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EVALUATION OF ANTI-QUORUM SENSING ACTIVITY OF N-HEXADECANOIC ACID PRODUCED BY *PSEUDOMONAS STUTZERI* SJ4 – A MARINE EPIBIOTIC BACTERIUM

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ABSTRACT

Quorum sensing (QS) mechanism is cell communication that plays vital role in the development of infection by many pathogenic microorganisms. It controls multiple virulence factors such as pigmentation, biofilm formation, swarming motility, resistance towards antibiotics, extracellular polysaccharide production (EPS) and expression of several collective traits. The disruption of quorum sensing mechanism can be a solution to the emerging problem of multi-drug resistance among pathogenic bacteria. The effector molecule for Quorum sensing inhibition may be enzymatic or non-enzymatic in nature termed as Quorum Quenching (QQ) or Quorum sensing inhibitory (QSIs)/anti–QS compound, respectively. We used marine epibiotic bacteria as a source to obtained novel bacterial strain as QSI producer. One of the potent isolate, SJ4 was identified as *Pseudomonas stutzeri* SJ4, it is a short rod, gram negative bacterium. The ethyl acetate extract from *P. stutzeri* SJ4, showed highest QSI activity against monitor strain *Chromobacterium violaceum* (MTCC 5526). The extracted compound was tested against *P. aeruginosa* PAO1 at minimum inhibitiory concentration (MIC) and sub-MIC to study the effect on virulence factors. The significant inhibition of pycocyanin pigment, EPS production, rhamnolipid production and reduced swimming and swarming motility was observed. In addition, biofilm formation was notably inhibited which was confirmed by staining and spectometric method. Based on this observation, QS interption by extract which contain QSI remarkebly reduced the virulence of pathogen hence, can be use as therapeutic agents. The Thin Layer Chomatography (TLC) and Gas chrmoatography-Mass Spectrometry (GC-MS) identified major compound as *n*-Hexadecanoic acid. However, further research is required on purification of compound and its potential applications for the treatement of infections.

Keywords: Quorum sensing, Quorum sensing inhibition, marine bacteria, *Pseudomonas stutzeri*, *Pseudomonas aeruginosa*

INTRODUCTION

The discovery of antibiotics was a boon but its excessive use and decreased efficiency turned it into a bane. The pathogens Which are become completely or partially resistant to one or more antibiotics/antimicrobial compounds are referred to as Multi-Drug Resistant (MDR) microbes. This global issue drew attention of scientific community to search for a novel approach which can combat pathogenic infections without triggering selection pressure which leads to development of drug resistance (**World Health Organization, 2017; Reina *et al*., 2019**).

Many pathogenic bacteria, have mechanisms which control the expression of virulence genes such as pigmentation, antibiotic resistance, extracellular proteases and biofilm formation termed as Quorum sensing (QS) **(Whitehead *et al*., 2001; Quinones *et al*., 2005; Jayaraman and Wood, 2008; Garg *et al*., 2014; Papenfort *et al*., 2016; Whiteley *et al*., 2017**). It is a cell population dependent process where cells communicate, coordinate and express genes leading to the production of signalling molecules called as autoinducers in the surrounding environment (**Fuqua *et al*., 1994; Fuqua *et al*., 2001**).The concentration of signalling molecules inducing characteristic phenotypes is directly proportional to the cell density.Gram positive bacteria produce oligopeptides and Gram negative bacteria produce N-Acyl Homoserine Lactones (AHLs) as QS signal molecules (**Ng WL *et al*., 2009; Parker *et al*., 2009**).QS regulation plays vital role in establishment of infection within the host by several pathogens such as *P. aeruginosa*, *Staphylococcus aureus*, *Escherichia coli*, *Vibrio cholerae* and *Vibrio harveyi*, therefore the disruption or silencing of virulence gene expression can be a novel strategy to combat MDR. **(Ng WL *et al*., 2009; Kalia *et al*., 2011; Natrah *et al*., 2011; Lasrre *et al*., 2013; Grandclement *et al*., 2016; Defoirdt *et al*., 2018; Kalia *et al*., 2019; Zhao *et al*., 2019).**

*P. aeruginosa PAO*1 is an infectious strain associated with the urinary tract infections (UTIs) and hospital acquired infections. It is a Gram-negative, motile organism and becoming resistant towards conventional antibiotics. QS inhibitory approach can be an effective way to control MDR clinical strain *P. aeruginosa PAO*1. The four QS systems of *P.aeruginosa* PAO1 are divided braodly into two groups, (i) AHL-based signalling system namely LasI/LasR and RhlI/RhlR system and (ii) non-AHL or quinolone based such as *Pseudomonas* quinolone signal (PQS) and integrated QS signal (IQS) (**Rutherford and Bassler 2012; Pérez-Pérez *et al.*, 2017; Li *et al.*, 2018; Hemmati *et al.*, 2020**). LasI and rhlI encode a signal synthetase, which synthesizes the required signal molecules. lasR and rhlR code for a receptor protein that binds to the signal molecule and initiate transcription of QS genes.The LasIR system uses 3-oxo-C12-HSL as a cognate signal molecule, and RhlR utilizes C4-HSL. These two systems work in a hierarchical manner, as the las-encoded system affects the expression of the rhl-encoded system **(Hentzer and Givskov 2003; Mostafa *et al*., 2020)**. The PQS system is monitored by 2-alkyl-quinolones while IQS is controlled by 2-(2-hydeoxyphenyl)-thiazole-4-carbaldehyde **(Lasarre & Federle 2013; Li *et al.*, 2018)**.

Many natural and synthetic compounds are known to function as quorum sensing inhibitors (QSIs). QSIs are non-enzymatic compounds, which can be cyclic or linear peptides, amides, AHL analogs. Further, inactivation of QS signal molecules by enzymes (Acylases, AHL Lactonases, and AHL Reductase) are termed as Quorum Quenching molecules (QQs) (**Vadakkan *et al*., 2018**).

QSIs or QQs can be obtained naturally from plants such as *Myrmecodia pendans* (**Satari *et al*.,2021**), *Castanea sativa* (**Eksi *et al*., 2020**), *Passiflora edulis* (Fruit) (**Venkatramanan *et al*., 2020**); fungi *Daldinia eschscholtzii* (**Mishra *et al*., 2020**); host associated bacteria *Exiguobacterium indicum* (**Singh *et al*., 2019**), *Desemzia incerta* and *Bacillus* sp., (**Singh *et al*., 2020**); and corals *Sarcotragus spinosulus* (**Saurav *et al*., 2019**).

The marine environment has plethora of biological components which are not studied yet, therefore there are chances of getting novel bacterial species with potential anti-QS application (**Borges *et al*., 2019**). Some of the reports of QSI producing marine bacteria are *Bacillus cereus* from marine sediments of Rhode Island,USA (**Teasdale *et al*., 2009**), *Bacillus amyloliquefaciens* from mangrove rhiazosphere rhizosphere, Bay of Bengal, India (**Gowrishankar *et al*., 2014**, **Gowrishankar *et al*., 2019**) and *Marinobacter sp*. from Arabian costal region, Oman (**Abed *et al*., 2013).**Gujarat has vast coastal region and its microbial diveristy is yet to be explored. Hence, the present study is focused on the exploration of marine epibiotic bacteria, isolated from the surface of macro-

organism like algae, sponges and seaweeds, for QSI activity. Isolates were screened for the anti-QS activity using *C. violaceum* (MTCC 5526) as a monitor strain. Total 20 isolates showed anti-QS activity,isolate showing highest anti-QS activity was selected for further studies on clinical strain of *Pseudomonas aeruginosa* PAO1.

MATERIALS AND METHODS

Sample Collection

The marine seaweeds, algae and sponges were collected in sterile sample bottles from coastal site of Okha, Gujarat, India. (22°28'38.7"N 69°04'51.7"E). The samples were kept in the sea water at 4˚C until use.

Isolation of marine epibiotic bacteria

A 1 gram sample (marine seaweeds, algae and sponges) was washed thrice with sterile sea water. The washings were collected (Total 30 mL) and treated as samples. All the samples were diluted up to 10^6 times; 100 µL was spread on Zobell Marine Agar (ZMA, Himedia,Mumbai India) and incubated at 37 ºC for 5-7 days. Morphologically distinct colonies were transferred to the ZMA plates to obtaine pure colonies (**Singh *et al.*, 2020**)

Screening of epibiotic bacteria for anti-QS activity

Isolates were screened for anti-QS activity by soft agar overlay method using *C. violaceium* (MTCC 2656) as a monitor strain (**McLean *et al.*, 2004**). The secondary screening was done by quantification of violacein pigment.The bacterial isolates (1×10^8CFU/mL cells) and *C. violaceium* (1×10^8CFU/mL cells) were inoculated in Luria broth (LB) and incubated at 37 ºC for 24 h under shaking condition. Next day, 1 mL sample was withdrawn and centrifuged at 10,000 rpm for 15 min., pellet was dissolved in DMSO and centrifuged again at 10,000 rpm for 15 min. Absorbance of the supernatant was measured at 585 nm. Cell density was also measured at 600 nm to check the growth of isolates (**Choo *et al.*, 2006; Musthafa *et al.*, 2011).**

Extraction of Anti-QS compounds

The select marine isolates were grown in ZMB at 37 ºC for 24 h under shaking condition. The cultures were centrifuged and the supernatant was extracted with equal volume of organic solvents of varying polarity like hexane, chloroform, ethyl acetate , *n*-butanol, isopropanol and methanol .The extract was concentrated by evaporation and the residue was dissolved in 500 µL of respective solvent. The compound was used for *C. violaceum* based bioassay by well diffusion method (**Nithya and Pandian SK., 2009; Nithya *et al.*, 2010**).

Morphological characterization of bacterial isolate

Isolate SJ4 showing highest QS inhibition, was gram stained and its morphologies were examined at 100X magnification using Magnus MLM light microscope **.**

Biochemical characterization of bacterial isolate

Biochemical characterization of selceted bacteria was done, which included tests such as catalase, oxidase and sugar utilization (lactose, glucose, sucrose, mannitol, rhamnose and arabinose).

Molecular identification of bacterial isolate

Genotypic characterization of SJ4 was by sequencing its 16S rDNA gene.The extracted genomic DNA was shipped to Eurofins Scientific India Pvt Ltd. (Banglore, India) and served as a template for PCR amplification of the nearly complete 16S rRNA gene using the universal oligonucleotide primer pair 27F (5'-AGA GTT TGA TCC TGG CTC AG-3') and 1492R (5'-TAC GGT TAC CTT GTT ACG ACT T-3'), as recommended by Lane **(Lane,1991).**

Testing QSI activity of extract

QSI activity of the extract was determined using well diffusion method. LB agar plates were inoculated with 100 µL of *C. Violaceum.*50 µL of extract was added into the wells. The plates were then incubated at 37 °C for 24 h. Extract showing zone of pigment inhibition in *C. violaceum* were shortlisted and then tested against *P. Aeruginosa* PAO1.

Determination of Minimum Inhibition Concentration (MIC) of QSI extract

The MIC was determined by broth dilution method using *P. aeruginosa* PAO1 as as a test strain. The extract was serially diluted to make different concentrations from 1 $mg.mL^{-1}$ to 0.098 $mg.mL^{-1}$. Test strain was inoculated in LB broth and incubated at 37 °C for 18 h. The control was prepared without extract. The tube showing no turbidity was considered as MIC. Consequently, sub-MICs were selected for further studies (**CLSI, 2012**).

Inhibition of virulence factors in *P. aeruginosa*

Pyocyanin inhibition

Pyocyanin inhibition was assayed by growing *P. aeruginosa* overnight with QSI extract (MIC and sub-MIC) and without extract at 37 °C for 24 h. After incubation, 5.0 mL culture supernatantwas extracted with 2.5mL of chloroform and was centrifuged at 3000 rpm for 2 min for separation of chloroform layer. 500µL of 0.2N HCL was added to the separated layer and vortexed vigorously which produced a red colored thick ring. The ring was carefully removed, the absorbance was measured at 520 nm and inhibition percentage was calculated **(Ganesh and Rai, 2016).**

Inhibition of Exopolysaccharide (EPS)

EPS was quantified from supernatant of *P. aeruginosa*. EPS was precipitated by three volumes of iso-propyl-alcohol (100%) and incubated for 24 h at room temperature. The precipitated EPS was pelleted by centrifugation (10000 rpm, 15 min) and dissolved in distilled H_2O. EPS was quantified using the phenol-sulphuric acid method, wherein 1 mL of 5% cold phenol and 5 mL of conc. H_2SO_4 were mixed with 1 mL of EPS suspension, which was measured spectrophotometrically at 490 nm **(Packiavathy *et al.*, 2014)**

Rhamnolipid inhibition

A 1 mL cell free supernatant was extracted with twice the volume of ethyl acetate and dried. The dried extract was suspended in 900 µL of orcinol solution (0.19% orcinol dissolved in 53% (v/v) sulfuric acid). The mixture was incubated for 30 min at 80 °C and quantified at 421 nm spectrophotometrically (**Packiavathy *et al.*, 2014**).

Biofilm inhibition

The test strain was mixed with QSI extract (MIC and sub-MIC) and incubated for 24 h at 37 °C; a control was prepared without QSI extract. After incubation, the wells were washed with sterile phosphate-buffered saline (PBS) to remove unadhered cells. The biofilm was stained with 1% crystal violet for 5 min and again washed with sterile PBS to remove excess stain. The crystal violet stained biofilm was dissolved with 33% acetic acid and was quantified by absorbance at 595 nm (**Luo *et al.*, 2016**).

Motility inhibition

Swimming and swarming was studied as described by **Rashmi *et al.* (2018)**. Growth media used for swimming assay comprised nutrient broth along with extract or without extract. Actively growing culture was point inoculated and plates were incubated at 37 °C for 24-48 h. In Swarming, test strain was inoculated in nutrient agar with or without QSI extract. Actively growing culture was point inoculated and plateswere incubated at 37 °C for 24-48 h.

Identification of active compound(s) in EA extract of *P.stutzeri* SJ4

Thin layer chromatography (TLC) was performed to partially purify active compound (QSI) from Ethyl acetate crude extract *of P.stutzeri* SJ4 using chloroform: methanol (10:1) as solvent system (**Basaran *et al.*, 2020**).The eluted bands were checked for the anti-QS activity. The band showing highest activity was analysed by Gas chromatography-Mass Spectrometry (GC-MS) (Perkin Elmer, Autosystem XL GC with Turbomass). The chromatogram was matched with NIST library database in order to predict the compound.

Anti-QS activity of n-Hexadecanoic acid

QS inhibitory activity of different concentrations of commercially available *n*-hexadecanoic acid (HDA) (Himedia, Mumbai, India) was compared with the crude extract from *P. stutzeri* SJ4 against *C. violaceum* MTCC 2656. The concentration showing highest decolorization zone was used for further studies**.**

RESULTS AND DISCUSSION

Epibiotic bacterium *P. stutzeri* SJ4 as a QSI producer

Total 60 bacteria having distinct morphology were isolated from the surfaces of 8 samples of seaweed, algae and sponges. All isolates were screened for QSI activity using *C. violaceum* MTCC 2656 as a monitor strain. 20 isolates showed QSI activity by inhibiting the violacein pigment production. SJ4 showing highest activity was selected for further study (figure 1).

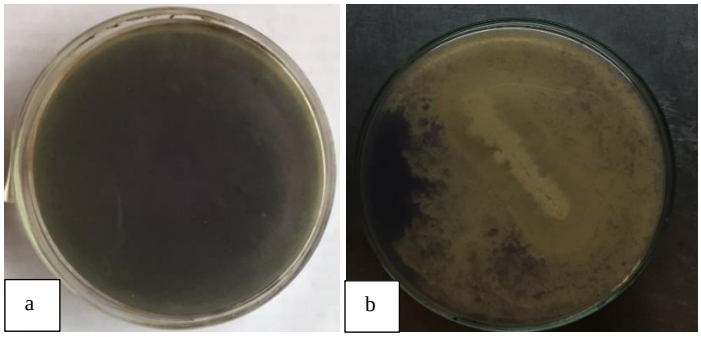

Figure 1 Primary screening by soft agar overlay method using C. violaceium as a monitor strain (a) Control (b) SJ4.

The colony of SJ4 was small, reddish brown, dry, and irregular in shape. It was gram negative, short rod, arranged singly (figure 2 a&b). SJ4 was catalase negative, oxidase positive, fermented lactose, glucose, sucrose, mannitol, rhamnose and arabinose sugars. Apart from morphological and biochemical characterisation, molecular identification was carried out by 16S rDNA sequencing, SJ4 was identified as *P. stutzeri* SJ4. The nucleotide sequences were submitted to Genebank (Accession No. MW547117) and analysed using NCBI Blast tool (https://blast.ncbi.nlm.nih.gov/Blast.cgi). Phylogenetic analysis was done using MegaX (Downloaded from https://www.megasoftware.net/) software. The phylogenetic tree was constructed by Neighbor-Joining method with the bootstrap value of 1000 (figure 2c).

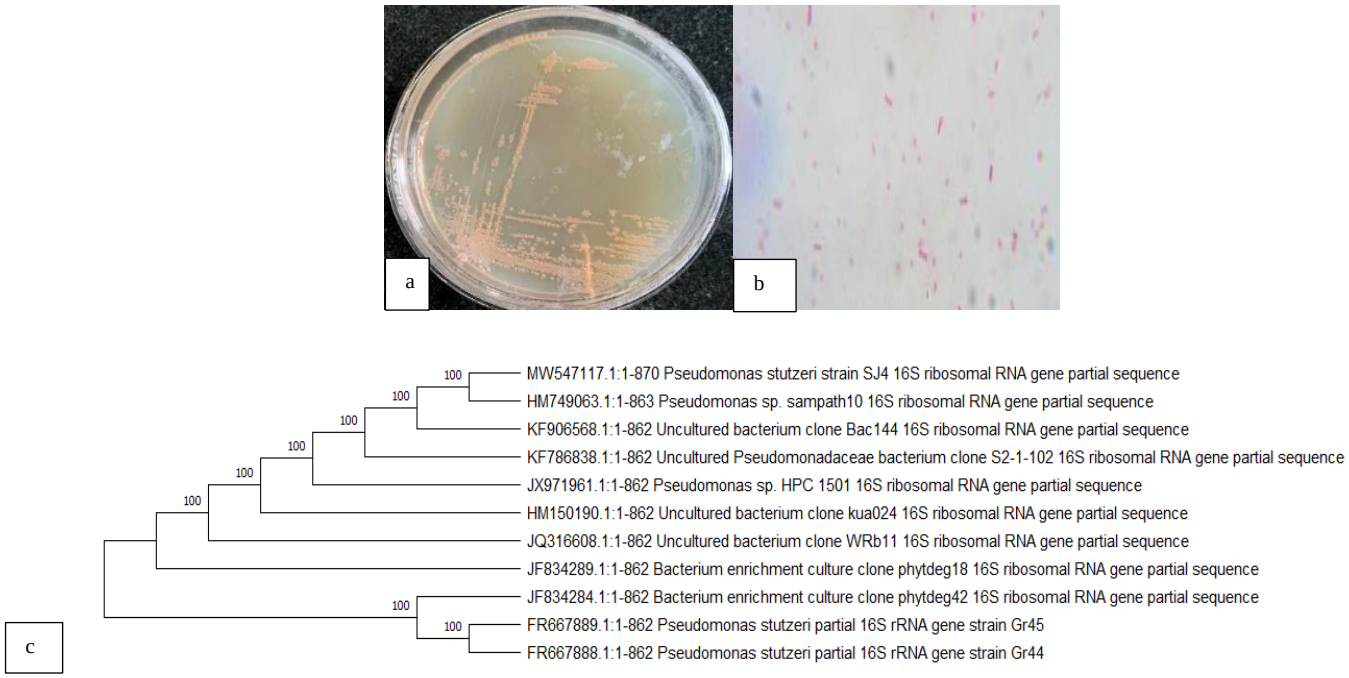

Figure 2 Identification and phylogenetic analysis of P.stutzeri SJ4 (a) The colony morphology (b) Gram's Reaction (c) Phylogenetic tree using Neighbor-Joining method. The percentage of replicate trees in which the associated taxa clustered together in th bootstrap test (1000 replicates) are shown next to the branches. Evolutionary analyses were conducted in MEGA X.

Extraction of QSI Substances

The supernatant was extracted with different organic solvents such as hexane, chloroform, ethyl acetate, n-butanol, isopropanol and methanol. The extracts were checked for the QSI activity against *C. violaceium* by well diffusion method. The zone of pigment inhibition was highest in ethyl acetate extract compared to other solvents (Table 1). **Teasdale *et al.*, (2009)**, also found highest anti-QS activity of Halobacillus *salinus* C42 in the ethyl acetate extract. On the other hand, **Abudoleh *et al.*, (2017)**, reported more anti-QS activity from three isolates in butanol, chloroform and hexane extracts. Hence, further virulence assays of *P. aeruginosa* were performed using ethyl acetate (EA) extract.

Table 1 QSI activity of compound extracted with various solvents

Sr. No.	Solvent	Diameter of Zone of Pigment Inhibition (mm)
1.	Hexane	5
2.	Chloroform	7
3.	Ethyl acetate	15
4.	n-Butanol	10
5.	Isopropanol	4
6.	Methanol	2

Inhibition of virulence factor in *P. aeruginosa*

Pyocyanin is a green, fluorescent compound produced by *P. aeruginosa* which plays a vital role in the infection To investigate, the effect on pyocyanin and other parameters, the MIC of QSI extract was tested and it was found to be 6.25 mg.mL^{-1} and the sub-MIC was 3.125 mg .mL^{-1}. These two concentration were used for the study. QSI extract inhibited pyocyanin production up to 79% and 34% at 6.25 and 3.125 mg.mL^{-1} concentration respectively (figure 3).

In a previous study, **Venkatramanam *et al.*, (2020)** reported 2 mg.mL^{-1} and 1mg.mL^{-1} as MIC and sub-MIC respectively, 75.8% inhibition of violacein pigment in *C. violaceum* was observed**. Busetti *et al* (2015)** have reported significant decrease in pyocyanin production (60%) at 1 mg.mL^{-1} concentration from *Pseudoalteromonas* sp.

EPS is a fundamental component of the biofilm formation and it is crucial to maintain integrity of biofilm.EPS production was reduced by 77% at MIC while 19.23% at sub-MIC (figure 3). **Mishra *et al.*, (2018)** studied effect of extract of *Alternaria alternata* on EPS production of *P. aeruginosa* PAO1; 66.6% reduction was observed.

Rhamnolipid is one of the important aspects in the formation of biofilm. In the present study, QSI reduced rhamnolipid production upto 45% at MIC; while 28% reduction was observed at sub-MIC (figure 3). **Rajkumari *et al.*, (2018)** observed 16.51% reduction in rhamnolipids of P. *aeruginosa* when cinnamic acid was used as QSI.

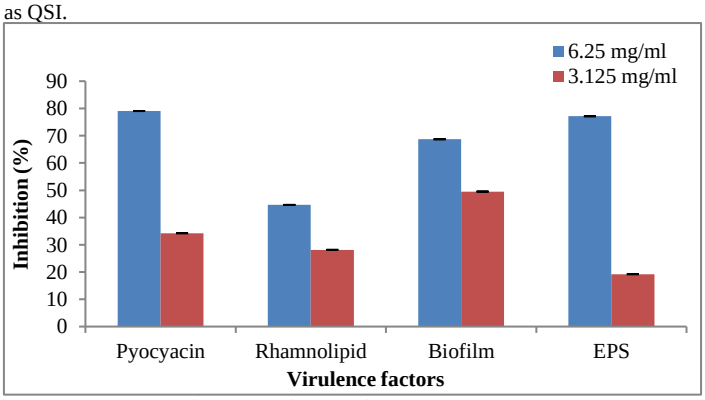

Figure 3 Inhibition of virulence factors of *P. aeruginosa* PAO1 by EA extract of *P.stutzeri* SJ4 at MIC (6.25 mg.mL^{-1}) and sub-MIC (3.125 mg.mL^{-1})

Effect of EA extract of *P. stutzeri* SJ4 on Biofilm formation of *P.aeruginosa*

Biofilm formation is most important factor responsible for the virulence of any pathogenic bacteria. It majorly contributes in antibiotic resistance and development of severe infection. Our study showed 69% reduction in biofilm formation in comparison with control. The staining with crystal violet showed marked difference in the color intensity in the presence and absence of EA extract (figure 4). In a study by **Christiaen *et al.* (2014),** *Diaphorobacter* sp. and *Delftia* sp., had reduced *P. aeruginosa* PAO1 biofilm formation by 20–30% whereas marine derived strain Rhizobium spi. NAO1, reduced biofilm formation by 72.3% in *P. aeruginosa* PAO1 (**Chang *et al.*, 2017**).

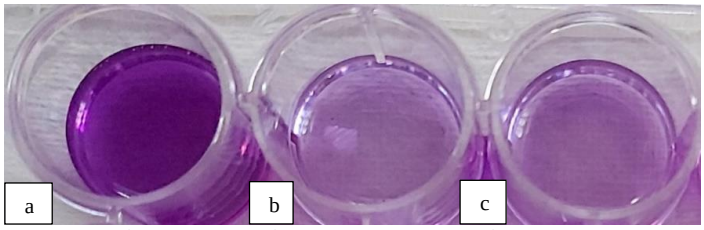

Figure 4 Biofilm inhibition of *P. aeruginosa* PAO1 after staining with crystal violet (a) control (without extract) (b) at 6.25 mg.mL^{-1} (MIC) (c) at 3.125 mg .mL^{-1} (Sub-MIC).

Motility helps in attachment to host cell surface and responsible for the movement. *P.aeruginosa* is a highly motile strain so swimming and swarming patterns of mobility were studied in the absence and presence of QSI. A significant decrease in the colony diameter was noticed in the presence of QSI compound (figure 5). According to earlier studies, aspirin inhibited the swimming motility of *P. aeruginosa* by 34% **(El-Mowafy *et al.*, 2014**).

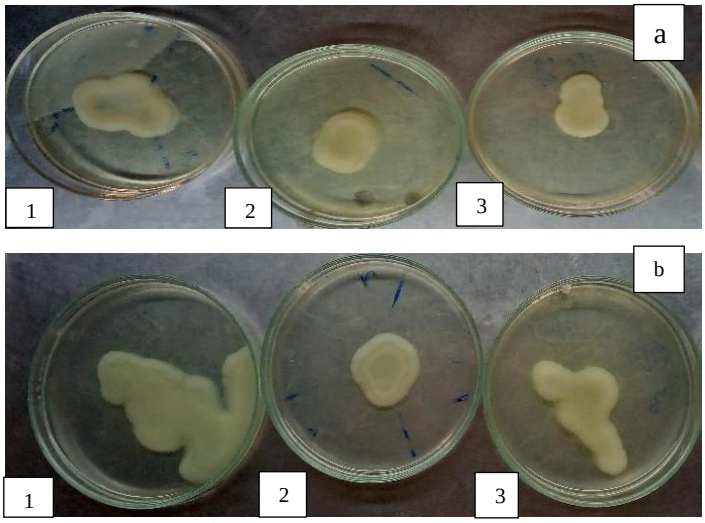

Figure 5 Inhibition of Motility in *P. aeruginosa PAO1* (a) Swimming (b) Swarming (1a & 1b) control, (2a&2b) extract concentration 6.25 mg.mL^{-1} (3a &3b) extract concentration 3.125 mg.mL^{-1}

Characterization of anti-QS compound of EA extract of *P. stutzeri* SJ4

A total of 4 prominent bands were observed on TLC plate under the UV light. QS inhibition was observed in only two eluted bands, one band with highest anti-QS activity was selected for the GC-MS analysis. The chromatogram of partially purified extract showed 15 peaks with different retention time (RT) (figure 6); 3 peaks were selected for MS based on the percentage of area. According to NIST library, major compound was *n*-hexadecanoic acid (18.47%) at 23.307 RT followed by Undecanoic acid (12.18%) at 21.451 RT and Hexadecane (7.82) at 25.903 RT (table 2).

Basaran *et al.* (2020), obtained 7 bands after TLC separation of EA extract of *Natrinema versiforme*. **Venkatramanam *et al.*, (2020)** also reproted *n*-hexadecanoic acid as the main QSI compound after GC-MS analysis of *Passiflora edulis* extract.

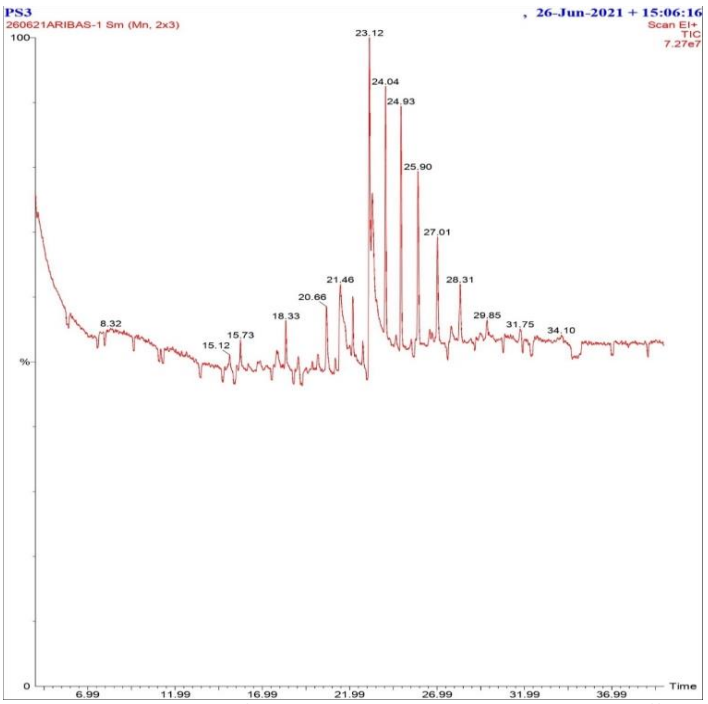

Figure 6 Chromatogram of Gas Chromatography showing 15 peaks at different retention time

Table 2 List of compounds at 23.307 RT obtained after searching in NIST library

PS3 — 260621ARIBAS-1

Hit	REV	for	Compound Name	M.W.	Formula	CAS
1	923	863	N-HEXADECANOIC ACID	256	C16H32O2	57-10-3
2	922	837	UNDECANOIC ACID	186	C11H22O2	112-37-8
3	898	845	N-DECANOIC ACID	172	C10H20O2	334-48-5
4	897	829	NONADECANOIC ACID	298	C19H38O2	646-30-0
5	896	809	PENTADECANOIC ACID	242	C15H30O2	1002-84-2
6	892	840	UNDECANOIC ACID	186	C11H22O2	112-37-8
7	891	834	N-DECANOIC ACID	172	C10H20O2	334-48-5
8	891	806	OCTADECANOIC ACID	284	C18H36O2	57-11-4
9	889	791	OLEIC ACID	282	C18H34O2	112-80-1
10	884	755	EICOSANOIC ACID	312	C20H40O2	506-30-9
11	882	738	DODECYL ACRYLATE	240	C15H28O2	2156-97-0
12	879	784	HEPTADECANOIC ACID	270	C17H34O2	506-12-7
13	878	792	TRIDECANOIC ACID	214	C13H26O2	638-53-9
14	876	664	DODECYL ACRYLATE	240	C15H28O2	2156-97-0
15	875	744	4-TETRADECANOL	214	C14H30O	1653-33-4
16	871	797	N-DECANOIC ACID	172	C10H20O2	334-48-5
17	871	745	NONADECANOIC ACID	298	C19H38O2	646-30-0
18	870	815	N-DECANOIC ACID	172	C10H20O2	334-48-5
19	870	751	OCTADECANOIC ACID	284	C18H36O2	57-11-4
20	870	771	TRIDECANOIC ACID	214	C13H26O2	638-53-9

Comparative study of crude and standard compound

Different concentrations (1 mg.ml^{-1}, 0.5 mg.ml^{-1}and 0.25 mg.ml^{-1} of HDA was tried, figure 7a represents that crude extract showed 12 mm zone of inhibition and HDA showed 9 mm zone. Further, quantification was done using 1 mg.mL^{-1} HDA where as the concentration of crude extract was 6 mg.mL^{-1}.As shown in the figure 7b, the percentage production of violacein pigment was reduced to 71.06% and 90.32% with HDA and crude extract respectively. A significant difference in the activity of the two compounds was observed, however the concentrations were also different. Since the purity of the standard and test compound is different their potency can not be compared. Chemmugil and co-workers have also reported the anti-QS activity of HDA against *Staphylococcus aureus* using qualitative method. They observed 12 mm zone of inhibition which is similar to our results (**Chemmugil *et al.*, 2019**).

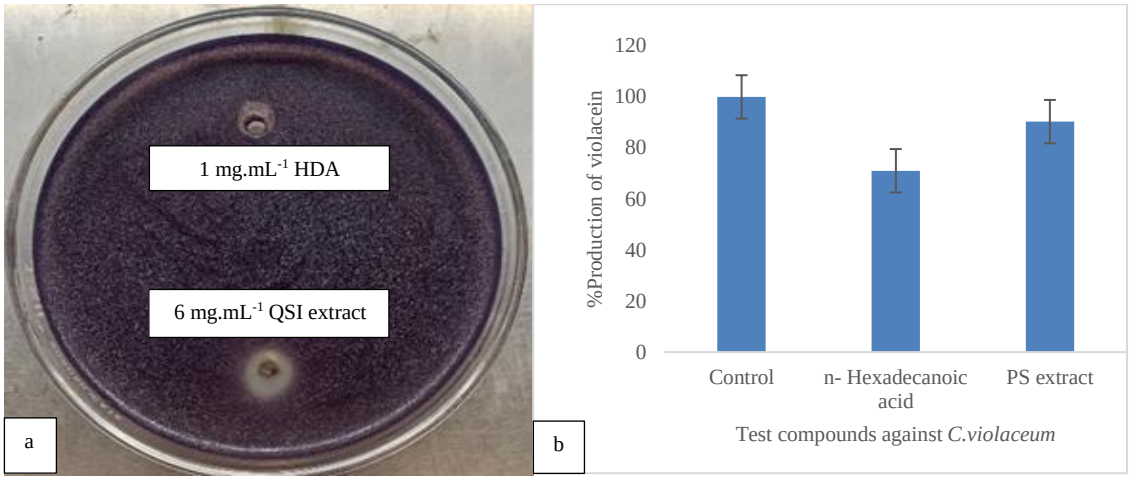

Figure 7 Qualitative and quantitative estimation of QSI activity of crude and standard compound (a).Well diffusion method against C.violaceum (b). Violacein inhibition assay

CONCLUSION

A total of 60 marine epibiotic bacteria were screened for the anti-QS activity. *Pseudomonas stutzeri* SJ4 significantly inhibitied the virulence factors which are associated with the quorum sensing of clincial strain of *Pseudomonas aeruginosa* PAO1. The active anti-QS compound present in the crude exract is tentatively identified as *n*-hexadecanoic acid. Further investigations regarding the mechanism of inhibition and genetic level expression is required.

Conflict of interest: The authors declare that there is no potential conflict of interest.

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