IJPSR (2019), Volume 10, Issue 3 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 01 July 2018; received in revised form, 17 September 2018; accepted, 22 September 2018; published 01 March 2019

ANALYSIS OF QUORUM QUENCHING POTENTIAL OF *EUPHORBIA TRIGONA* MILL.

K. Marathe *, N. Nashikkar, S. Bundale and A. Upadhyay

Hislop School of Biotechnology, Hislop College, Temple Road, Civil Lines, Nagpur - 440001, Maharashtra, India.

Keywords:

Quorum Sensing (QS), Fatty acid derivatives, 9,12-Octadecadienoyl chloride (Z, Z)-, Swarming, Biofilm

Correspondence to Author:
K. Marathe

Hislop School of Biotechnology, Hislop College, Temple Road, Civil Lines, Nagpur - 440001, Maharashtra, India.

E-mail: kirtidubli@gmail.com

ABSTRACT: *S. marcescens* and *P. mirabilis* are gram-negative bacteria responsible for nosocomial infections. Quorum sensing regulates virulence in *S. marcescens* and *P. mirabilis*. Present study documents the potential of *E. trigona* extracts in inhibiting quorum sensing in both the bacterial species. Extracts of aerial part of the plant were prepared in petroleum ether, chloroform, ethyl acetate, acetone and methanol using soxhlet extraction technique. Petroleum ether extract, chloroform extract, and acetone extract were found to quench quorum in *S. marcescens*. These extracts were next subjected to column chromatography and assayed for its quorum quenching capacity. Later, thin layer chromatography was used to separate and identify the bioactive fraction responsible for quorum quenching. The fraction exhibiting quorum quenching capacity was subjected to GC-MS analysis. It revealed the presence of fatty acid derivatives in the bioactive fraction. A major part of the bioactive fraction was 9,12-Octadecadienoyl chloride (Z, Z), a derivative of linoleic acid, and, hence linoleic acid was also used for testing its effect on swarm motility of bacteria. Furthermore, the bioactive fraction was employed to study the inhibition of virulence factors in *S. marcescens* and *P. mirabilis*. Bioactive fraction lowered the prodigiosin production as well as reduced swarming motility and biofilm formation in *S. marcescens*. It was also found effective in decreasing urease synthesis, biofilm formation and swarming in *P. mirabilis*. Therefore, bioactive fraction isolated from *E. trigona* and linoleic acid both quenched quorum in *S. marcescens* and *P. mirabilis*.

INTRODUCTION: Bacterial communication involving the production of molecules called autoinducers and their detection by receptors is referred to as quorum sensing (QS). QS is a population density-dependent phenomenon responsible for regulating the behavior of bacterial cells in consortium [1]. It accounts for various attributes of bacterial behavior including swarming, luminescence, biofilm formation, sporulation, conjugation, and virulence.

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.10(3).1372-86

The article can be accessed online on www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.10(3).1372-86

Disruption of QS in bacteria, therefore, might serve as an effective strategy to deal with bacterial infections [2].

Serratia marcescens is a member of the Enterobacteriaceae family of gram-negative bacteria [3]. It causes nosocomial infections of respiratory tract, urinary tract [4], ocular region [5] and also septicemia and meningitis [6]. QS regulates the production of prodigiosin, chitinase, protease, hemolysin production and virulence factors like swarming motility, swimming motility, biofilm formation [7]. *Proteus mirabilis* is also a gram-negative bacterium belonging to the family of Enterobacteriaceae. This organism is primarily responsible for causing urinary tract infections comprising pyelonephritis as well as cystitis.

Besides, *P. mirabilis* is associated with infections of eyes, ears, respiratory tract, throat, skin burns, and wounds. *P. mirabilis* swarms on the surface of the agar in an extremely organized mode giving rise to the characteristic bull's eye pattern. Swarming of *P. mirabilis* is concomitantly associated with its virulence including urease synthesis, biofilm formation and host cell invasion [8]. QS regulates swarming and synthesis of virulence factors in *P. mirabilis* [9].

An alternative approach to treat *S. marcescens* and *P. mirabilis* infections is the need of the hour since many strains of these bacteria are highly resistant to antibiotics. Interference and inhibition of QS system of *S. marcescens* and *P. mirabilis* may, therefore, be a pertinent and proficient technique to reduce the pathogenicity of these bacteria.

Many species of Euphorbia (*E. hirta*, *E. nerifolia*, *E. antiquourm*, *E. tirucalli*, *E. trigona*) are employed in traditional ayurvedic medicines for the treatment of infections and inflammation [9]. They are also used for conditions like rheumatoid arthritis, gout, blood disorders, jaundice, tumors, insect bite and hepato-splenomegaly [10]. *Euphorbia trigona* Mill. is a tropical plant that yields milky latex [11]. Previous work in our lab has established anti-QS [9], anti-swarming, immunostimulatory [12] anti-fungal and antioxidant properties [10] of *E. trigona* Mill. The present study aims to isolate the anti-QS compound(s) from *E. trigona* Mill. and utilize it to study anti-QS effects against *S. marcescens* and *P. mirabilis*.

MATERIALS AND METHODS:

Bacterial Strains and Growth Conditions: Bacterial strains and their culture conditions are described in **Table 1**.

TABLE 1: BACTERIAL STRAINS AND GROWTH CONDITIONS

Bacterial Species	Strain (Based on 16SrRNA sequencing)	Growth Conditions
Serratia marcescens	21 – 3 (Accession: JF429937.1)	LB medium at 30 ºC
Proteus mirabilis	Isolate 3 – 1 – 32 (Accession: AB932526.1)	LB medium at 37 ºC

Collection of Plant Material and Preparation of Extract: The aerial part of the plant was collected from the garden of Hislop College, Nagpur and identified by comparison with reference material (accession no. 10081) at Department of Botany, RTMNU, Nagpur. Plant material was washed and shade dried. The dried plant material was powdered and subjected to soxhlet extraction using petroleum ether, chloroform, ethyl acetate, acetone, and methanol. Extracts obtained were dried using vacuum concentrator and re-suspended in a minimum volume of a suitable solvent and stored at 4 °C for further use.

Preliminary Phytochemical Analysis: The extracts obtained from soxhlet extraction were qualitatively analyzed for the presence of phenols [13], tannins, saponins, flavonoids, alkaloids [14], anthraquinones [15], steroids [16] and triterpenoids [17].

Analysis of Anti-QS Activity of Crude Extracts Against *S. marcescens* and Isolation of Bioactive Principle: 1% of the overnight grown culture of *S. marcescens* was added to molten nutrient agar and plated. The agar was allowed to solidify, and wells (diameter = 0.5 cm) were punched into it. 100 µl of concentrated extracts were added to these wells and incubated overnight at room temperature against a suitable control. Plates were observed the next day for the zone of pigment inhibition. The extracts inhibiting pigment production were subjected to column chromatography.

The column for chromatography was prepared using 7 g of silica mesh 60-120 pore size. 600-800 µl of concentrated extract was adsorbed onto 0.2 – 0.3 g of silica mesh 60-120 pore size and used for chromatographic extraction. Elution was carried out at the rate of 40-60 drops per minute using different solvents and solvent systems - chloroform, chloroform: acetone: acetic acid (75: 24:1, 75: 40: 1, 75: 50: 1, 75: 60: 1 and 75: 60: 5) and methanol. Different fractions were collected, concentrated and assayed for anti-QS activity by agar well diffusion method.

Preparative TLC of Bioactive Principle: The bioactive fractions isolated using column chromatography was subjected to TLC. The bioactive fractions were dissolved in 100 µl solvent, and 10µl was used to spot on TLC plate (silica gel 60 f_{254}). The plate was run using ethyl acetate: methanol: water (6: 2: 1) as the mobile phase. The separated bands on TLC were extracted from silica by scraping it and extracting using small

volumes of the solvent system until the silica was rendered completely colorless. The pooled extracts were concentrated, dried and then assayed for its anti-QS activity by agar well diffusion method. The TLC band which demonstrated anti-QS potential was labelled as F-b and was subjected to GC-MS analysis.

GC-MS Analysis: The F-b was dissolved in methanol and then subjected to GC-MS analysis at Sophisticated Analytical Instruments facility, IIT Powai, Mumbai. The instruments used for GC and MS were Agilent 7890 and Joel Accu TOF GCV respectively. The carrier gas was helium and a flow rate of 1ml/min was used. The length and internal diameter of HP5 column were 30 mm and 0.32 mm and the film were 0.25 mm. 5 µl of the sample was injected in split mode with injector split ratio 1:10. The temperature used was 80 °C to 200 °C at a rate of 8°/min and hold for 1 min. The temperature was raised to 275 °C at a rate of 8°/min and hold for 3 min. The temperature was then raised to 280 °C at the rate of 5°/min and hold for 5 min. The complete elution time was 35 min. Identification of compounds was done by comparing spectra of unknown compounds with known compounds via library search with HPCHEM software.

QS Inhibition in *S. marcescens*:

Prodigiosin Assay: 1% inoculum was added to a nutrient broth containing different concentrations of F-b (252-1020 µg/ml) and incubated overnight. After incubation, the cells were pelleted by centrifugation. The supernatant was discarded, and the pellet was re-suspended in 1 ml of 4% 1 M HCl for extracting prodigiosin from the bacterial cells. The mixture was again centrifuged to remove cell debris. The supernatant was collected, and its absorbance was read at 534 nm. [6] The relative prodigiosin concentration per cell was calculated [18] as:

$$\text{Relative Prodigiosin concentration per cell} = \frac{A_{534}\ ml^{-1}}{OD_{600}\ unit}$$

Swarm Assay: 5 µl of an overnight culture of *S. marcescens* was inoculated on dried 0.5% LB agar plates containing different concentrations of F-b (252-1020 µg/ml). The plates were incubated for 24 h and observed for swarming pattern changes against the control plate [19].

Biofilm Formation Assay: Effect of F-b on biofilm formation by *S. marcescens* was quantified using crystal violet. 1% inoculum was added to LB broth and 100 µl of this freshly inoculated broth was transferred to the wells of the microtiter plate. Different concentrations of F-b (252-1020 µg/ml) were then added to the wells and incubated overnight. After the completion of incubation, the planktonic cells were removed by washing with sterile water twice and biofilm was stained with 0.4% crystal violet. After two minutes, excess stain was removed by using sterile water. The cells bound to crystal violet were solubilized in 1 ml of ethanol, and its absorbance was read using spectrophotometer at 570nm. [20]

Air-Liquid Interface Assay: This assay was performed for microscopic analysis of bacterial cells forming biofilm in the presence of F-b. Briefly, a sterile 24-well microtiter plate was adjusted at an angle of 30 - 50° and inoculated with 250 µl broth containing 0.1% inoculum of *S. marcescens*. Different concentrations of F-b (252-1020 µg/ml) were then added to the wells and incubated overnight in the angled position. After incubation, spent medium was removed, followed by washing twice gently with sterile medium. 200 µl of medium was then added to the wells and plate was laid on the flat surface of an inverted microscope to visualize the cells [21] using suitable controls.

QS Inhibition in *P. mirabilis*:

Swarm Assay: 5 µl of an overnight culture of *P. mirabilis* was inoculated on dried 1.5% LB agar plates containing different concentrations of F-b (133, 333, 533 and 733 µg/ml). The plates were incubated for 24 h and observed for changes in swarming pattern against a suitable control.

Urease assay: Effect of F-b (133-733 µg/ml) on urease production was analyzed by determining the amount of un-hydrolyzed urea in urea-LB broth cultures (filter sterilized urea was added to LB broth with a final concentration of 0.5 mg/ml). Urea-LB broth culture containing bioactive principle was inoculated with 2% overnight culture of *P. mirabilis* and incubated for 48 h at 37 °C. The amount of urea remaining was estimated to ascertain the urease activity using suitable control [9].

Biofilm Formation Assay: Effect of the bioactive compound on biofilm formation by *P. mirabilis* was quantified using crystal violet. 1% inoculum was added to LB broth and 100 µl of freshly inoculated broth was transferred to the wells of a microtiter plate. Different concentrations of F-b (133-733 µg/ml) were then added to the wells and incubated overnight. After the completion of incubation, the planktonic cells were removed by washing twice with sterile water and biofilm was stained with 0.4% crystal violet. After two minutes, excess stain was removed with the help of sterile water. The cells bound to crystal violet were solubilized in 1 ml of ethanol and its absorbance was read using spectrophotometer at 570nm. [20]

Air-Liquid Interface Assay: Briefly, a sterile 24-well microtiter plate was adjusted at an angle of 30 – 50º and inoculated with 250 µl broth containing 0.1% inoculum of *P. mirabilis*. Different concentrations of F-b (133-733 µg/ml) were then added to the wells and incubated overnight in the angled position. The spent medium was removed after incubation, followed by washing gently twice with sterile medium. 200 µl of medium was then added to the wells and plate was laid on the flat surface of an inverted microscope to visualize the cells [21] using suitable controls.

Bacterial Growth Assay: The bacterial growth was monitored in the presence of F-b using growth curve assay described by Hall *et al*., [22] with few modifications. 0.1% of overnight cultures of *P. mirabilis* and *S. marcescens* were inoculated into sterile broth tubes containing different concentrations of F-b. 100 µl of this freshly inoculated broth was transferred to the different wells microtiter plate. The plates were covered, and immediately the absorbance of the plate was read at 600 nm using plate reader. The plates were now transferred to shaker incubator, and OD_{600} was read at 2, 4, 6, 8 and 24 h. The readings were recorded and compared with control.

Statistical Analysis: All the experiments were accomplished in triplicates. The results are calculated and expressed as mean ± standard deviation. The statistical significance was evaluated using Student's t-test ($p<0.05$) and ANOVA using Microsoft Excel (version 16.4.1).

RESULTS AND DISCUSSION: QS in *S. marcescens* and *P. mirabilis* is a population density-dependent phenomenon that regulates expression of virulence factors. It directs the production of antibiotics and exoenzymes, swarming, adhesion to the surfaces [23] and biofilm formation [24]. Thus, disruption of QS might prove to be a contemporary method of controlling virulence and pathogenesis in *S. marcescens* and *P. mirabilis*.

Preliminary Phytochemical Analysis: Preliminary phytochemical analysis revealed the presence of various phytoconstituents as shown in **Table 2**. Presence of flavonoids was detected in petroleum ether extract (PX), ethyl acetate extract (EX), acetone extract (AX) and methanolic extract (MX). PX, chloroform extract (CX), EX and AX also demonstrated the presence of alkaloids. The occurrence of phenols and saponins was observed in all extracts. The presence of tannins, flavonoids, alkaloids, triterpenoids, and saponins has been previously reported in plants belonging to the *Euphorbia* genus [12, 25]. Several reports suggest that phytochemicals can restrain QS [26]. Various phenolics, tannins, stilbenes, flavonoids, diarylheptanoids, triterpenoids, sulfur-containing phytochemicals, coumarins, quinones, and alkaloids have been described to possess QS inhibitory potential [27, 28]. Thus, phytoconstituents detected in extracts of *E. trigona* Mill. might be responsible for its QS inhibitory properties.

TABLE 2: PRELIMINARY PHYTOCHEMICAL ANALYSIS OF *EUPHORBIA TRIGONA* EXTRACTS

Test	Petroleum ether extract	Chloroform extract	Ethyl acetate extract	Acetone extract	Methanol extract
Phenols	+	+	+	+	+
Tannins	+	+	-	-	+
Saponins	+	+	-	+	+
Flavonoids	+	-	+	+	+
Steroids	+	+	+	-	-
Alkaloids	+	+	+	+	-
Anthraquinones	+	-	-	+	+
Triterpenoids	-	-	-	+	+

+: Phytochemical Present; -: Phytochemical absent

Analysis of Anti-QS Activity of Crude Extracts Against *S. marcescens*, Isolation of Bioactive Principle and Preparative TLC of Bioactive Principle: Quorum quenching potential of crude extracts of PX, CX, EX, AX, and MX was evaluated using *S. marcescens* as indicator strain [19, 29]. The work presented here reports the novel use of *S. marcescens* as indicator strain to identify QS inhibitor(s) from terrestrial plant *E. trigona* Mill. by agar well diffusion method. Synthesis of Prodigiosin pigment in *S. marcescens* is regulated by QS [7]. PX, CX, and AX demonstrated QS inhibitory potential, as no prodigiosin synthesis was observed around the wells in the plates, while EX showed no such effect as shown in **Fig. 1**. MX (as shown in **Fig. 1**) was found to enhance synthesis of pigment prodigiosin, in turn demonstrating QS augmenting potential. The activity of crude extracts is graphically represented in **Fig. 2**. Several extracts of plants like *K. Africana* [30], *T. chebula* [31], *P. nigrum*, *P. betle*, *G. gnemon* [32], *C. verum* [33] and many others have exhibited anti-QS potential.

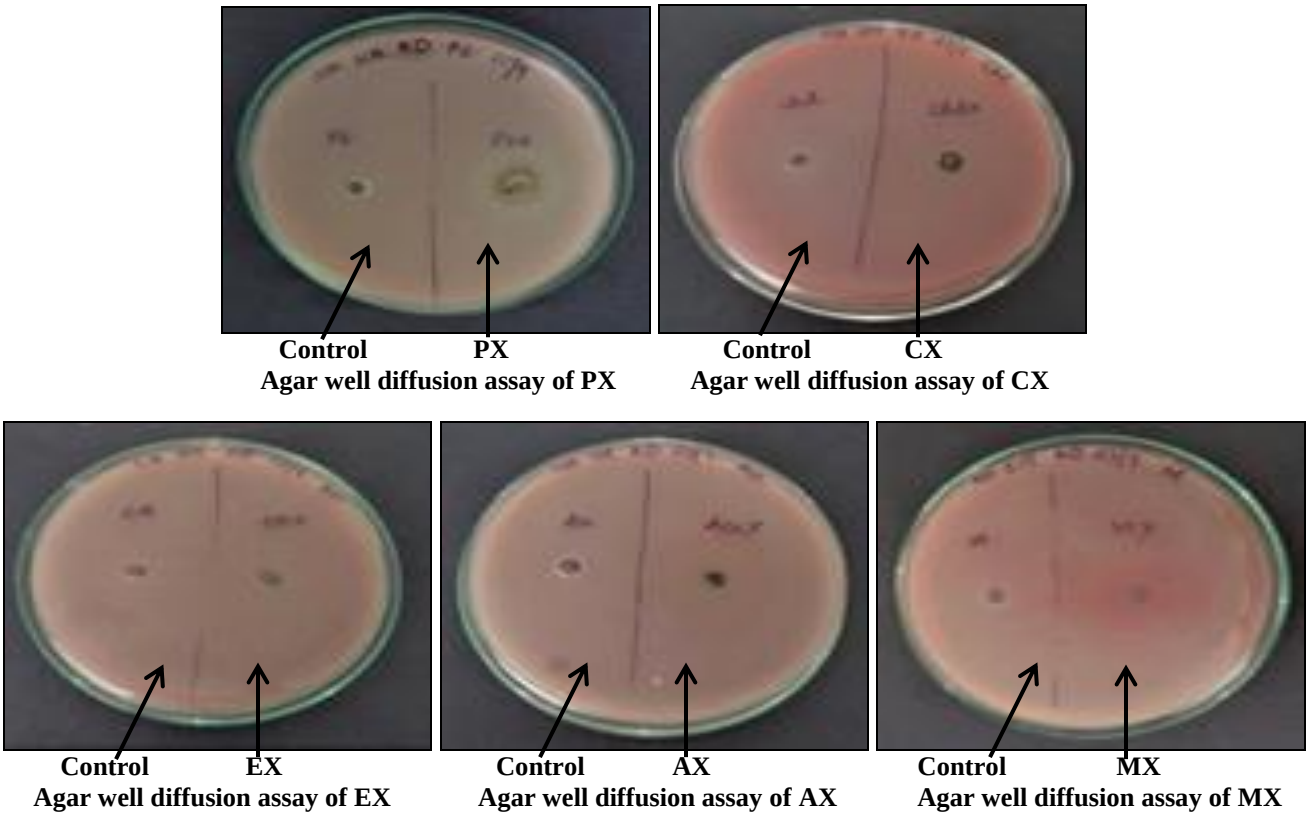


FIG. 1: AGAR WELL DIFFUSION ASSAY AGAINST *S. MARCESCENS* TO DETECT TO QUORUM SENSING INHIBITORY POTENTIAL OF CRUDE EXTRACTS

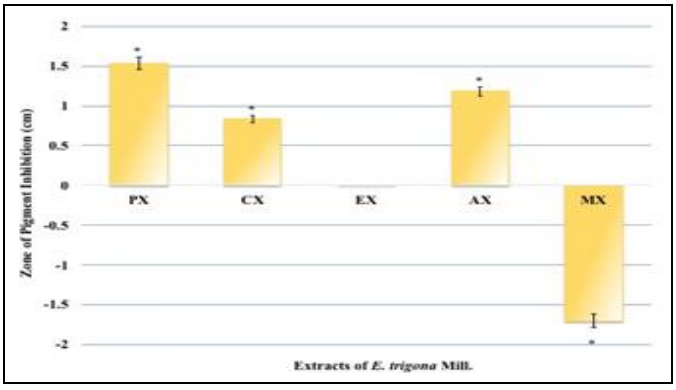


FIG. 2: EFFECT OF CRUDE EXTRACTS ON PIGMENT INHIBITION IN *S. MARCESCNES*. The error bars shown indicate that the experiments are carried out in triplicates and $p<0.001$ as compared with control

Crude PX, CX, and AX demonstrating QS inhibitory potential were subjected to column chromatography. Different fractions of column chromatography of each extract were collected, concentrated and examined for QS inhibitory property using *S. marcescens* as an indicator organism. PX yielded 12 fractions (named as PXF1 – PXF12) while CX and AX both yielded 13 fractions each (named as CXF1 – CXF13 and AXF1 – AXF13 respectively) when subjected to column chromatography. The effect of these fractions of column chromatography of PX, CX, and AX are shown in **Fig. 3**.

Those fractions that inhibited prodigiosin synthesis in *S. marcescens* were subjected to TLC. R_f values of bands obtained on TLC plates were calculated, and each of the bands was scraped, extracted and then checked for its anti - QS activity against *S. marcescens* using agar well diffusion assay. Fraction PXF5, CXF11, and AXF12 demonstrated the presence of common bands on TLC and also showed QS inhibitory potential. Various bands obtained of PXF5, CXF11 and AXF12 on TLC, their R_f values are shown in **Fig. 4**.

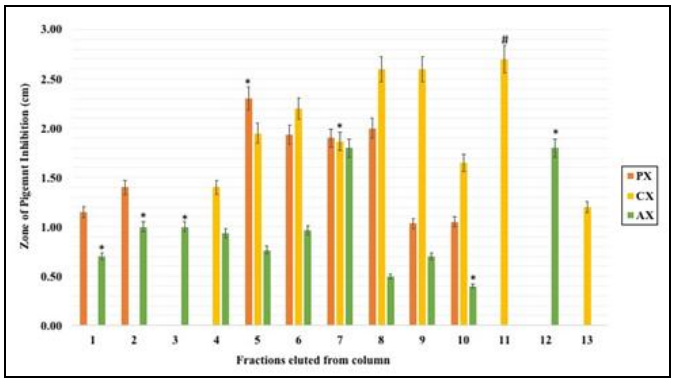


FIG. 3: EFFECT OF DIFFERENT FRACTIONS OF COLUMN CHROMATOGRAPHY OF PX, CX AND AX. The error bars indicate that experiments were carried out in triplicates ($^{\#}p<0.02$ and $^{*}p<0.03$ as compared with control)

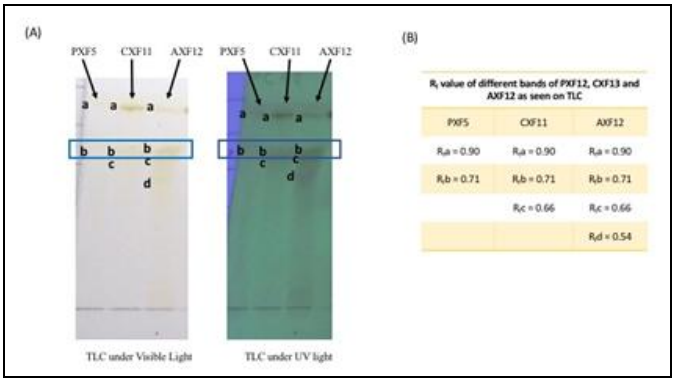


FIG. 4: (A) TLC OF PXF5, CXF11 AND AXF12 AS SEEN IN VISIBLE LIGHT AND UV LIGHT – 254 nm RESPECTIVELY AND (B) R_f VALUES OF DIFFERENT BANDS OF PXF5, CXF11 AND AXF12 AS SEEN ON TLC

Only a single band each from PX, CX, and AX (as indicated by the blue rectangle), with common R_f value showed inhibition of prodigiosin synthesis in *S. marcescens* and hence this band was selected and named as F-b for further assays.

GC-MS Analysis: GC-MS analysis of F-b in methanol yielded a total 12 peaks, of which 4 were major peaks and the rest of them were minor peaks. Comparison of mass spectra of peaks with the database led to the identification of various compounds present in F-b. Remarkably, compounds detected in F-b also displayed bio-activities. The details of compounds identified, nature of the compound, molecular weight, molecular formula, retention time, peak area (%), NIST match factor are as indicated in **Table 3**. Reported biological activities/functions of these compounds are shown in **Table 4**.

TABLE 3: COMPOUNDS IDENTIFIED FROM F-B BY GC-MS ANALYSIS

Name of Compound	Nature of Compound	Molecular weight	Molecular Formula	Retention time	Peak Area (%)	NIST Match Factor
Icosapent	Long Chain Fatty acid	302	$C_{20}H_{30}O_2$	3.17	2.04	333529
Ethanol 2(2-butoxyethoxy)	Ethylene glycols	162	$C_8H_{18}O_3$	7.12	5.04	133143
N, N-dimethyloctylamine	Volatile Organic Compound	157	$C_{10}H_{32}N$	12.02	0.90	233924
Phenol 2, 4 – bis (1,1-dimethylethyl)-	Alkylbenzene	206	$C_{14}H_{22}O$	12.45	1.71	228966
Oxalic acid, allyl dodecyl ester	Alkyl ester	298	$C_{17}H_{30}O_4$	19.47	2.57	309240
13-Hexyloxacyclotridec-10-en-2-one	Ketone	280	$C_{18}H_{32}O_2$	21.00	4.46	192992
Oleic acid	Fatty acid	282	$C_{18}H_{34}O_2$	22.56	1.61	154664
Hexadecanoic acid, 2-hydroxy-(1-hydroxymethyl) ethyl ester	Fatty acid ester	330	$C_{19}H_{38}O_4$	24.42	9.12	15400
Oleic acid	Fatty acid	282	$C_{18}H_{34}O_2$	26.05	1.66	154664
9,12-Octadecadienoyl chloride (Z, Z)-	Linoleic acid chloride	298	$C_{18}H_{31}ClO$	26.61	24.84	76312
Oleic acid	Fatty acid	282	$C_{18}H_{34}O_2$	27.20	5.15	154664
9,12,15-Octadecatrienoic acid, 2-(acetyloxy)-1- [(acetyloxy)methyl] ethyl ester, (Z, Z, Z)-	Linolenic acid ester	436	$C_{25}H_{40}O_6$	27.56	1.22	17550
(E)- 9- Octadecenoic acid ethyl ester	Fatty acid	310	$C_{20}H_{38}O_2$	28.45	1.90	130916
7-Methyl-Z-tetradecen-1-ol acetate	Acetate ester	268	$C_{17}H_{32}O_2$	28.75	7.82	130996
9,12-Octadecadienoyl chloride (Z, Z)-	Linoleic acid chloride	298	$C_{18}H_{31}ClO$	29.13	25.54	76312
9,12-Octadecadienoyl chloride (Z, Z)-	Linoleic acid chloride	298	$C_{18}H_{31}ClO$	29.88	1.56	76312
9,12-Octadecadienoyl chloride (Z, Z)-	Linoleic acid chloride	298	$C_{18}H_{31}ClO$	30.11	2.88	76312

TABLE 4: BIOLOGICAL ACTIVITY/FUNCTIONS OF COMPOUNDS IDENTIFIED BY GC-MS ANALYSIS OF F-B

Name of the Compound	Functions
Icosapent	The precursor of Thromboxane – 3 and Prostaglandin – 3 molecules, anti-inflammatory, anti-thrombotic and immunomodulatory [34]
Ethanol 2(2-butoxyethoxy)	Solvents, Pigments, viscosity adjustors [35]
N, N-dimethyloctylamine	Anti-*Pseudogygmnoascus destructans* activity [36]
Phenol 2, 4 – bis (1,1-dimethylethyl)-	Anti-bacterial and anti-inflammatory [37], anti-QS [38]
Oxalic acid, allyl dodecyl ester	-
13-Hexyloxacyclotridec-10-en-2-one	Protein kinase C gamma inhibitor [39],
Oleic acid	Anti-bacterial [40], anemiagenic insectifuge, dermatitigenic, cancer preventive and anti-androgenic [41]
Hexadecanoic acid, 2-hydroxy-(1-hydroxymethyl) ethyl ester	Hemolytic, pesticide, flavor, antioxidant [42]
9,12-Octadecadienoyl chloride (Z, Z)-	Anti-secretory, anti-tonsillitic, anti-spermigenic, choloretic, anti-tubercular, contraceptive [42], antimicrobial [43]
9,12,15-Octadecatrienoic acid, 2-(acetyloxy)-1-[(acetyloxy)methyl] ethyl ester, (Z, Z, Z)-	Cancer preventive, Anti-inflammatory and hepato-protective [44]
(E)- 9- Octadecenoic acid ethyl ester	Perfumery [45]
7-Methyl-Z-tetradecane-1-ol acetate	Anti-inflammatory, hepatoprotective and Anti-cancer [46]

GC-MS analysis of F-b revealed the presence of various fatty acids and their derivatives. 9,12-Octadecadienoyl chloride (Z, Z)-, was found to be the highest component, 54.82%, in F-b followed by Hexadecanoic acid, 2 - hydroxyl - (1 - hydroxymethyl) ethyl ester 9.12%, oleic acid 8.42% and 7 - Methyl - Z - tetradecane - 1 - ol acetate 7.82%. Different peaks obtained by GC-MS analysis of F-b can be seen in **Fig. 5**. The mass spectra of different compounds identified are shown in **Fig. 6**.

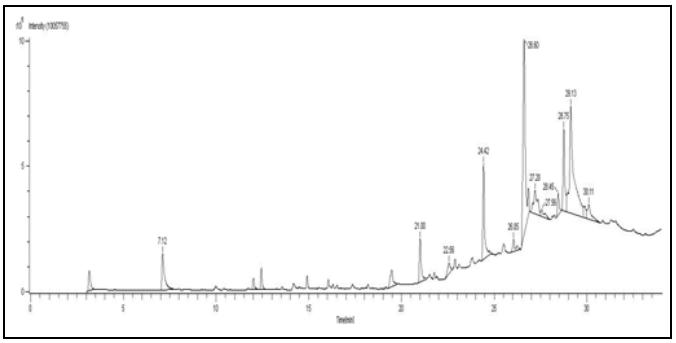
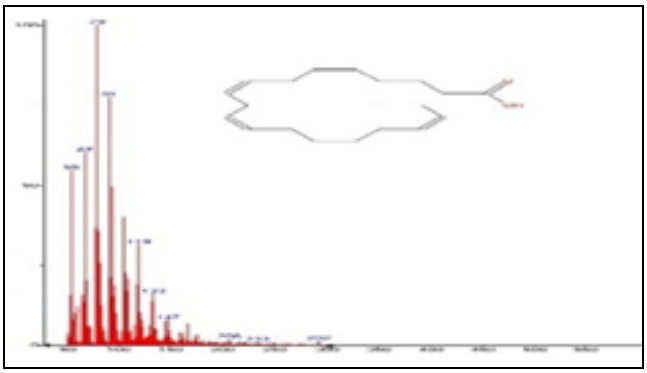
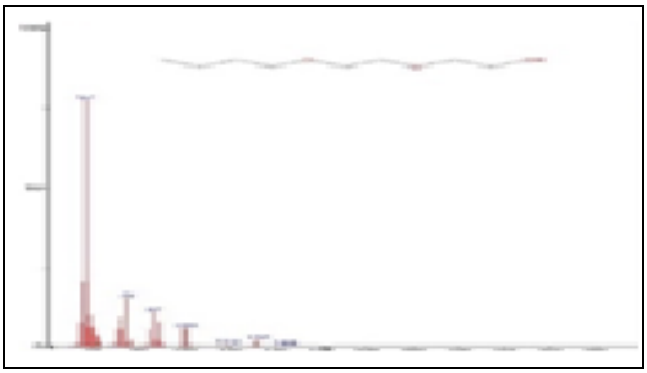


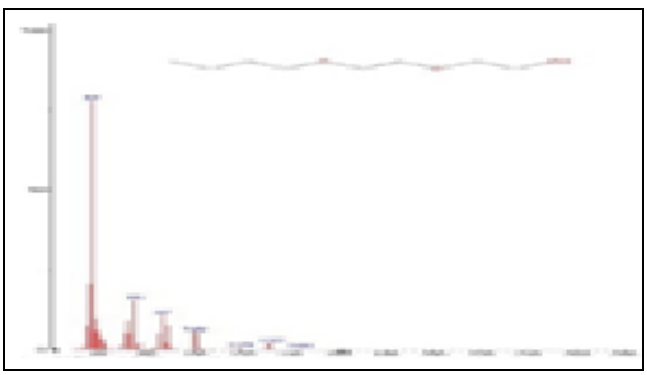
FIG. 5: GC-MS CHROMATOGRAM OF F-B DISSOLVED IN METHANOL



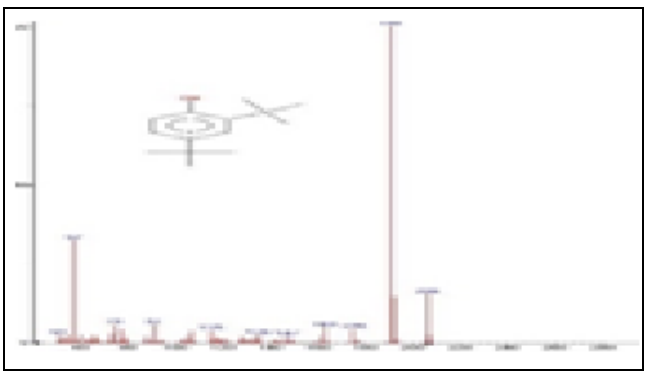
Icosapent



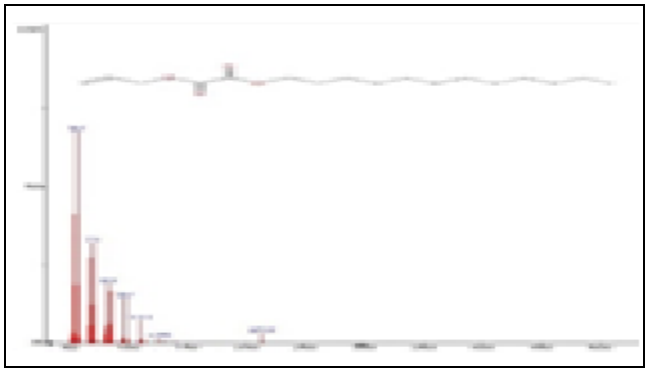
Ethanol 2(2-butoxyethoxy)



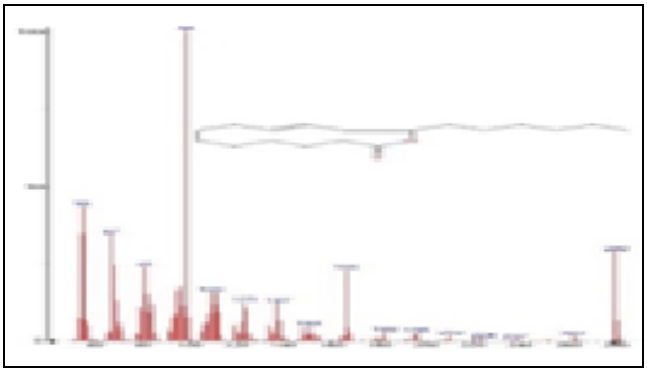
N, N – dimethyloctylamine



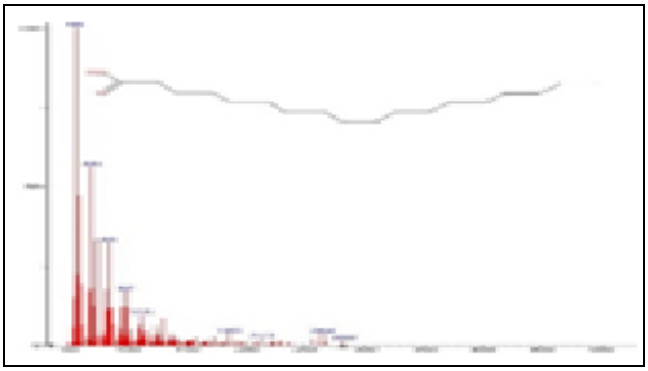
Phenol 2, 4 – bis (1, 1 – dimethylethyl) -



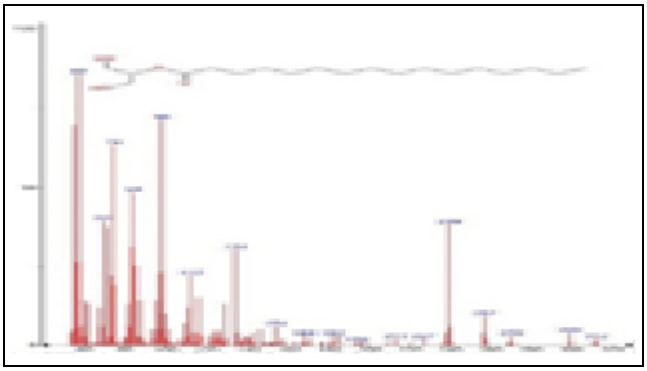
Oxalic acid, allyl dodecyl ester



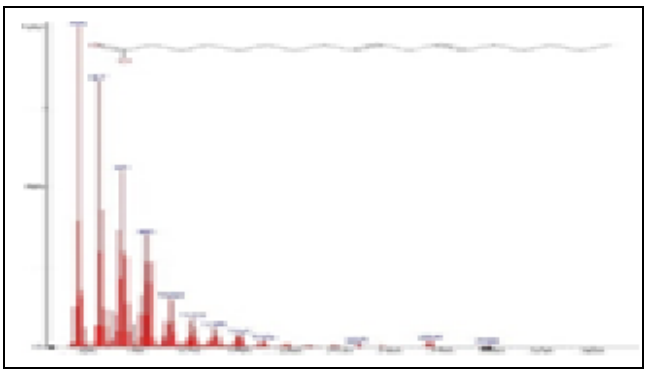
13-Hexyloxacyclotridec-10-en-2-one



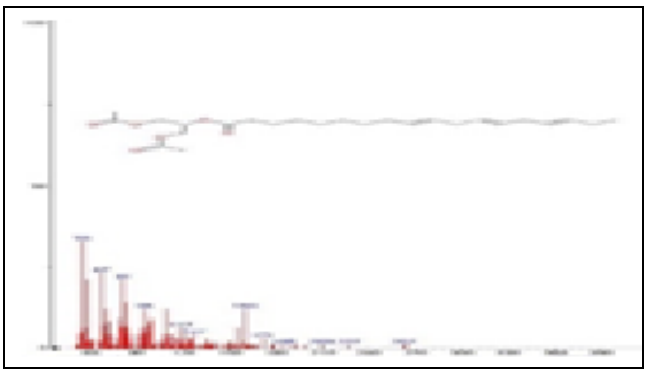
Oleic acid



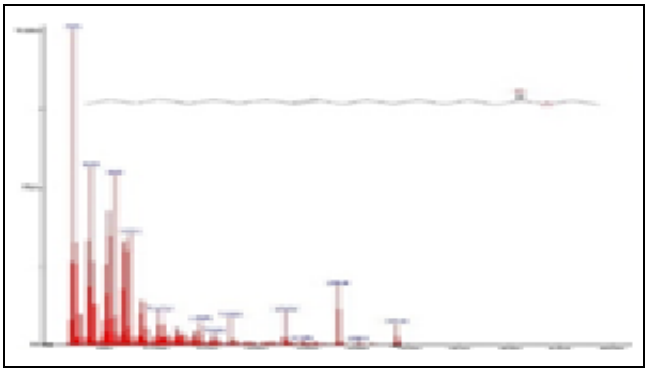
Hexadecanoic acid, 2-hydroxy-(1-hydroxymethyl) ethyl ester



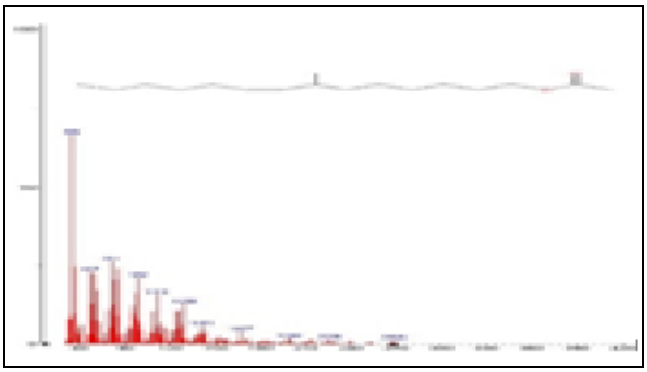
9, 12 -Octadecadienoyl chloride (Z, Z) -



9, 12, 15 -Octadecatrienoic acid, 2-(acetyloxy) -1-[(acetyloxy)methyl] ethyl ester, (Z, Z, Z) -



(E)-9-Octadecenoic acid ethyl ester



7-Methyl-Z- tetradecane-1-ol acetate

FIG. 6: MASS SPECTRA OF COMPONENTS IDENTIFIED IN F-B DISSOLVED IN METHANOL

Various components of F-b have been reported to possess antibacterial properties as well as QS inhibitory properties. 9, 12-Octadecadienoyl chloride (Z, Z)- [derivative of linoleic acid], present in the highest concentration, might be responsible for QS inhibition of virulence factors seen in *S. marcescens* and *P. mirabilis*. Linoleic acid has been reported to possess antimicrobial

activity in addition to bacteriostatic functions [47]. Linoleic acid and oleic acid have also been earlier reported as QS inhibitors against *Vibrio harveyi* and *Escherichia coli* [48]. Inhibitory action of fatty acids is a result of inhibition of FabI, in turn inhibiting fatty acid synthesis in bacteria [49]. Work presented in this research paper reports fatty acids and their derivatives as QS inhibitors isolated from *E. trigona* Mill. against *S. marcescens* and *P. mirabilis* responsible for causing nosocomial infections. As per our knowledge, it is one of the primary reports of fatty acids and their derivatives as QS inhibitors from the terrestrial succulent plant. Hence, the identified factors might be useful to develop drugs for effective infection control and elimination of pathogens.

Prodigiosin Assay: QS regulates the synthesis of secondary metabolite-prodigiosin in *S. marcescens* [7]. When the effect of F-b was studied on prodigiosin synthesis in *S. marcescens*, it was found that prodigiosin synthesis decreased in a dose-dependent manner as shown in **Fig. 7**. The highest inhibition was seen at a concentration of 1020 µg/ml demonstrating the least prodigiosin synthesis of 24.66%.

The concentrations of 252 µg/ml, 508 µg/ml and 764 µg/ml resulted in 52.05%, 45.21% and 35.62% of prodigiosin synthesis by bacteria. Similar results were also observed in QS inhibition studies of plants like *Cinnamomum verum* [33] and *Anethum graveolens* [20] which resulted in 38.3% and 29% of prodigiosin synthesis in the bacterium, indicating effectivity of F-b in inhibiting prodigiosin synthesis. Thus, F-b was effective in inhibiting prodigiosin synthesis, in turn, influencing QS in *S. marcescens*.

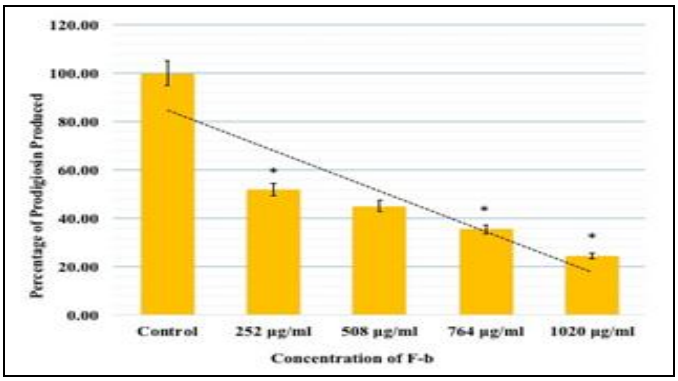


FIG. 7: EFFECT OF F-B ON PRODIGIOSIN SYNTHESIS IN *S. MARCESCNES*. All the experiments were performed in triplicates and *p<0.001.

Urease Assay: Urease is a nickel metalloenzyme produced by *P. mirabilis*. Urea is hydrolyzed by enzyme urease to yield ammonia and carbon dioxide, subsequently increasing local pH of urine. This increased alkaline pH ensues precipitation of calcium and magnesium ions, consequently forming apatite (calcium phosphate) and struvite (magnesium ammonium phosphate), *i.e.,* urinary stones, respectively [8]. Stone formation and alkaline pH facilitates the bacteria to adhere to cell surfaces of host and form a biofilm. Besides, stone formation benefits the bacteria as it protects the host immune system. It also triggers ammonia toxicity, the direct damage of tissues and blocking of ureters [50].

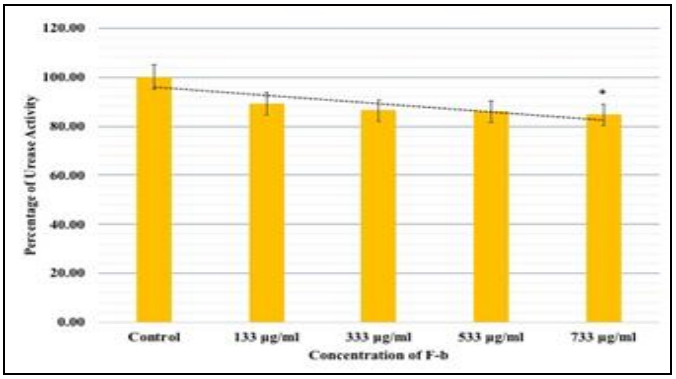


FIG. 8: EFFECT OF F-B ON UREASE ACTIVITY OF *P. MIRABILIS*. Error bars indicate that the experiment was performed in triplicate and *p<0.01 concerning control

Study of the effect of F-b on the urease activity of *P. mirabilis* yielded in steadily decreased urease activity with increasing concentration of F-b as seen in **Fig. 8**. Urease activity was found to be 89.25%, 86.46%, 86.01% and 84.69% at concentrations of 133, 333, 533 and 733µg/ml respectively. There was a decrease of urease activity at a concentration of 133µg/ml followed by a gradual reduction in enzyme activity at increased concentrations of F-b. Inhibition of urease activity has also been revealed by the study of the effect of allicin from garlic [51], and antibacterial agents like fluoroquinolones [52]. Thus, inhibition of urease activity would prevent struvite and apatite formation and accordingly benefit in the elimination of pathogens by the host immune system.

Swarm Assay: Swarming in bacteria is a multicellular behavior characterized by movement over surfaces using rotating flagella [53]. Swarming motility assists bacteria in better proliferation by

mechanisms like the production of antimicrobial surfactants, prevention of engulfment by macrophages, toxin production [54], biofilm formation and invading eukaryotic cell [55].

Work reported here establishes concentration-dependent inhibition of swarming in *S. marcescens*. Swarm diameter was reduced to 5.00 cm, *i.e.* 61.54% at a concentration of 1020 µg/ml concerning control. Effect of F-b on swarming is graphically represented in **Fig. 9**. Swarm diameter was found to be 7.70 cm, 6.68 cm and 6.00 cm signifying 94.77%, 82.15% and 73.85% swarming at concentrations of 252 µg/ml, 508 µg/ml and 764 µg/ml, thus indicating a consistent dose-dependent reduction. Studies conducted using *Plectranathus ambonicus* [56], *Capparis spinosa* [57] and petroselinic acid [58] have similarly demonstrated swarm inhibition in *S. marcescens*.

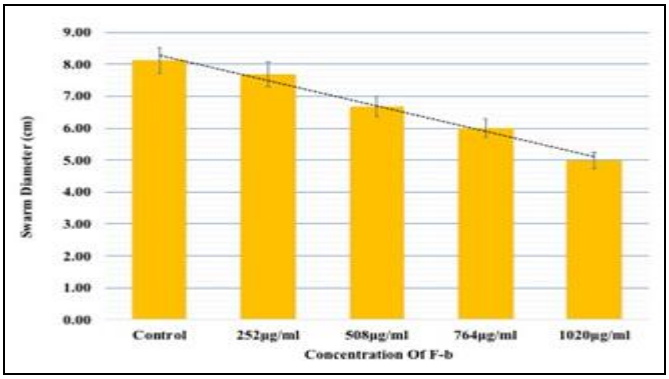
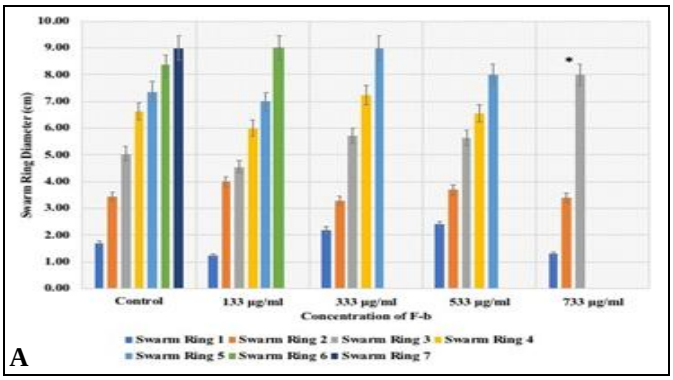


FIG. 9: GRAPHICAL REPRESENTATION OF EFFECT OF F-B ON SWARMING IN *S. MARCESCENS*. Experiments were performed in triplicates and p<0.001.

Swarming in *P. mirabilis* is unique with a distinctive bull's eye pattern [8]. The current study analyses change in swarming pattern of *P. mirabilis* in the presence of F-b. The changes in swarm pattern of *P. mirabilis* are represented in **Fig. 10**. As the concentration of F-b increased, the no. of swarm rings formed by bacterium decreased accompanied by a reduction in swarm diameter. The decline in swarm diameter was gradual, but the no. of swarm rings reduced to 50% at the highest concentration of 733 µg/ml. Swarm inhibition and changes in swarm diameter have also been previously documented in work conducted using thioridazine [59], bacteriocins from probiotic bacteria [60], lauric acid, myristic acid and palmitic acid [61]. F-b was more effective in inhibiting swarming of *S. marcescens*. While F-b inhibited swarming in *P. mirabilis*, it decreased the ability to form more swarm rings, a representative attribute of QS and virulence. Thus, additional insight into the process at the molecular level might facilitate a better understanding of inhibition observed.



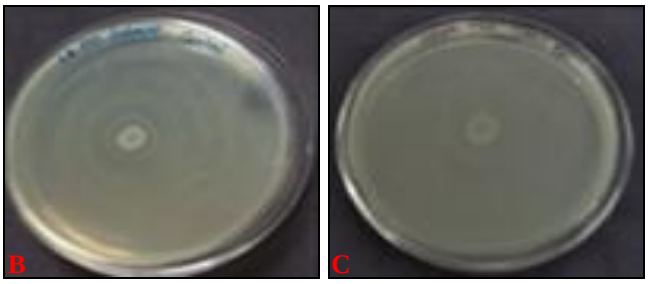


FIG. 10: EFFECT OF F-B ON SWARMING IN *P. MIRABILIS*. A: GRAPHICAL REPRESENTATION OF EFFECT OF F-B ON SWARMING IN *P. MIRABILIS*. EXPERIMENTS WERE PERFORMED IN TRIPLICATES AND *P<0.005. B: CONTROL SWARM PLATE OF *P. MIRABILIS*. C: SWARM INHIBITION PLATE OF *P. MIRABILIS* CONTAINING 733 µg/ml OF F-B

Biofilm Formation: Pathogenic bacteria adhere to surfaces and propagate to form a biofilm. Formation of biofilm results in altered gene expression as well as modified phenotype. Development of biofilm provides an advantage of the decreased vulnerability to antibiotics [33] and increased ability to survive against host defenses. Consequently, biofilm formation on medical devices such as catheters and implants result in long-term or persisting infections. Hence, inhibiting biofilm formation would help in the control of bacterial pathogenesis.

S. marcescens forms a biofilm of undifferentiated cells arranged as micro-colonies, regulated by QS [62]. Biofilm formation reduced with increasing concentration of F-b. Least biofilm formation was observed to be 61.61% at a concentration of 1020 µg/ml while, the concentrations of 252 µg/ml, 508 µg/ml and 764 µg/ml resulted in the formation of 88.89%, 86.11% and 79.17% biofilm concerning control as shown in **Fig. 11**.

The results observed were coherent with the QS inhibition studies of plants like *Vitex trifolia* [63], *Cinnamomum verum* [33] and *Anethum graveolens* [20], thus indicating that F-b was able to reduce the biofilm formation ability of *S. marcescens*.

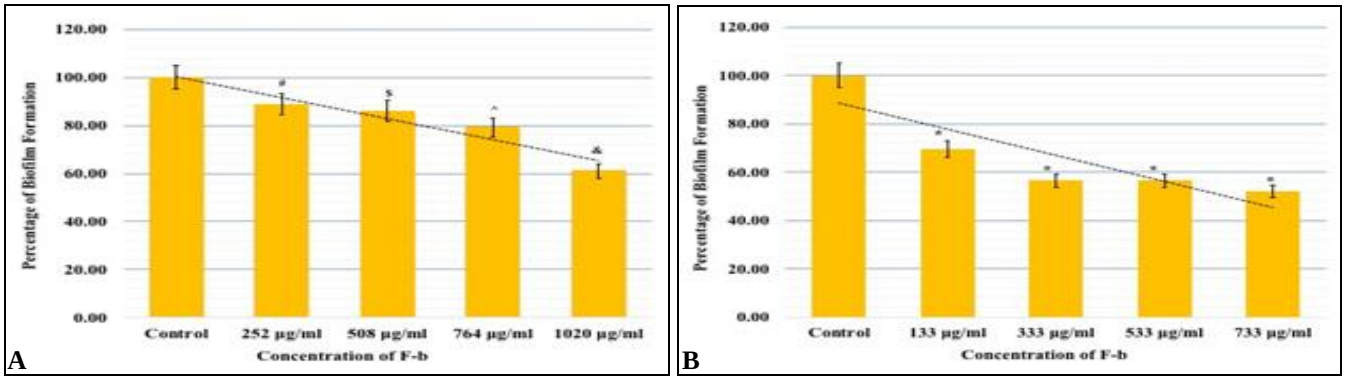


FIG. 11: EFFECT OF F-B ON BIOFILM FORMATION

(A) GRAPHICAL REPRESENTATION OF BIOFILM FORMATION IN *S. MARCESCENS* IN PRESENCE OF F-B. ERROR BARS INDICATE THAT EXPERIMENTS HAVE BEEN PERFORMED IN TRIPLICATE (#P<0.02, $P<0.007, ^P<0.01 AND & P<0.005). (B) GRAPHICAL REPRESENTATION OF BIOFILM FORMATION IN *P. MIRABILIS* IN PRESENCE OF F-B ERROR BARS INDICATE THAT EXPERIMENTS HAVE BEEN PERFORMED IN TRIPLICATE (*P<0.001)

P. mirabilis forms crystalline biofilms, which is mushroom-shaped. These crystalline biofilms instigate various problems like kidney and bladder stones, further worsening the infection [64]. Biofilm formation is also regulated by QS; hence, the effect of F-b was analyzed on biofilm formation in *P. mirabilis*. 733 µg/ml diminished the biofilm formation to 52.17%, while 133, 333 and 533 µg/ml reduced biofilm formation to 69.57%, 56.52%, and 56.52% respectively as depicted in the **Fig. 11**. F-b actively inhibited biofilm formation in *P. mirabilis* demonstrating the potential of fatty acid and their derivatives to interfere with QS. Investigations made by Liaw *et al*., on the effect of fatty acids- stearic acid and palmitic acid also demonstrate their QS inhibitory potential [61]. Likewise work described by Widmer *et al*., also establishes the QS inhibitory nature of linoleic acid mediated by AI - 2 inhibition [48]. Thus, the presence of fatty acids and their derivatives in F-b corroborates its QS inhibitory potential.

Growth Curve Assay: Growth curve analysis was performed to assess whether changes seen in QS-regulated properties of *S. marcescens* and *P. mirabilis* was a consequence of QS inhibition by F-b and not its antibacterial activity. Analysis indicated no significant change in cell densities at 24 h when compared to control, as shown in **Fig. 12** and **Fig. 13**, indicating that changes in various virulence factors observed ensued from QS inhibition by F-b.

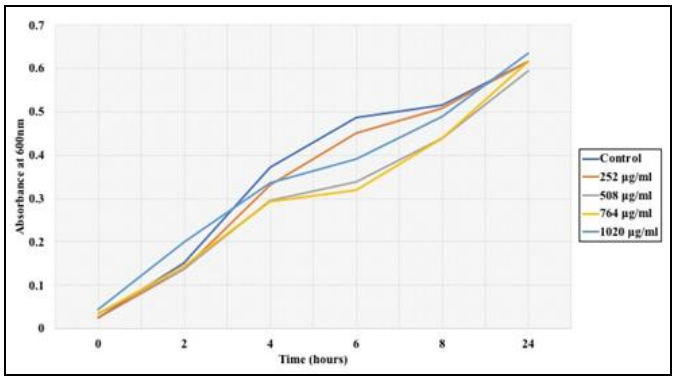


FIG. 12: EFFECT OF F-B ON GROWTH OF S. *MARCESCENS*

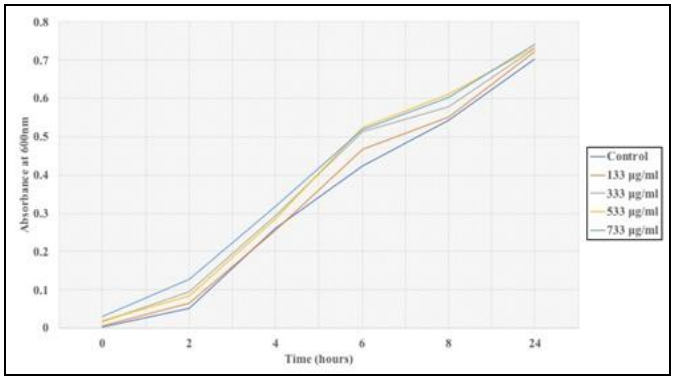


FIG. 13: EFFECT OF F-B ON GROWTH OF *P. MIRABILIS*

Air-Liquid Interface Assay: Observation of biofilm of *S. marcescent* and *P. mirabilis* under the inverted microscope in the presence of F-b (1020 µg/ml and 733 µg/ml) revealed diminished biofilm when compared to control **Fig. 14**. This observation further ascertains the QS inhibitory capacity of F-b.

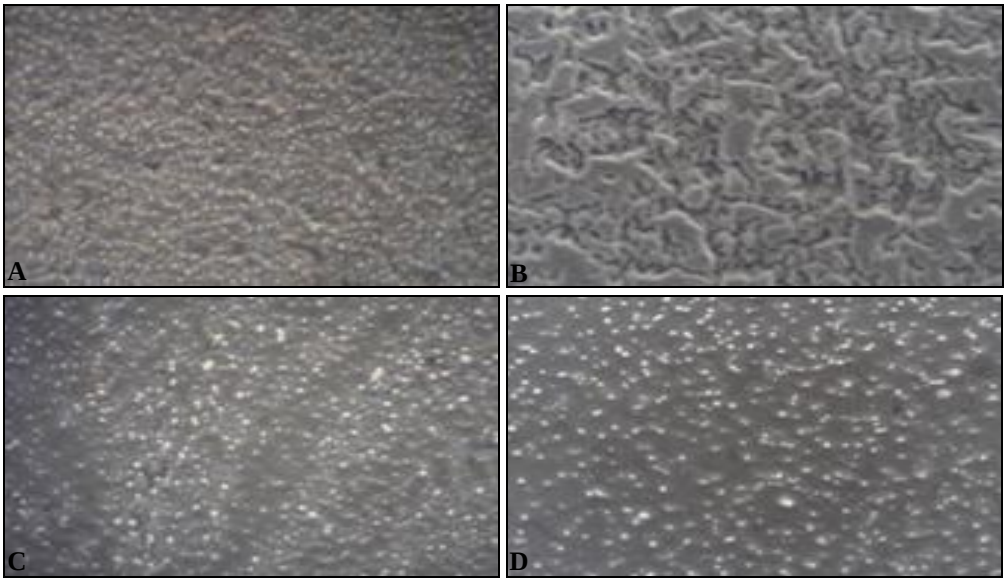
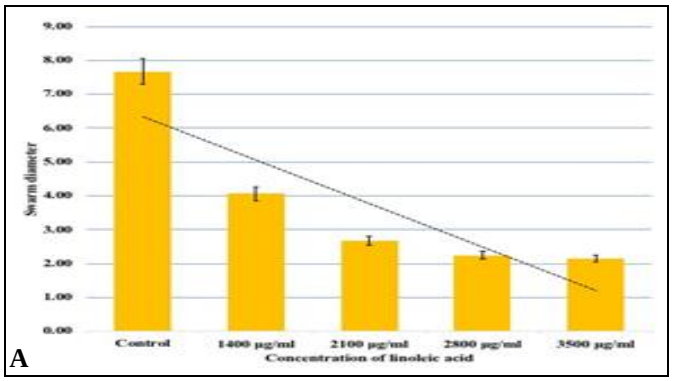


FIG. 14: AIR LIQUID INTERFACE ASSAY OF BIOFILM

A. CONTROL BIOFILM OF *S. MARCESCENS*
B. *S. MARCESCENS* BIOFILM IN PRESENCE OF 1020 µg/ml OF F-B
C. CONTROL BIOFILM OF *P. MIRABILIS*
D. *P. MIRABILIS* BIOFILM IN PRESENCE OF 733 µg/ml OF F-B

As 9,12-Octadecadienoyl chloride (Z, Z)- [chloride derivative of linoleic acid] formed the primary component of F-b, hence, linoleic acid was used to assess QS inhibition, by analyzing swarm pattern changes in *S. marcescens* and *P. mirabilis*.



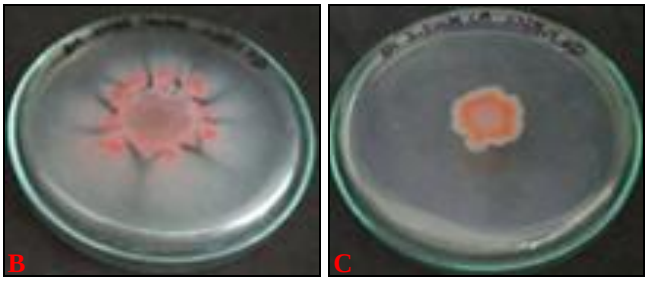


FIG. 15: EFFECT OF LINOLEIC ACID ON SWARMING IN *S. MARCESCENS*. A: GRAPHICAL REPRESENTATION OF EFFECT OF LINOLEIC ACID ON SWARMING OF *S. MARCESCENS*. ERROR BARS INDICATE THAT THE EXPERIMENT WAS PERFORMED IN TRIPLICATES AND P<0.001. B: CONTROL - SWARM PLATE OF *S. MARCESCENS*. C: SWARM PLATE OF *S. MARCESCENS* CONTAINING 3500 µg/ml OF LINOLEIC ACID

Concentrations of 1400, 2100, 2800 and 3500 µg/ml of linoleic acid were used to analyze its swarm inhibitory potential. The effect of linoleic acid on swarm motility of *S. marcescens* and *P. mirabilis* is represented in **Fig. 15** and **16**. It was more effective in inhibiting swarm motility of *S. marcescens* than *P. mirabilis*. Linoleic acid reduced swarming to 53.06% at 1400 µg/ml and 28.03% at 3500 µg/ml in *S. marcescens*. The pattern of anti-swarm ability of linoleic acid was comparable to that of F-b, however, F-b was effective in bringing about QS inhibition as evident from the concentrations used for investigation, implying higher QS inhibitory ability of fatty acid derivatives.

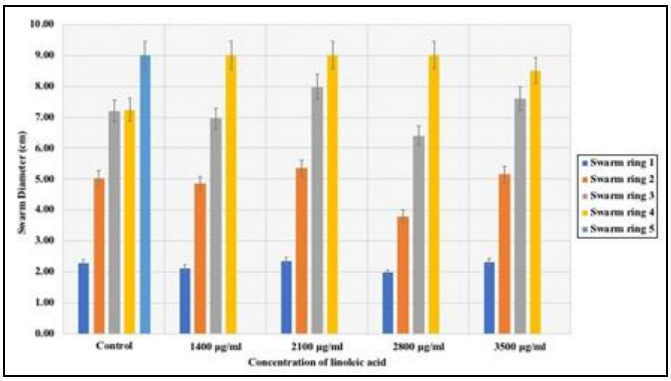


FIG. 16: EFFECT OF LINOLEIC ACID ON SWARMING OF *P. MIRABILIS*. Error bars indicate that the experiment was performed in triplicates and p<0.05

CONCLUSION: The work presented here depicts QS inhibitory effects of fatty acid derivatives on *S. marcescens* and *P. mirabilis* isolated from terrestrial plant *E. trigona* Mill. While the exact mechanism of QS inhibition by fatty acids or their derivatives is not known, this study paves the way for developing drugs, containing fatty acid derivatives and promulgating the importance of lipids. Further, analysis of the interaction of fatty acid derivatives with different receptors and its effect on various genes of bacteria might furnish a better understanding of impeding bacterial pathogenesis and contribute to better treatment of infections.

ACKNOWLEDGEMENT: We would like to acknowledge CSIR for funding the study in the form of Fellowship [File no. 08/611(0001)2013-EMR-I]. Further, we would also like to express our gratitude to IIT-POWAI SAIF for helping us with GC-MS analysis.

CONFLICT OF INTEREST: No Conflict of Interest.

REFERENCES:

1. Rémy B, Mion S, Plener L, Elias M, Chabrière E and Daudé D: Interference in Bacterial Quorum Sensing : A Biopharmaceutical Perspective. Front Pharmacol. 2018; 9: 1-17. doi:10.3389/fphar.2018.00203
2. Oliveira BDA, Rodrigues AC, Bertoldi MC, Taylor JG and Pinto UM: Microbial control and quorum sensing inhibition by phenolic compounds of acerola (*Malpighia emarginata*). Int Food Res J 2017; 24(5): 2228-2237.
3. Iguchi A, Nagaya Y and Pradel E: Genome evolution and plasticity of serratia marcescens, an important multidrug-resistant nosocomial pathogen. Genome Biol Evol 2014; 6(8): 2096-2110. doi: 10.1093/gbe/evu160
4. Srinivasan R, Mohankumar R and Kannappan A: Exploring the anti-quorum sensing and antibiofilm efficacy of phytol against *Serratia marcescens* associated acute pyelonephritis infection in Wistar rats. Front Cell Infect Microbiol 2017. doi: 10.3389/fcimb.2017.00498
5. Hover T, Maya T and Ron S: Mechanisms of bacterial (*Serratia marcescens*) attachment to, migration along, and killing of fungal hyphae. Appl Environ Microbiol 2016; 82(9): 2585-2594. doi: 10.1128/AEM.04070-15
6. Sethupathy S, Ananthi S and Selvaraj A: Vanillic acid from *Actinidia deliciosa* impedes virulence in Serratia marcescens by affecting S-layer, flagellin and fatty acid biosynthesis proteins. Sci Rep 2017; 7(1): 16328. doi: 10.1038/s41598-017-16507-x
7. Koul S, Prakash J, Mishra A and Kalia VC: Potential Emergence of Multi-quorum Sensing Inhibitor-Resistant (MQSIR) Bacteria. Indian J Microbiol 2016; 56(1): 1-18. doi: 10.1007/s12088-015-0558-0
8. Schaffer JN and Pearson MM: Proteus mirabilis and Urinary Tract Infections. Microbiol Spectr 2015; 3(5): 212-263. doi: 10.1128/microbiolspec.UTI-0017-2013. Proteus
9. Nashikkar N, Begde D, Bundale S, Pise M, Rudra J and Upadhyay: Inhibition of swarming motility, biofilm formation and virulence factor expression of urinary pathogens by *Euphorbia trigona* latex extracts. Int J Pharm Res 2011; 2(3): 558-566.
10. Upadhyay M, Nashikkar N and Begde D: Study of antimicrobial, antioxidant and antiquourm sensing properties of *Euphorbia trigona*. Glob J Res Med Plants Indig Med 2013; 2(9): 630-641. doi: 10.7897/2277-4343. 04135
11. Tada M and Seki H: Toxic Diterpenes from *Euphorbia trigona* (Saiunkaku : an Indoor Foliage Plant in Japan). Agric Biol Chem. 1989; 53(2): 425-430. doi: 10.1080/ 00021369.1989.10869285
12. Nashikkar N, Begde D, Bundale S, Mashitha P, Rudra J and Upadhyay A: Evaluation of the Immunomodulatory Properties of *Euphorbia trigona* - an *in-vitro* study. Int J Institutional Pharm Life Sci. 2012; 2(1): 88-105.
13. Santhi K and Sengottuvel R: Qualitative and quantitative phytochemical analysis of *Moringa concanensis* Nimmo. Int J Curr Microbiol Appl Sci 2016; 5(1): 633-640. doi:10.20546/ijcmas.2016.501.064
14. Prabhavathi R, Prasad M and Jayaramu J: Studies on qualitative and quantitative phytochemical analysis of *Cissus quadrangularis*. Adv Appl Sci Res 2016; 7(4): 11-17.
15. Ghalem BR and Ali B: Preliminary phytochemical screening of five commercial essential oils. World J Appled Chem 2017; 2(4): 145-151. doi: 10.11648/j.wjac. 20170204.16
16. Rajesh KD, Vasantha S, Rajesh NV and Panneerselvam A: Qualitative and quantitative phytochemical analysis in four pteridophytes. Int J Pharm Sci Rev Res 2014; 27(2): 408-412. doi:10.13040/IJPSR.0975-8232.6(1).393-98
17. Tadesse S, Ganesan K, Nair SKP, Letha N, Gani SB and Sciences M: Preliminary phytochemical screening of *Euphorbia cotinifolia* L. (Family : Euphorbiaceae) by using different solvents. World J Pharm Pharm Sci 2016; 5(3): 1176-1183.
18. Elkenawy NM, Yassin AS, Elhifnawy HN and Amin MA: Optimization of prodigiosin production by *Serratia marcescens* using crude glycerol and enhancing production using gamma radiation. Biotechnol Reports 2017; 14: 47-53. doi:10.1016/j.btre.2017.04.001
19. Morohoshi T, Shiono T and Takidouchi K: Inhibition of quorum sensing in *Serratia marcescens* AS-1 by synthetic analogs of N-acylhomoserine lactone. Appl Environ Microbiol 2007; 73(20): 6339-6344. doi: 10.1128/AEM. 00593-07
20. Salini R and Pandian SK: Interference of quorum sensing in urinary pathogen *Serratia marcescens* by Anethum graveolens. FEMS Pathog Dis 2015; 73: 1-8. doi: 10.1093/femspd/ftv038
21. Merritt JH, Kadouri DE and O'Toole GA: Growing and Analyzing Static Biofilms. Curr Protoc Microbiol 2005; 1(1B.1): 1-29. doi: 10.1002/9780471729259.mc01b01s00. Growing.
22. Hall BG, Acar H, Nandipati A and Barlow M: Growth rates made easy. Mol Biol Evol. 2014; 31(1): 232-238. doi: 10.1093/molbev/mst187
23. Ding T, Li T, Wang Z and Li J: Curcumin liposomes interfere with quorum sensing system of *Aeromonas sobria* and *in-silico* analysis. Sci Rep 2017; 7(1): 1-16. doi: 10.1038/s41598-017-08986-9

24. El-Hamid MLA: A new promising target for plant extracts : inhibition of bacterial quorum sensing. J Mol Biol Biotechnol 2016; 1(1:4): 1-3.
25. Bigoniya P, Agrawal S and Verma NK: Potential wound healing activity of *Euphorbia hirta* Linn. total flavonoid fraction. Int J Pharm Sci Rev Res 2013; 22(2): 149-156.
26. Nazzaro F, Fratianni F and Coppola R: Quorum sensing and phytochemicals. Int J Mol Sci 2013; 14(6): 12607-12619. doi: 10.3390/ijms140612607
27. Ta CAK and Arnason JT: Mini review of phytochemicals and plant taxa with activity as microbial biofilm and quorum sensing inhibitors. Molecules 2016; 21(1): 1-26. doi:10.3390/molecules21010029
28. Bouyahya A, Dakka N, Et-Touys A, Abrini J and Bakri Y: Medicinal plant products targeting quorum sensing for combating bacterial infections. Asian Pac J Trop Med 2017; 10(8): 729-743. doi: 10.1016/j.apjtm.2017.07.021
29. Yamazaki G, Nishimura S and Ishida A: Effect of salt stress on pigment production of *Serratia rubidaea* N-1 : A potential indicator strain for screening quorum sensing inhibitors from marine microbes. J Gen Appl Microbiol 2006; 52: 113-117. doi: 10.2323/jgam.52.113
30. Chenia HY: Anti-quorum sensing potential of crude *Kigelia africana* fruit extracts. Sensors 2013; 13: 2802-2817. doi: 10.3390/s130302802
31. Sarabhai S, Sharma P and Capalash N: Ellagic acid derivatives from *Terminalia chebula* Retz . downregulate the expression of quorum sensing genes to attenuate *Pseudomonas aeruginosa* PAO1 Virulence. PLoS One 2013; 8(1): 1-11. doi: 10.1371/journal.pone.0053441
32. Tan LY, Yin WF and Chan KG: *Piper nigrum*, *Piper betle* and *Gnetum gnemon*- Natural food sources with anti-quorum sensing properties. Sensors 2013; 13: 3975-3985. doi: 10.3390/s130303975
33. Yamarthi A, Narayanan L and Jonnalgadda SV: Quorum quenching ability of dietary spice *Cinnamomum verum* on pathogenic bacteria. Int J Pharm Sci Res 2014; 5(12): 5216-5223. doi:10.13040/IJPSR.0975-8232.5(12).5216-23
34. PubChem. Eicosapentaenoic Acid. NCBI. http://pubchem.ncbi.nlm.nih.gov/summary/summary.cgi?cid=446284#itabs-2d. Published in 2008. Accessed November 6, 2017.
35. PubChem. 2-(2-Butoxyethoxy)ethanol _ C8H18O3 - PubChem. NCBI. https://pubchem.ncbi.nlm.nih.gov/compound/8177. Published in 2005. Accessed November 6, 2017.
36. Cornelison CT, Gabriel KT, Barlament C and Crow Jr. SA: Inhibition of *Pseudogymnoascus destructans* Growth from Conidia and Mycelial Extension by Bacterially Produced Volatile Organic Compounds. Mycopathologia 2014; 177: 1-10. doi: 10.1007/s11046-013-9716-2
37. Chandrasekar T, Rao MRK, Kumar RV, Prabhu K, Kumar SN and Divya D: GC-MS analysis, antimicrobial, the antioxidant activity of Ayurvedic medicine, *Nimbapatradi Choornam*. J Chem Pharm Res 2015; 7(8): 124-136.
38. Padmavathi AR, Abinaya B and Pandian SK: Phenol, 2,4-bis(1,1-dimethylethyl) of marine bacterial origin inhibits quorum sensing mediated biofilm formation in the uropathogenic *Serratia marcescens*. Biofouling 2014; 30(9): 1111-1122. doi: 10.1080/08927014.2014.972386
39. Igwe KK, Nwankwo PO, Otuokere IE, Ijioma SN, Amaku FJ and Chika I: Identification of the phytocomponents in *Loranthus micranthus* methanolic extract by Gas Chromatography-Mass Spectrometry. Int J Res Sci Manag 2015; 2(12): 53-57. doi: 10.1089/cmb.2012.0251
40. Abubakar M and Majinda R: GC-MS analysis and preliminary antimicrobial activity of *Albizia adianthifolia* (Schumach) and *Pterocarpus angolensis* (DC). Medicines 2016; 3(1): 3. doi: 10.3390/medicines3010003
41. Gnanavel V and Saral MA: GC-MS analysis of petroleum ether and ethanol leaf extracts from *Abrus precatorius* linn. Int J Pharma Bio Sci 2013; 4(3): 37-44.
42. Murugan K, Sangeetha S, Kalyanasundaram VB and Al-sohaibani S: *In-vitro* and *in-silico* screening for *Andrographis paniculata* quorum sensing mimics: New therapeutic leads for cystic fibrosis Pseudomonas aeruginosa biofilms. Plant Omics 2013; 6(5): 340-346.
43. Santhi V and Kannagi A: Bioactive potential of *Purpura persica*. J Chem Pharm Res 2016; 8(3): 700-707.
44. Bharathy V, Sumathy BM and Uthayakumari F: Determination of Phytocomponents by GC–MS in leaves of *Jatropha gossypifolia* L. Sci Res Report 2012; 2(3): 286-290. http://www.jsrr.in/Vol.2 No.3/23 Bharathy 104-108.pdf.
45. Gideon VA: GC-MS analysis of phytochemical components of *Pseudoglochidion anamalayanum* Gamble : An endangered medicinal tree. Asian J Plant Sci Res 2015; 5(12): 36-41.
46. Hameed IH, Hussein HJ, Kareem MA and Hamad NS: Identification of five newly described bioactive chemical compounds in methanolic extract of *Mentha viridis* by using gas chromatography-mass spectrometry (GC-MS). J Pharmacogn Phyther 2015; 7(7): 107-125. doi: 10.5897/JPP2015.0349
47. Porter E, Ma DC, Alvarez S and Faull KF: Antimicrobial lipids: Emerging effector molecules of innate host defense. World J Immunol. 2015; 5(2): 51. doi:10.5411/wji.v5.i2.51
48. Widmer KW, Soni KA, Hume ME, Beier RC, Jesudhasan P and Pillai SD: Identification of poultry meat-derived fatty acids functioning as quorum sensing signal inhibitors to autoinducer-2 (AI-2). J Food Sci 2007; 72(9). doi: 10.1111/j.1750-3841.2007.00527.x
49. Zheng CJ, Yoo JS, Lee TG, Cho HY, Kim YH and Kim WG: Fatty acid synthesis is a target for antibacterial activity of unsaturated fatty acids. FEBS Lett 2005; 579(23): 5157-5162. doi: 10.1016/j.febslet.2005.08.028
50. Baldo C, Paulo S, Rocha D and Rocha SPD: Virulence factors of uropathogenic *Proteus mirabilis* - A Mini Review. Int J Sci Technol Res 2014; 3(11): 1-4.
51. Ranjbar-Omid M, Arzanlou M, Amani M, Shokri Al-Hashem SK, Mozafari NA and Doghaheh HP: Allicin from garlic inhibits the biofilm formation and urease activity of Proteus mirabilis *in-vitro*. FEMS Microbiol Lett. 2015; 362(9): 1-9. doi: 10.1093/femsle/fnv049
52. Abdullah MAA, El-baky RMA, Hassan HA, Abdelhafez A-SMN and Abduo-Rahma GE-DA: Fluoroquinolones as Urease Inhibitors : Anti- Proteus mirabilis Activity and Molecular Docking Studies. Am J Microbiol Res 2016; 4(3): 81-84. doi: 10.12691/ajmr-4-3-3
53. Morales-Soto N, Anyan ME and Mattingly AE: Preparation, imaging, and quantification of bacterial surface motility assays. J Vis Exp 2015; 98: 1-10. doi: 10.3791/52338
54. Kearns DB: A field guide to bacterial swarming motility. Nat Rev Microbiol 2010; 8(9): 634-644. doi: 10.1038/nrmicro2405.A
55. Sharma M and Anand SK: Swarming: A coordinated bacterial activity. Curr Sci 2002; 83(6): 707-715.
56. Mary RNI and Banu N: Screening of antibiofilm and anti-quorum sensing potential of *Vitex trifolia* in *Pseudomonas aeruginosa*. Int J Pharm Pharm Sci. 2015; 7(8): 242-245.
57. Abraham SVI, Palani A, Ramaswamy BR, Shunmugaiah KP and Armugam VR: Antiquorum sensing and

antibiofilm potential of *Capparis spinosa*. Arch Med Res 2017; 42(8): 658-668.
58. Ramanathan S, Ravindran D, Arunachalam K and Arumugam VR: Inhibition of quorum sensing-dependent biofilm and virulence genes expression in environmental pathogen *Serratia marcescens* by petroselinic acid. Antonie Van Leeuwenhoek 2017; 1-15. doi: 10.1007/ s10482-017-0971-y
59. Nzakizwanayo J, Scavone P and Jamshidi S: Fluoxetine and thioridazine inhibit efflux and attenuate crystalline biofilm formation by *Proteus mirabilis*. Sci Rep 2017; 7(1): 1-14. doi: 10.1038/s41598-017-12445-w
60. Goudarzi L, Kermanshahi RK, Mousavinezhad Z and Dallal MMS: Antimicrobial and anti-swarming effects of bacteriocins and biosurfactants from probiotic bacterial strains against *Proteus* sp. Journal of Med Bacteriol 2016; 5(5, 6): 1-12.
61. Liaw SJJ, Lai HCC and Wang WBB: Modulation of swarming and virulence by fatty acids through the RsbA protein in *Proteus mirabilis*. Infect Immun 2004; 72(12): 6836-6845. doi: 10.1128/IAI.72.12.6836-6845.2004
62. Van HR, Givskov M and Michiels CW: Quorum sensing in Serratia. FEMS Microbiol Rev 2007; 31: 407-424. doi: 10.1111/j.1574-6976.2007.00071.x
63. Mary RNI and Banu N: Inhibition of antibiofilm mediated virulence factors production in urinary pathogen *Serratia marcescens* by *Vitex trifolia* 2014; 3(8): 2012-2014.
64. Mathur S, Udgire M and Khambhapati A: Effect of essential oils on biofilm formation by *Proteus mirabilis*. Int J Pharma Bio Sci 2013; 4(4): 1282-1289.

How to cite this article:
Marathe K, Nashikkar N, Bundale S and Upadhyay A: Analysis of quorum quenching potential of *Euphorbia trigona* Mill. Int J Pharm Sci & Res 2019; 10(3): 1372-86. doi: 10.13040/IJPSR.0975-8232.10(3).1372-86.