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Research paper

Evaluation of anti-quorum sensing activity of indigenous dietary plants against *Pseudomonas aeruginosa*

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ABSTRACT

Introduction: Increasing antibiotic resistance in *Pseudomonas aeruginosa* has encouraged the search for alternative means which can aid in reducing the virulence of the pathogen. Quorum sensing (QS) is considered to be an attractive target to limit infections caused by this bacterium. The limited reports on the screening of anti-QS activity from locally available vegetables and spices against *P. aeruginosa* prompted us to take up the present investigation.
Methods: Initial screening of antibacterial activity against *P. aeruginosa* was carried out by agar well diffusion method. Assessment of anti-QS activity was performed against *C. violaceum* MTCC 2656. Virulence assays against *P. aeruginosa* ATCC 15692 were accomplished in the presence of sub-MIC values of the crude extracts.
Results: Of the 95 samples initially screened, only 11 extracts with a significant antibacterial activity were selected for MIC determination against *P. aeruginosa* and *C. violaceum*. 6 extracts with a MIC value in the range of 0.5–1.0 mg/ml were picked for anti-QS screening. The highest violacein inhibition (70.5%) and inhibition of elastase (81.41%), pyocyanin (94%), swarming motility (71%) and biofilm formation (80.3%) was found in *Cinnamomum verum* leaf extract.
Conclusions: The present investigation reveals the anti-QS activity in the indigenous vegetables and spices in effectively reducing the virulence factor production in *P. aeruginosa*. It also emphasizes the importance of evaluating the anti-QS property of un-explored locally available vegetables and spices for isolating novel anti-QS compounds which might lead to new therapeutic strategies.

1. Introduction

Pseudomonas aeruginosa is an important human opportunistic pathogen. It is one of the most important causes of hospital-acquired infections worldwide. Infections caused by this organism are often severe, life-threatening and difficult to treat because of the emergence of antibiotic-resistant strains. Innate and acquired resistance of this pathogen to many of the available antibiotics and its ability to survive in different environments has made them the commonly occurring pathogens in hospitals, particularly in intensive care units [1]. The pathogenicity of this bacterium depends on the production and secretion of multiple virulence factors that are regulated at the transcriptional level by a cell to cell signalling system known as quorum sensing (QS) [2]. Bacteria communicate with the production of small signal molecules called autoinducers. Synchronization of their behaviour based on the population density is due to these autoinducers produced by them. Literature supports the involvement of bacterial QS in the regulation of virulence gene expression, biofilm formation, resistance to antibiotics, and motility [3]. This makes QS a novel target for bacterial virulence attenuation. As anti-QS compounds from the plant sources are generally considered to be safe with least side effects on the eukaryotic cell, the discovery of such compounds is the need of the hour. In addition to medicinal plants, dietary plants also have an alluring repertoire of phytochemicals and have attracted considerable interest recently. Though extensive work has been done on the antimicrobial properties of dietary plants, the literature on dietary phytochemicals as a source of QS modulator is very limited. Against this background, our study aimed at the screening of indigenous vegetables and spices from Mangaluru region, India, for their anti-QS activity on *P. aeruginosa*.

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Table 1
List of vegetables used in the study.

Sl.No	Common name of the vegetables with voucher numbers	Scientific name of vegetables	Parts of vegetable used in the study
1.	Ash gourd (NUCSER 025)	*Benincasa hispida*	Whole Fruit
2.	Baby corn (NUCSER021)	*Zea mays*	Fruit
3.	Basale (NUCSER 028)	*Basella alba*	Stem, leaf
4.	Beetroot (NUCSER 027)	*Beta vulgaris*	Whole fruit, Peel
5.	Bimbuli (NUCSER 029)	*Averrhoa bilimbi*	Whole fruit
6.	Bird's eye chilli (NUCSER 013)	*Capsicum frutescens*	Whole fruit
7.	Bitter gourd (NUCSER 036)	*Momordica charantia*	Fruit peel, seed
8.	Bottle gourd (NUCSER 020)	*Lagenaria siceraria*	Fruit peel, seed
9.	Brahmi(NUCSER 044)	*Centella asiatica*	Leaf, Whole plant
10.	Cabbage (NUCSER 037)	*Brassica oleracea* var. capitata	Modified leaf
11.	Capsicum (NUCSER 019)	*Capsicum annum*	Whole fruit
12.	Carrot (NUCSER 045)	*Daucus carota*	Whole root
13.	Cauliflower (NUCSER 014)	*Brassica oleracea* var. botrytis	Flower
14.	Cinnamon (NUCSER 026)	*Cinnamomum verum*	Leaf
15.	Clove (NUCSER 057)	*Syzygium aromaticum*	Leaf
16.	Cluster beans (NUCSER 015)	*Cyamopsis tetragonoloba*	Whole pod
17.	Coccinia (NUCSER 046)	*Coccinia grandis*	Whole fruit
18.	Coriander (NUCSER 022)	*Coriandrum sativum*	Leaf
19.	Cowpea (NUCSER 065)	*Vigna unguiculata*	Whole fruit
20.	Curry leaves (NUCSER 017)	*Murraya koenigii*	Leaf
21.	Dill (NUCSER 058)	*Anethum graveolens*	Leaf
22.	Drum stick (NUCSER 023)	*Moringa oleifera*	Leaf, Fruit
23.	Elasuru(NUCSER 059)	*Brassica juncea*	Leaf
24.	Fenugreek (NUCSER 018)	*Trigonella foenum-graecum*	Leaf
25.	Flat beans (NUCSER 047)	*Vicia faba*	Pod peel, seed
26.	French beans (NUCSER 024)	*Phaseolus vulgaris*	Whole pod
27.	Frutescens chilli (NUCSER 008)	*Capsicum frutescens*	Whole fruit
28.	Garlic (NUCSER 009)	*Allium sativum*	Modified root
29.	Ginger (NUCSER 011)	*Zingiber officinale*	Rhizome
30.	Gooseberry (NUCSER 038)	*Phyllanthus emblica*	Fruit
31.	Green brinjal (NUCSER 012)	*Solanum melongena*	Fruit, leaf
32.	Green chilli (NUCSER 048)	*Capsicum annum*	Fruit
33.	Green Colocasia (NUCSER 039)	*Colocasia esculenta*	Root, leaf, tuber
34.	Green cucumber (NUCSER 056)	*Cucumis sativus*	Fruit
35.	Green Mango (NUCSER 010)	*Mangifera indica*	Unripe fruit, Leaf
36.	Green peas (NUCSER 049)	*Pisum sativum*	Pod peel, seed
37.	Green tomato (NUCSER 060)	*Solanum lycopersicum*	Whole fruit
38.	Harive (NUCSER 061)	*Amaranthus viridis*	Stem, whole root
39.	Indian borage (NUCSER 030)	*Plectranthus amboinicus*	Stem, Leaf, Whole
40.	Kasaraka(NUCSER 040)	*Strychnine nux-vomica*	Stem, Leaf
41.	Kokum (NUCSER 064)	*Garcinia indica*	Stem, Seed, Leaf, Fruit pulp
42.	Lady's finger (NUCSER 031)	*Abelmoschus esculentus*	Fruit
43.	Lemon (NUCSER 055)	*Citrus limon*	Fruit
44.	Mangalore cucumber (NUCSER 032)	*Cucumis maderaspatensis*	Fruit peel, pulp, seed
45.	Mint (NUCSER 062)	*Mentha piperita*	Leaf
46.	Navilukosu (NUCSER 006)	*Brassica caulorapa*	Leaf
47.	Nellabasale (NUCSER 033)	*Talinum fruticosum*	Leaf
48.	Onion (NUCSER 051)	*Allium cepa*	Bulb
49.	Panchapatre(NUCSER 007)	*Aremisia absinthium*	Leaf
50.	Parwal (NUCSER 052)	*Trichosanthes dioica*	Fruit
51.	Plantain (NUCSER 005)	*Musa acuminata*	Flower
52.	Potato (NUCSER 053)	*Solanum tuberosum*	Tuber
53.	Pumpkin (NUCSER 034)	*Cucurbita pepo*	Fruit peel, pulp,seed
54.	Radish (NUCSER 050)	*Raphanus sativus*	Modified root, Leaf
55.	Red Colocasia(NUCSER 001)	*Colocasia antiquorum*	Tuber, leaf
56.	Ridge gourd (NUCSER 063)	*Luffa acutangula*	Fruit, Seed
57.	Sambrani (NUCSER 035)	*Plectranthus amboinicus*	Leef
58.	Snake gourd (NUCSER 002)	*Trichosanthes cucumerina*	Fruit peel, pulp, seed
59.	Spinach (NUCSER 054)	*Spinacia oleracea*	Leaf
60.	Spring onion (NUCSER 016)	*Allium fistulosum*	Leaf
61.	Squash (NUCSER 041)	*Sechium edule*	Fruit
62.	Sweet potato (NUCSER 003)	*Ipomoea batatas*	Tuber
63.	Turmeric (NUCSER 004)	*Curcuma longa*	Whole root, leaf, Rhizome
64.	Vitamin greens (NUCSER 042)	*Sauropus androgynus*	Leaf
65.	Yam (NUCSER 043)	*Amorphallus paeoniifolius*	Modified root

2. Methods

2.1. Bacterial strains and culture conditions

Pseudomonas aeruginosa, ATCC 15692, a reference strain was used in this study. *Chromobacterium violaceum* MTCC2656, a biomonitor strain was employed for anti-QS assay and was subcultured in nutrient broth at 26 °C.

2.2. Plant material and preparation of extracts

Fresh material from sixty-five dietary plants which were collected from local vendors and farm owners of Mangaluru, situated in South Karnataka from Jan-Dec 2016 were screened for their anti-QS potentials. Different parts of the plants were considered as separate samples, and in total 95 samples were used in this study. A thorough wash with distilled water was given to the collected plant material. It was dried at 40 °C for 48–72 h in a hot air oven, powdered and used for extraction. The list of dietary plants with the parts used is provided in Table 1. Solvent extraction was performed according to a previously described method of Harborne et al [4] with slight modifications. Briefly, 50 g of the powdered material was mixed with 100 ml of acetone, methanol, ethanol, and water separately and kept in a shaker incubator at 37 °C for 24 h. The extract thus obtained was filtered using sterile Whatman filter paper. Using the same amount of solvent, second and third extraction was carried out. The extracts were later pooled and concentrated under reduced pressure using a rotary flash evaporator (Rotek, India). The extract thus obtained was weighed and preserved at 4 °C until further use.

2.3. Assessment of antibacterial activity against P. aeruginosa

Antibacterial activity of 95 samples was tested against *P. aeruginosa* by agar well diffusion method. Ten mg of the dried extracts were freshly dissolved in 1 ml of their respective solvents. Approximately 1×10^6 CFU of the 24 h old culture of *P. aeruginosa* was used to lawn Luria Bertani agar plates. The plates were dried for 15 min and wells were punched in the plates (6 mm) in which 20 μl of sample extracts were placed. 20 μl of the respective solvent served as the negative control and streptomycin (300 μg/well) served as the positive control. The plates were incubated at 37 °C for 24 h. Formation of a clear zone of inhibition around the sample extract compared to its control was considered to be positive.

2.4. Determination of Minimum Inhibitory Concentration (MIC) of extracts against P. aeruginosa and C. violaceum

Different concentrations of the dried extracts ranging from 0.25 mg/ml – 10 mg/ml in their respective solvents were prepared on the day of the experiment. Approximately 1×10^6 CFU/ml of the 24 h old culture of *P. aeruginosa* and *C. violaceum* were used to lawn the agar plates and wells were punched. To the wells, 20 μl of the sample extract and 20 μl of the control solvent were loaded and the plates were incubated. The clear zone of inhibition (mm) at different concentrations around the sample extract was noted down. The least concentration giving a zone of inhibition was considered as the MIC value.

2.5. Assessment of anti-QS activity of the extracts using biomonitor strain of C. violaceum

Different concentrations of the extracts (20 μl) at their subMIC levels were loaded into the wells of previously inoculated nutrient agar plates. Incubated plates were checked for the pigment inhibition around the well. The zone of pigment inhibition was measured in millimeters.

2.6. Quantitative estimation of violacein inhibition in the presence of plant extracts

Amount of violacein production by *C. violaceum* in the presence of sub-MICs of plant extracts was studied by extracting and quantifying violacein photometrically according to the method described by Pantanella et al. [5]. One ml of bacterial culture was grown in the presence of 100 μl of plant extract. One tube without extract served as the control. Lysis of the treated and untreated cultures was carried out with 10% SDS and incubated for 10 min at room temperature. To the cell lysate, butanol was added, vortexed and centrifuged at 10,000 rpm for 10 min. The absorbance of the collected butanol phase containing violacein was read at 585 nm in a spectrophotometer (Metertech, Taiwan). Reduction in pigment production in the presence of plant extracts was measured in terms of percent inhibition as [(OD of control – OD of treated)/OD of control] x100.

2.7. Assessment of P. aeruginosa virulence factors in the presence of plant extracts

Virulence factor assessment in *P. aeruginosa* was carried out using Las B elastolytic assay, pyocyanin quantification assay, swarming motility assay and biofilm formation. All the assays were carried out at sub-MIC values of the selected plant extracts. The control which was maintained in all the assays was without the plant extract.

2.7.1. Las B elastolytic assay

The method of elastin congo red according to a previously described protocol of Pearson et al. [6] was used with some modifications. The cells were grown at 37 °C for 14 h in LB broth, centrifuged at 10,000 rpm for 10 min and the supernatant thus obtained (50 μl) was mixed with 10 mM $NaHPO_4$ (2 ml) containing 30 mg of elastin congo red. The mixture was incubated at 37 °C for 14 h, centrifuged and the released congo red was measured at 495 nm in a spectrophotometer.

2.7.2. Pyocyanin quantification assay

The assay was performed according to the method reported by Raoof et al. [7] with slight modifications. The cells were grown in LB broth at

37 °C for 24–48 h and centrifuged. 5 ml of the culture supernatant was mixed with 3 ml of chloroform and vortexed. The separated lower organic phase was collected to which 2 ml of 0.2 M HCl was added. The lower organic phase thus obtained was measured at 520 nm in a spectrophotometer. Pyocyanin produced was expressed as μg/ml of culture supernatant and was determined by multiplying the optical density with 17.072.

2.7.3. Swarming motility assay

Method of Tremblay et al. [8] with minor modification was employed. 5 μl of the overnight culture was inoculated onto 0.4% nutrient agar and incubated for 24 h. The diameter of the swarm on the agar plates was measured in millimeters.

2.7.4. Biofilm formation

Biofilm quantification was carried out according to the method of O'Toole et al. [9] with a small modification. 100 μl of the diluted culture was taken in a microtitre plate and incubated for 24 h at 37 °C. The adherent cells were washed thrice with PBS of pH 7.4. 125 μl of 0.1% freshly prepared crystal violet was added to the dried pellet and incubated for 10 min. To the stained and washed pellet, 200 μl of 30% acetic acid was added and incubated for 15 min for stain solubilization. 100 μl was transferred to the fresh plate and optical density was measured at 600 nm in an ELISA reader (Biorad, USA). Reduction in biofilm formation in the presence of plant extracts was measured in terms of percent inhibition as [(OD of control – OD of treated)/OD of control] x100.

2.8. Statistical analysis

The data were subjected to one sample *t*-test using SPSS, 16.0 software and data was considered to be significant at $P \leq 0.05$.

3. Results

3.1. Assessment of antibacterial activity against P. aeruginosa

95 extracts each of acetone, methanol, ethanol, and water were assessed for their antibacterial activity against *P. aeruginosa*. There was a zone of inhibition ranging from 3 mm to 12 mm in 6 acetone, 3

Table 2
Zone of inhibition of the test samples against *P. aeruginosa.*

Sl. No.	Name of the sample	Acetone extract (zone of inhibition in mm)	Methanol extract (zone of inhibition in mm)	Ethanolextract (zone of inhibition in mm)
	Streptomycin (Drug control)	10 ± 0.57		
1	*Amaranthus viridis* (Whole root)	–	–	11 ± 1
2	*Amorphophallus paeoniifolius* (Modified root)	–	–	9 ± 1
3	*Averrhoa bilimbi* (Fruit)	–	–	3 ± 1.24
4	*Basella alba* (Leaf)	–	–	4 ± 0.18
5	*Benincasa hispida* (Pulp)	–	–	11 ± 0.18
6	*Beta vulgaris* (Peel)	–	–	11.33 ± 0.157
7	*Capsicum annum* (Fruit)	–	–	9.33 ± 0.57
8	*Cinnamomum verum* (Leaf)	–	–	10 ± 1.24
9	*Colocasia esculenta* (Root)	–	9 ± 1	–
10	*Colocasiae sculenta* (Leaf)	8 ± 0.57	–	–
11	*Cucurbita pepo* (Pulp)	–	–	9 ± 1
12	*Cucurbita pepo* (Seed)	–	–	8 ± 1
13	*Cucurma longa* (Leaf)	–	–	12.33 ± 0.57
14	*Cucurma longa* (Rhizome)	–	–	4 ± 1
15	*Garcinia indica* (Seed)	9 ± 1	–	11.33 ± 1.52
16	*Lagenaria siceraria* (Seed)	–	–	7.33 ± 0.15
17	*Luffa acutangula* (Fruit)	–	–	10.33 ± 057
18	*Mangifera indica* (Unripe fruit)	–	–	10.33 ± 1.52
19	*Moringa olifera* (Fruit)	–	–	9 ± 0.18
20	*Murraya koenigii* (Leaf)	–	–	4 ± 1.24
21	*Phyllanthus emblica* (Fruit)	5.66 ± 0.157	5 ± 0.18	6.33 ± 0.15
22	*Pisum sativum* (Seeds)	–	–	3 ± 1.24
23	*Plectranthus amboinicus* (Leaf)	–	–	10.33 ± 0.57
24	*Raphanus sativus* (Modified root)	–	–	7.33 ± 0.57
25	*Raphanus sativus* (Leaf)	9.33 ± 0.57	–	–
26	*Solanum lycopersicum* (Fruit)	–	–	8 ± 1
27	*Solanum melongena* (Fruit)	–	–	10 ± 1
28	*Solanum tuberosum* (Tuber)	7 ± 1	–	8.33 ± 0.57
29	*Strychnine nux-vomica* (Leaf)	–	–	8 ± 0.57
30	*Syzygium aromaticum* (Leaf)	–	–	10 ± 1.24
31	*Trichosanthes cucumerina* (Pulp)	–	–	7.33 ± 0.57
32	*Trichosanthes cucumerina* (Seed)	–	–	8.33 ± 0.57
33	*Trichosanthes dioica* (Fruit)	–	–	8 ± 0.57
34	*Vicia faba* (Pods)	6 ± 0.57	–	–
35	*Zea mays* (Fruit)	–	–	3 ± 0.57
36	*Zingiber officinale* (Rhizome)	–	3 ± 0.57	–

methanol and 31 ethanol extracts. None of the 95 aqueous extracts did show a zone of inhibition. Only those extracts with a significant zone of inhibition as compared to the solvent control (2 mm) at $p < 0.05$ and the drug control, streptomycin (300 μg/ well, 10 mm) were selected for further work. The data was considered significant based on one sample *t*-test. In total, 11 ethanol extracts were picked for the MIC determination. The diameter of the zone of inhibition of all the 36 extracts has been illustrated in Table 2.

3.2. MIC determination

For the MIC determination against *P. aeruginosa* and *C. violaceum*, 11 plant extracts selected from the preliminary antibacterial screening were used. Concentrations ranging from 0.25 to 10 mg/ml were employed. The least MIC value at which a zone of inhibition was observed was found to be in the range of 0.5–1.0 mg/ml (Table 3). Accordingly, 6 plants *Viz*, *Cinnamomum. verum* leaf, *Cucurma longa* leaf, *Garcinia indica* seed, *Luffa acutangula* fruit, *Solanum melongena* fruit, *and Syzygium aromaticum* leaf were chosen to carry out the anti-QS activity assay.

3.3. Assessment of anti-QS activity

6 plants selected based on their MIC values were further evaluated for the anti-QS activity against *C. violaceum*. Concentration less than their MIC values were used for the assay. Although 5 plant extracts inhibited the pigment production, the diameter of zone of inhibition was highest in *C. verum* leaf, *S. melongena* fruit, *and S. aromaticum* leaf (Table 4). Plant extracts with the lowest sub-MIC value exhibiting the highest zone of pigment inhibition were further assessed.

Table 3
MIC of plant extracts against *P. aeruginosa* and *C. violaceum.*

Sl. No	Name of the plant	Minimum inhibitory concentration (mg/ml)	
		P. aeruginosa	*C. violaceum*
1	*Amaranthus viridis* (Root)	7.0	3.0
2	*Benincasa hispida* (pulp)	5.0	5.0
3	*Beta vulgaris* (peel)	3.0	3.0
4	*Cinnamomum verum* (leaf)	0.5	0.5
5	*Cucurma longa* (leaf)	0.5	1.0
6	*Garcinia indica* (seed)	1.0	0.75
7	*Luffa acutangula* (fruit)	0.5	0.5
8	*Mangifera indica* (unripe fruit)	7.0	3.0
9	*Plectranthus amboinicus* (leaf)	2.0	2.0
10	*Solanum melongena* (fruit)	0.5	0.5
11	*Syzygium aromaticum* (leaf)	0.5	1.0

3.4. Quantitative estimation of violacein inhibition in the presence of plant extracts

All the three extracts of *C. verum*, *S. melongena*, *and S. aromaticum* at the concentration of 0.25 mg/ml were able to exhibit inhibition of violacein (Fig. 1). Percent reduction of violacein in

C. verum leaf, *S. melongena* fruit and *S. aromaticum* leaf was 70.52%, 64.52%, and 65.7% respectively.

Table 4
Anti-QS activity of the extracts against *C. violaceum*.

Sl. No	Name of the sample	Ethanol extract (mg/ml)	Zone of pigment inhibition (mm)
1	*Cinnamomum verum* (leaf)	0.25	10.33 ± 0.57
2	*Cucurma longa* (leaf)	0.25	8.33 ± 0.57
3	*Garcinia indica* (seed)	0.5	7.66 ± 0.57
4	*Luffa acutangula* (fruit)	0.25	7.66 ± 0.57
5	*Solanum melongena* (fruit)	0.25	11 ± 0.57
6	*Syzygium aromaticum* (leaf)	0.25	11 ± 1

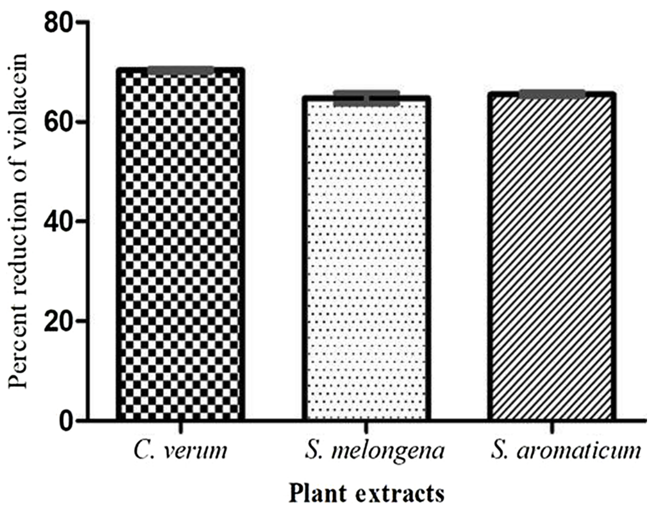

Fig. 1. Percent inhibition of violacein production in the presence of sub-MIC of plant extracts.

3.5. Assessment of P. aeruginosa virulence factors in the presence of plant extracts

All the 3 ethanolic plant extracts were able to reduce elastase production, pyocyanin production, biofilm formation and swarming motility in *P. aeruginosa* (Fig. 2). There was 81.41% elastase inhibition with *C. verum* leaf extract followed by *S. melongena* fruit (76.55%) and *S. aromaticum* leaf (78.8%) extracts (Fig. 2a). All the extracts were able to inhibit the production of pyocyanin (Fig. 2b). The percent inhibition was more than 90 in all the extracts. The highest inhibition was in the *C. verum* leaf extract (94%). There was no major difference between the extracts in reducing swarming motility in *P. aeruginosa*. But, there was a significant difference in the swarming motility exhibited by the control and the treated ones (Fig. 2c). Percent inhibition of swarming motility in *C. verum* leaf extract was 71%. Both *S. melongena* fruit and *S. aromaticum* leaf extracts exhibited inhibition of around 66%. There was a remarkable reduction in the biofilm formed in the presence of plant extracts (Fig. 2d). *P. aeruginosa* grown in the absence of extracts showed high biofilm formation. *C. verum* leaf extract significantly reduced the biofilm formation by 80.3%. The inhibition of biofilm formation with the other two extracts were slightly lower than that of *C. verum*. Based on the results of the virulence assays, *C. verum* leaf extract was considered to possess good anti-QS activity against *P. aeruginosa* ($p < 0.05$).

4. Discussion

Due to the extensive use of antibiotics, drug resistance in pathogenic bacteria has become a threat worldwide. To overcome this problem, several new strategies are being tried, and one such strategy is the attenuation of the quorum sensing system using naturally occurring compounds. Studies have been carried to report the anti-QS activity of medicinal plants, essential oils, edible fruits and wild berries [10–12]. But, there are limited studies that have exploited locally available vegetables and spices as QS inhibitors. Moreover, vegetables are generally considered to be safe and rarely associated with side-effects as seen with many antibiotic regimens [13].

Initially, different solvent extracts of plants were screened for their antibacterial activity against *P. aeruginosa*. Only 37% of the samples taken in the present study displayed the antibacterial activity. This is comparatively lower than that of the previous studies on medicinal plants which have shown the antibacterial activity of 50–70% in the plants tested [14,15]. The lower antibacterial activity might be due to the constituent phytochemicals found in the plant species and also the kind of test bacteria used. The variation in the antibacterial activity of the extracts with the solvents tested can be explained in terms of solvent polarity, the polarity of the compounds being extracted, loss of active compounds during extraction, and the ability of the compound to dissolve or diffuse in the media used in the assay [16,17]. Ethanol was concluded as the best solvent for extracting antibacterial compounds. Earlier studies have also shown a greater antibacterial activity of ethanol extract than other solvents [18,19]. Selection of plant extracts with a zone of inhibition of ≥ 10 mm was according to a previous report of Leite [20] where concentrations of 10 mg/ml with an inhibition zone of 10 mm or greater was considered to indicate good antibacterial activity. Plant extracts with the MIC below 1 mg/ml selected for anti-QS activity screening were based on the study by Rios and Recio [21] who reported avoiding of extracts showing MIC values of more than 1 mg/ml because of their poor activity.

C. violaceum employed in the present research is the commonly used model for screening compounds that can interfere with AHL system in bacteria as indicated by a decrease in violacein production. AHL antagonistic activity of various plants such as *Mangifera indica, Vanilla planifolia, Punica granatum, Tremella fuciformis, Hemidesmus indicus, Zingiber officinale, Ocimum basilicum, Brassica oleracea* etc. have been discovered using this bacterium [22]. Earlier studies on medicinal plants have shown a positive correlation between antibacterial and anti-QS activity [10,11]. Considering this, in the present study, plants with significant antibacterial activity based on their MIC values were screened for their anti-QS activity. Similar to previous findings, we were able to establish a positive correlation between antibacterial and anti-QS activity. Plants with significant antibacterial activity were proved to have good anti-QS activity.

Selection of plant extracts for the further assessment of virulence activity in *P. aeruginosa* was based on the extent of violacein inhibition and absence of growth restriction in *C. violaceum*. As the amount of violacein inhibition in the presence of *C. verum* leaf, *S. melongena* fruit and *S. aromaticum* leaf was greater than the other extracts tested, only these 3 plant extracts were further tested for their anti-QS activity on *P. aeruginosa*. Anti-QS activity of the selected plant extracts in reducing the virulence of *P. aeruginosa* was assessed by quantifying the reduction in swarming motility, pyocyanin, elastase and biofilm production.

P. aeruginosa is known to produce a large number of virulence factors including elastase and pyocyanin [23]. Elastase is one of the elastolytic zinc metalloproteinases causing damage to the host tissue through the breakdown of extracellular matrix thus attacking intercellular tight junctions [24]. Pyocyanin is a secondary metabolite which interferes with numerous cellular functions by producing reactive oxygen species (ROS) and generating oxidative stress in the host [25]. In the present investigation, incubation of *P. aeruginosa* with the extracts revealed inhibition of elastase and pyocyanin production. The data corroborated with the earlier studies where, elastase activity and pyocyanin production were decreased by essential oil and medicinal herbs [26,27].

Biofilm produced by *P. aeruginosa* helps the bacterium to adhere to the surfaces of the host cell. AHLs released by *P. aeruginosa* play an

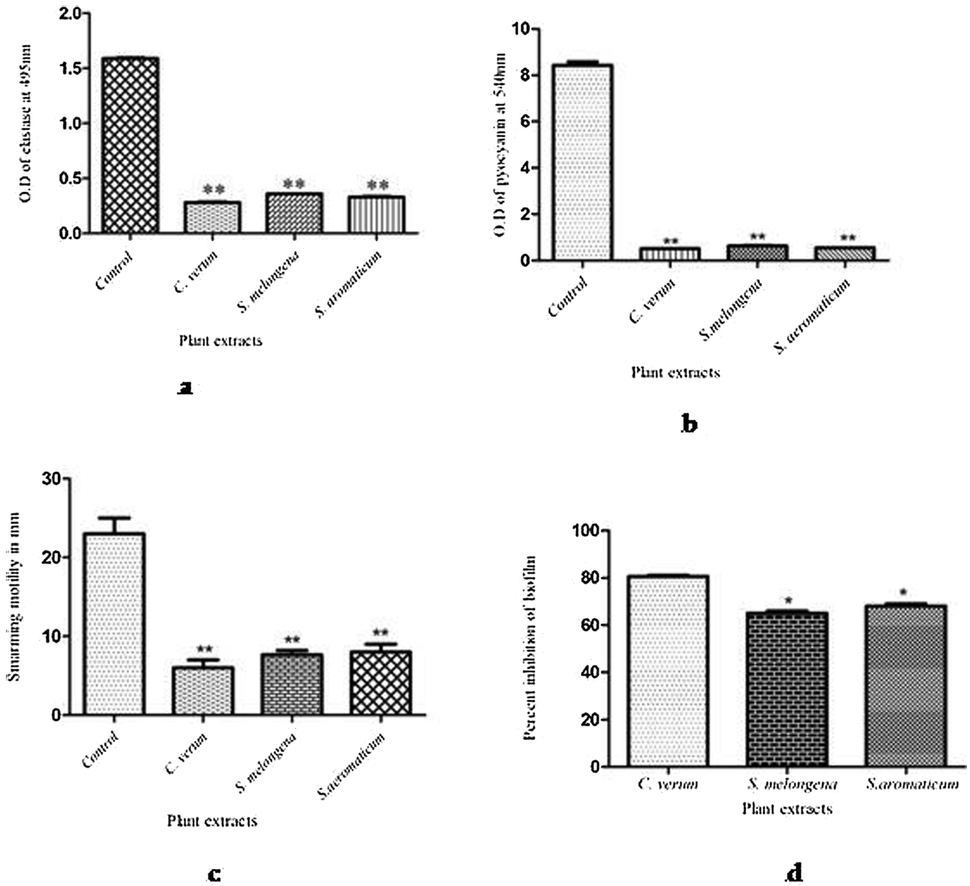

Fig. 2. Reduction in virulence factors of *P. aeruginosa* in the presence of plant extracts. a) *LasB* assay, b) Pyocyanin assay, c) Swarming motility assay, and d) Biofilm assay. (* $p < 0.05$; ** $p < 0.01$)

important role in biofilm formation [28]. Extracts of *C. verum* leaf, *S. melongena* fruit, *and S. aromaticum* leaf used in the current investigation significantly reduced biofilm formation in *P. aeruginosa*. The results from the present study are in agreement with similar studies on extracts of *Sclerocarya birrea* [29], *Trigonella foenum-graecum* [30] and *Terminalia bellerica* [31]. Reduction in the surface adhesion or subsequent steps in biofilm formation might be the possible ways of interference that has led to the reduced biofilm formation [32].

Swarming motility, the co-ordinated translocation of the bacterial population across a solid or semisolid surface requires recognition of extracellular signals (QS molecules) and also a functional lipopolysaccharide [33]. Inhibition of swarming motility in the presence of selected plant extracts, as noticed in the present investigation, is also reported from other investigators using extracts of Cranberry [34] and *Poria cum Radix pini*, *Angelica dahurica*, *Rhizoma cibotii* and *Schizonepeta tenuifoli* [35]. The significant role of swarming motility exhibited by *P. aeruginosa* in the early stages of biofilm formation has been described [36]. Reduction in swarming motility of *P. aeruginosa* as observed with the plant extracts might have caused a notable reduction in the formation of biofilm also.

The 3 selected extracts of *C. verum* leaf, *S. melongena* fruit, *and S. aromaticum* leaf showed a considerable reduction in the QS activity of *P. aeruginosa* as recognized by the inhibition of swarming motility, limiting pyocyanin, elastase and biofilm production at a concentration of 0.25 mg/ml. More significantly, the concentration represents that of crude extracts, and therefore, concentration would be much lower when the putative anti-QS compounds from these plants are isolated. The anti-QS activity of these plant extracts could be due to the presence of phenolics, tannins, flavonoids, saponins, terpenoids or a combination of some of these [37]. The mechanism of action of these plant extracts as QS inhibitors can be hypothesized to the reduction in the signalling molecules from being synthesized or modification or degradation of signalling molecules and/or targeting the *LuxR* signal receptor [22]. This study was the initial part of the Ph.D. work of the first author intended to isolate a putative anti-QS compound/s from vegetables and spices against *P. aeruginosa*. Further work on isolation and characterization of the biomolecule responsible for the observed interference is being carried out.

5. Conclusion

It is well known that plants with anti-QS activity can serve as a promising tool for the management of infections caused by multidrug resistant bacteria. The study highlights the importance of evaluating the unexplored anti-QS property of locally available vegetables and spices, besides the usual evaluation of traditionally used medicinal plant sources. The present investigation envisages the use of dietary plants which could effectively be used in future as QS inhibitors with minimal toxicity to combat infections caused by *P. aeruginosa*, a known multidrug resistant bacterium. The presence of active QS inhibitory compounds as observed in the present investigation in the extracts of *C. verum* leaf, *S. melongena* fruit, *and S. aromaticum* leaf effectively inhibits QS and QS associated virulence in *P. aeruginosa*. The deeper understanding of the mechanism of action of these QS inhibitors on microbial cell communication promises novel therapeutic applications in eliminating the pathogen.

Author contributions

Ramya Premanath conceived and supervised the work. Prathiksha P and Sarika S performed the experiments. Gururaj MP analyzed the data. All authors contributed to the final version of the manuscript.

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Declaration of Competing Interest

The authors declare that there are no known conflicts of interest associated with this publication.

References

[1] N. Hoiby, Recent advances in the treatment of *Pseudomonas aeruginosa* infections in cystic fibrosis, BMC Med. 9 (2011) 32.
[2] R.S. Smith, B.H. Iglewski, *P. aeruginosa* quorum-sensing systems and virulence, Curr. Opin. Microbiol. 6 (2003) 56–60.
[3] P.N. Jimenez, G. Koch, J.A. Thompson, K.B. Xavier, R.H. Cool, W.J. Quax, The multiple signaling systems regulating virulence in *Pseudomonas aeruginosa*, Microbiol. Mol. Biol. Rev. 76 (2012) 46–65.
[4] J.B. Harborne, Phytochemical Methods, Chapman and Hall Ltd., London, 1973, pp. 49–88.
[5] F. Pantanella, F. Berlutti, C. Passariello, S. Sarli, C. Morea, S. Schippa, Violacein and biofilm production in *Janthinobacterium lividum*, J. Appl. Microbiol. 102 (2007) 992–999.
[6] J.P. Pearson, E.C. Pesci, B.H. Iglewski, Roles of *Pseudomonas aeruginosa las* and *rhl* quorum-sensing systems in control of elastase and rhamnolipid biosynthesis genes, J. Bacteriol. 179 (1997) 5756–5767.
[7] W.M. Raoof, I.A. Latif, In vitro study of the swarming phenomena and antimicrobial activity of pyocyanin produced by *Pseudomonas aeruginosa* isolated from different human infections, Eur. J. Sci. Res. 47 (2010) 405–421.
[8] J. Tremblay, E. Deziel, Improving the reproducibility of *Pseudomonas aeruginosa* swarming motility assays, J. Basic Microbiol. 48 (2008) 509–515.
[9] G.A. O'Toole, R. Kolter, Initiation of biofilm formation in *Pseudomonas fluorescens* proceeds via multiple, convergent signaling pathways: a genetic Analysis, Mol. Microbiol. 28 (1998) 449–461.
[10] D. Damte, E. Gebru, S.J. Lee, J.W. Suh, S.C. Park, Evaluation of anti-quorum sensing activity of 97 indigenous plant extracts from Korea through bioreporter bacterial strains *Chromobacterium violaceum* and *Pseudomonas aeruginosa*, J. Microb. Biochem. Technol. 5 (2013) 42–46.
[11] S.K. Namasivayam, J.M. Vivek, Screening of quorum sensing (QS) modulatory effect of medicinal plant extracts against quorum sensing mediated virulence factors of human pathogenic gram negative bacteria, Int. J. Pharmacogn. Phytochem. Res. 8 (2016) 263–271.
[12] H.Z. Asfour, Anti-quorum sensing natural compounds, J. Microsc. Ultrastruct. 6 (2017) 1–10.
[13] D.A. Vattem, K. Mihalik, S.H. Crixell, R.J. McLean, Dietary phytochemicals as quorum sensing inhibitors, Fitoterapia 78 (2007) 302–310.
[14] R. Dabur, A. Gupta, T.K. Mandal, D.D. Singh, V. Bajpai, A.M. Gurav, G.S. Lavekar, Antimicrobial activity of some Indian medicinal plants, Afr. J. Tradit. Complement. Altern. Med. 4 (2007) 313–318.
[15] D. Maharjan, A. Singh, B. Lekhak, S. Basnyat, L.S. Gautam, Study on antibacterial activity of common spices, Nepal J. Sci. Technol. 12 (2011) 312–317.
[16] P. Sampathkumar, B. Dheeba, Z.V. Vidhyasagar, T. Arulprakash, R. Vinothkannan, Potential antimicrobial activity of various extracts of *Bacopa monnieri* (Linn.), Int. J. Pharmacol. Res. 4 (2008) 230–232.
[17] S. Anjana, V. Rani, R. Padmini, Antibacterial activity of some medicinal plants used by tribals against UTI causing pathogens, World Appl. Sci. J. 7 (2009) 332–339.
[18] F. Aqil, I. Ahmad, Broad-spectrum antibacterial and antifungal properties of certain traditionally used Indian medicinal plants, World J. Microbiol. Biotechnol. 19 (2003) 653–657.
[19] P. Kannan, S.R. Ramadevi, W. Hopper, Antibacterial activity of *Terminalia chebula* fruit extract, Afr. J. Microbiol. Res. 3 (2009) 180–184.
[20] S.P. Leite, J.R. Vieira, P.L. de Medeiros, R.M. Leite, V.L. De Menezes Lima, H.S. Xavier, E. de Oliveira Lima, Antimicrobial activity of Indigofera suffruticosa, Evid. Complement. Alternat. Med. 3 (2006) 261–265.
[21] J.L. Rios, M.C. Recio, Medicinal plants and anti-microbial activity, J. Ethnopharmacol. 100 (2005) 80–84.
[22] C.L. Koh, S. Choon-Kook, Y. Wai-Fong, T. Li Ying, K. Thiba, C. Yee Meng, C. Kok-Gan, Plant-derived natural products as sources of anti-quorum sensing compounds, Sensors 13 (2013) 6217–6228.
[23] A. Annapoorani, B. Kalpana, K.S. Musthafa, S.K. Pandian, A.V. Ravi, Antipathogenic potential of *Rhizophora* spp. Against the quorum sensing mediated virulence factors production in drug resistant *Pseudomonas aeruginosa*, Phytomedicine 20 (2013) 956–963.
[24] K.S. McIver, E. Kessler, D.E. Ohman, Identification of residues in the *Pseudomonas aeruginosa* elastase propeptide required for chaperone and secretion activities, Microbiology 150 (2004) 3969–3977.
[25] T. Das, M. Simone, A.I. Ibugo, P.K. Witting, M. Manefield, J. Manos, Glutathione enhances antibiotic efficiency and effectiveness of DNase I in disrupting *Pseudomonas aeruginosa* biofilms while also inhibiting pyocyanin activity, thus facilitating restoration of cell enzymatic activity, confluence and viability, Front. Microbiol. 8 (2017) 2429.
[26] P.S. Ganesh, R.V. Rai, Inhibition of quorum-sensing-controlled virulence factors of *Pseudomonas aeruginosa* by *Murraya koenigii* essential oil: a study in a *Caenorhabditis elegans* infectious model, J. Med. Microbiol. 16 (2016) 1528–1535.
[27] V. Karbasizade, P. Dehghan, M.M. Sichani, K. Shahanipoor, S. Sepahvand, R. Jafari, R. Yousefian, Evaluation of three plant extracts against biofilm formation and expression of quorum sensing regulated virulence factors in Pseudomonas aeruginosa, Pak. J. Pharm. Sci. 30 (2017) 585–589.
[28] L. Chen, Y. Wen, The role of bacterial biofilm in persistent infections and control strategies, Int. J. Oral Sci. 3 (2011) 66–73.
[29] R. Sarkar, S.K. Chaudhary, A. Sharma, K.K. Yadav, N.K. Nema, M. Sekhoacha, S. Karmakar, F.C. Braga, M.G. Matsabisa, P.K. Mukherjee, T. Sen, Anti-biofilm activity of Marula–a study with the standardized bark extract, J. Ethnopharmacol. 154 (2014) 170–175.
[30] F.M. Husain, I. Ahmad, M.S. Khan, N.A. Al-Shabib, *Trigonella foenum-graceum* (seed) Extract Interferes With Quorum Sensing Regulated Traits and Biofilm Formation in the Strains of *Pseudomonas aeruginosa* and *Aeromonas hydrophila*, Evidence-Based Complementary and Alternative Medicine, 2015, p. 879540.
[31] P.S. Ganesh, V.R. Rai, Attenuation of quorum-sensing-dependent virulence factors and biofilm formation by medicinal plants against antibiotic resistant *Pseudomonas aeruginosa*, J. Tradit Complement. Med. 8 (2018) 170–177.
[32] F.M. Husain, I. Ahmad, A.S. Al-thubiani, H.H. Abulreesh, I.M. AlHazza, F. Aqil, Leaf extracts of *Mangifera indica* L. Inhibit quorum sensing–regulated production of virulence factors and biofilm in test bacteria, Front. Microbiol. 8 (2017) 727.
[33] T. Lindhout, P.C. Lau, D. Brewer, J.S. Lam, Truncation in the core oligosaccharide of lipopolysaccharide affects flagella-mediated motility in *Pseudomonas aeruginosa* PAO1 via modulation of cell surface attachment, Microbiol. 155 (2009) 3449–3460.
[34] C.O.' May, N. Tufenkji. The swarming motility of *Pseudomonas aeruginosa* is blocked by cranberry proanthocyanidins and other tannin-containing materials, Appl. Environ. Microbiol. 77 (2011) 3061–3067.
[35] Y. Chong, K. How, W. Yin, K. Chan, The effects of chinese herbal medicines on the quorum sensing-regulated virulence in *Pseudomonas aeruginosa* PAO1, Molecules 23 (2018) 972.
[36] J.D. Shrout, D.L. Chopp, C.L. Just, M. Hentzer, M. Givskov, M.R. Parsek, The impact of quorum sensing and swarming motility on *Pseudomonas aeruginosa biofilm* formation is nutritionally conditional, Mol. Microbiol. 62 (2006) 1264–1277.
[37] L. Mayaud, A. Carricajo, A. Zhiri, G. Aubert, Comparison of bacteriostatic and bactericidal activity of 13 essential oils against strains with varying sensitivity to antibiotics, Lett. Appl. Microbiol. 47 (2008) 167–173.