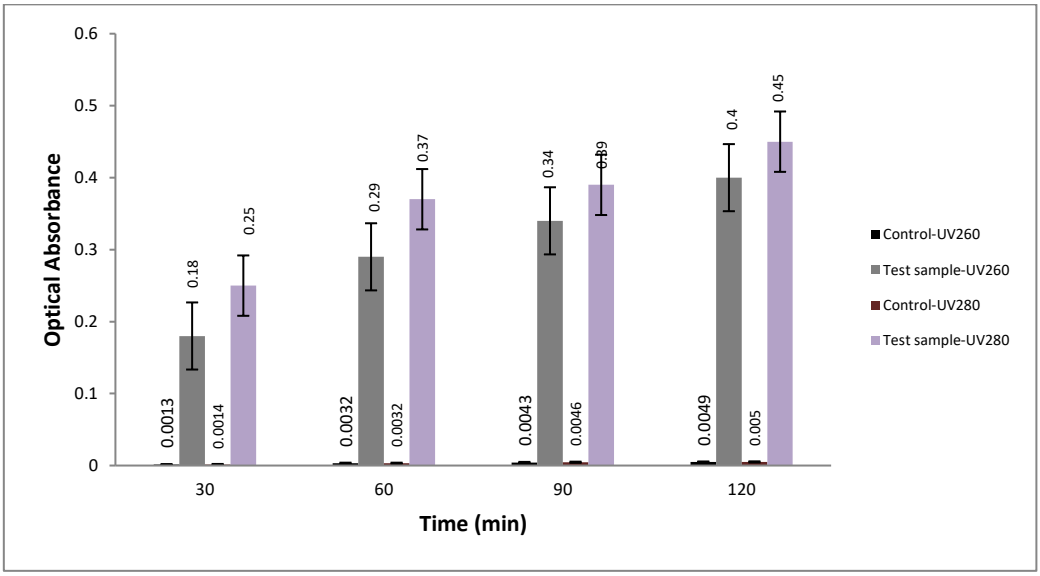

Figure 2. Leakage of UV_{260}/ UV_{280} from essential oil treated *Pseudomonas aerugenosa* (Mean value ± Standard error)

Gram-negative bacteria are generally considered as less sensitive towards essential oil (Azhdarzadeh and Hojjati, 2016) [20]. The increased resistance is attributed to the presence of hydrophilic lypopolysaccharides (LPS) in the outer membrane of Gram-negative bacteria. LPS proffer increased tolerance by generating a barrier against the penetration of cell membrane by hydrophobic antimicrobial components in the essential oil (Nazzaro *et al*. 2013)[21]. Ultee and team showed membrane damage and death of *E. coli* treated with black pepper essential oil in his studies (Ultee et al., 1999)[22]. In another study, Cinnamon essential oil destroyed *E. coli* and *S.aureus* cell membrane at MIC level and caused cell death at MBC (Horvath *et al*. 2009)[23]. Essential oil extracted from *Dipteracarpus gracilis* was reported to suppress cultures of *Bacillus cereus* and *Proteus mirabilis* by exerting inhibitory effects on the cell membrane as one of its targets (Kolli *et al*. 2016) [24].

Electron microscopic (SEM) analysis further supports the leakage of reducing sugar and $UV_{260/280}$ solutes via damage of cell membrane. The membrane disruption, distorted cell shape and clumbing in essential oil treated samples of *Staphylococcus aureus* was apparant, whereas shape and membrane found intact in control (Fig. 3). The results thus propose *Jasminum sambac* essential oil exerts inhibition on bacteria by disturbing peptidoglycan alignment, weakening cell wall and possibly cell death by osmotic lysis. A similar observation was reported by Li and team in *Citrus medica* L. var. *sarcodactylis* essential oil treated bacteria *E. coli* and *S. aureus* (Li *et al*. 2019) [25]. It is inferred that essential oil and its constituents target cell wall and interfere with its formation by blocking N-acetyl muramic acid (Goldbeck *et al*. 2014) [26].

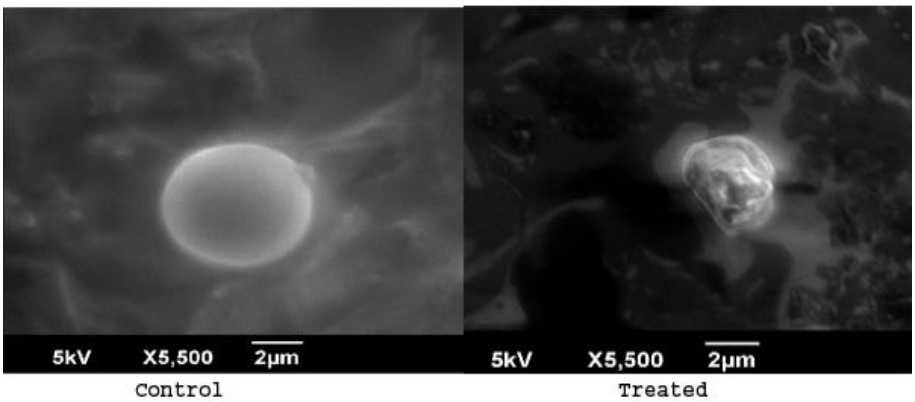

Figure 3. SEM analysis of essential oil treated *Staphylococcus aureus* and control.

CONCLUSION

Present study has attempted to isolate essential oil from *Jasminum sambac* flower and evaluated its biological potential in terms of antibacterial activity. Disc diffusion assay produced significant inhibition zones against bacteria such as *Staphylococcus aureus* and *Pseudomonas aerugenosa.* MIC, MBC and AI further substantiated the disc diffusion result. Estimation of leakage of reducing sugar, UV_{260} and UV_{280} absorbing materials suggested the probable membrane damage caused by the essential oil. The membrane damage is confirmed by studies on bacterial cell morphology using scanning electron microscopic method. Further studies are required to identify the active principle and to understand the underlying molecular mechanism of membrane damage caused by *Jasminum sambac* essential oil.

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