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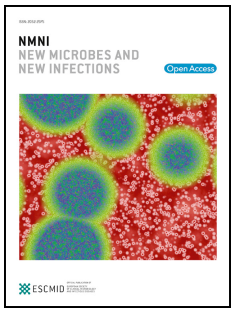

Evaluation of Quorum Sensing inhibitory effects of the extracts of Three traditional Medicine plants with Known antibacterial Properties

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Nahal Hadi: Study concept and design, expanding, revision and editing the article. **Farhad Moradi:** Sample collection, laboratory examinations, interpretation of data, Literature search and writing the first draft of the article. **Abdollah Bazargani:** Definition of intellectual content and Critical revision of the manuscript.

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Author contributions:

NH Study concept and design, expanding, revision and editing the article. **FM** Sample collection, laboratory examinations, interpretation of data, Literature search and writing the first draft of the article. **AB** Definition of intellectual content and Critical revision of the manuscript.

Evaluation of Quorum Sensing Inhibitory Effects of the Extracts of Three Traditional Medicine Plants with Known Antibacterial Properties

Abstract

Background: Today an alternative approach to control bacterial infections is the usage of natural and traditional plant compounds to interfere with their Quorum Sensing (QS) systems. In this study, antibacterial and anti-QS sensing activity of *Syzygium aromaticum*, *Dionysia revolute* Boiss and *Eucalyptus camaldulensis* Dehnh were evaluated. These plants are local to the Middle East region and has been used for their antibacterial activity by ancient people for many years.

Method: Plant compounds were extracted with n-hexane, methanol and 96% ethanol mixed solvent. Antibacterial activity of this herbal extracts against five gram negative and positive bacteria were assessed. Therefore, the effective sub minimum inhibitory concentration (MIC) of this extract on bacterial QS systems were investigated by Violacein quantification assay in *Chromobacterium violaceum* CV026 biosensor strain, inhibition of exogenously QS signal molecules in *Aeromonas veronii* bv. Sobria strain BC88 and *Pseudomonas aeruginosa* isolated from patient with cystic fibrosis *in vitro.*

Results: According to the results, *Syzygium aromaticum* by (0.39 to 0.048 mg/ml), *Dionysia revolute* Boiss by (3.1 to 0.39 mg/ml) and *Eucalyptus camaldulensis* by (0.78 to 0.097 mg/ml) showed anti-QS activities through reducing the violacein formation depletion of QS signals produced in *A. veronii* and *P. aeruginosa* at sub-MIC concentrations.

Conclusion: Regarding the anti-QS effect of this herbal extracts in, the effective component of them can be a candidate to use for combating bacterial infection at sub-MIC concentrations.

Keywords: *Syzygium aromaticum*, *Dionysia revolute*, *Eucalyptus camaldulensis*, anti-quorum sensing, plants extract

1. Introduction

In recent times controlling bacterial infections is very problematic because the excessive use of antibiotics in human medicine therapy is considered the major factor for the widespread bacterial resistance and the formation of biofilm significantly reduces the sensitivity of bacteria to antibacterial agents [1-3]. Today an alternative approach to control bacterial infections is to interfere with their quorum sensing (QS) mechanisms by decreasing the production, diffusion, and destroying of QS signals or inhibition of their receptors through imitating of signal structures [4]. In bacterial populations, quorum sensing regulates the biological behavior including biofilm formation, bioluminescence, and enzymes secretion at a threshold population density through signal molecules [5, 6]. Finding new ways to target QS in bacteria is a known strategy to find and develop new antibiotics, which might provoke a response within the host defense system, even while bacteria are in low density [7, 8]. Many eukaryotes, fungi, medicinal plants, fruit, and vegetables, in particular, secrete compounds that can interfere with the QS-regulated gene expression in the invading organism [9-13]. One of the recommended methods to deal with drug-resistant bacteria is the usage of natural antimicrobial agents such as natural plant compounds. For instance, in this process, the medicinal plant extracts have an important role in combating bacterial infection by producing a diverse antimicrobial compound, such as phenolic, terpenoids, flavanones, quinones, due to similarity in their chemical structure to those of QS signals (AHL) and ability to degrade signal receptors [4, 14, 15]. Antimicrobial activities of herbal medicine against some bacterial species were reported, especially from the extracts and fraction of *Syzygium aromaticum*, *Dionysia revolute*, and *Eucalyptus camaldulensis* [16-19]. Furthermore, some of the researches have reported antifungal, anti-inflammatory, anti-cancer, and antioxidant effects of these herbal medicines [18, 20-22]. Hence,

the aim of this study was to evaluate antibacterial effects and the role of sub-minimum inhibitory (sub-MIC) concentration of these herbal extracts to interfere in the QS system of *Chromobacterium violaceum* CV026 biosensor strain, *Aeromonas veronii* bv. Sobria strain BC88 and *Pseudomonas aeruginosa* which isolated from the hospitalized patient as a clinical sample.

2. Materials and methods

2.1. Collection of plant material and extract preparation

Plant Materials were obtained from a local market located in Shiraz, Iran. A voucher specimen and scientific identity of the plants compound were confirmed at the Shiraz University of Medical Sciences Herbarium department. A voucher specimen was deposited in the Herbarium of Shiraz Faculty of Pharmacy, Shiraz, Iran. At first, the plant samples were washed twice with distilled water and dried in the dark place at 25°C for 72 hours. The dried plant samples were powdered and submerged in 600 ml of n-hexane, methanol, and 96% ethanol mixture solvent (ratio 1:1 w/v) in a rotary for 72 hours. The percentage yields of different extracts were calculated using the formula (Table1):

Yield (%) = [dry crude extract / dry initial plant material before extraction] × 100

The plant extracts were filtered and concentrated with a rotary evaporator and solved in appropriate concentrations of dimethyl sulfoxide ((DMSO), 50 to 0.048 mg/ml) until further analysis.

2.2. Bacterial strain and culture conditions

For the antibacterial assays, five clinical islolates [*Klebsiella pneumoniae, Staphylococcus aureus, Acinetobacter baumannii, Serratia marcescens,* and *Pseudomonas aeruginosa* (isolated from a patient with cystic fibrosis)], were provided from clinical bacteriology laboratory of Shahid Faghihi hospital, Shiraz, Iran. *Aeromonas veronii bv. Sobria* strain BC88, *Chromobacterium violaceum* CV026 (mini-Tn5 mutant of the wild type strain, National Collection of Type Culture 13278), -80° C stock in the Bacteriology and Virology department of Shiraz University of Medical Sciences were used for QS inhibition assay. The C6-AHL (N-Hexanoyl-L-Homoserine Lactone) was used in this study as a signal molecule purchased from Sigma Aldrich (56395-10MG Germany). *C. violaceum* CV026 cultured in Luria-Bertani medium (1% w/v NaCl, 1% w/v tryptone, 0.5% w/v yeast extract) supplemented with kanamycin (30 µg/mL) and chloramphenicol (30 µg/mL) with shaking at 220 rpm, 28°C.

2.3. Antibacterial properties

In this study, we used the disc diffusion method based on (Murray et al., 2016) to determine the antibacterial activity of herbal extracts on bacterial strains [37]. Herbal extracts were dissolved in DMSO (50-0.39mg/ml) and Mueller Hinton Agar Plates (Merck, Germany) were inoculated uniformly with 1 ml of each bacterial suspensions (108 CFU/ml). Therefore, sterile paper discs (6 mm) loaded by herbal extracts dilutions (50 µl) and set on the bacterial cultured plate and then incubated at 37°C for 48 hrs. Tetracycline (30 µg) and DMSO were used as positive and negative controls. The antibacterial activity of herbal extracts was determined by measuring the diameter of the inhibition zone in mm.

2.4. Minimal inhibitory concentrations (MICs)

Before the anti-QS assay, The MBC (minimal bactericidal concentrations), MIC, and sub-MIC value of herbal extracts were determined using serial macro dilution assay in control and test tubes according to Laboratory Standards Institute (CLSL, 2015) guidelines [41]. At first *A. veronii* bv. Sobria BC88, *P. aeruginosa* isolated from a patient, and *C. violaceum* CV026 were cultivated overnight at 37°C in LB broth. All extracts were initially tested at 25 mg/ml and serially diluted in two-fold (control and test tube), to 0.048 mg/ml. Each test tube contained 0.5 ml of each concentration and inoculated with equal volumes of the microbial suspension (106 CFU/ml). Whiles control tubes just contained herbal extracts concentration and broth media without bacterial inoculum to be used as a blank. This was done to eliminate plant extracts and broth media turbidity to follow bacterial growth in the test tube in the spectrophotometric assay at 600 nm. An extract-free tube containing microbial suspension was used as a positive control. All of the tubes were incubated at 37°C for 24. The tubs without bacterial growth showed bactericidal effects and the lowest concentration of the plant extracts that could at least inhibited 50% of microorganism's growth was considered as MIC by the use of the following equation [23]:

Inhibition % = [(OD C – OD T) / OD C] × 100

OD C is OD600 nm to track bacterial growth in the positive control tube, and OD T is OD600 nm to estimate bacterial growth in the test tubes. To ensure the presence or absence of bacterial growth in the test tubes, a standard loop of the suspensions in each tube was inoculated on 3mm MHA and incubated overnight at 37ºC.

2.5. Violacein Quantification Assay

To explore the anti-quorum sensing potential of sub-MIC concentration of the herbal extracts in a 96 well microplate, assays were performed to examine the inhibition of violacein production in *C. violaceum* CV026**.** An overnight culture of C. *violaceum* CV026 diluted 1:50 in 4 mL of fresh LB broth, supplemented with (0.25 µg/ml) N-acyl homoserine lactone (C6-AHL) and different sub-MIC concentrations of herbal extracts. In this experiment, we used control wells containing *C. violaceum* CV026 and LB broth to manage bacterial population and without pigment production (negative control, OD600 =1) and C. *violaceum* CV026, AHL signal without herbal extracts to evaluate violacein production in the constant bacterial population (positive control). Microplates were incubated for 24 hours at 28°C in a shaking incubator and the absorbance of each well was read in 585 and 600nm for violacein production and bacterial population growth, respectively by Polar Star Omega Microplate reader (BMG LAB TECH, Germany) (Fig. 1). The experiment was repeated in triplicate and the percentage of violacein pigment inhibition, when compared with control wells, was calculated through the following equation [24, 25]:

Inhibition (%) = [(OD 585 C – OD585 T) / OD585 C] × 100.

In which OD C is OD585 for violacein production in control wells (without herbal extracts) and OD T is OD585 for violacein production in presence of herbal extracts by *C. violaceum* CV026. To ensure that the inhibition of violacein production was not due to antibacterial activity, the effect of herbal extracts on the growth of *C. violaceum* CV026 was further examined by comparison bacterial population in all of the wells with the controls (without herbal extracts) in OD600=1. The constant of the bacterial population in all of the wells indicating that the inhibition of violacein production is due to anti-quorum sensing effects.

2.6. *C. violaceum* CV026 Assay

For *C. violaceum* CV026 assay, an overnight culture of CV026 was diluted to (OD600 = 1), and 200 µl of it was spread circularly in the middle of LB agar plates that were supplemented with C6-AHL (0.25 µg/ml). Wells was punched in the center of the culture plate after incubating it for 30 min. the sub-MIC concentration of plant extracts (50 µL) with maximum anti QS effects were obtained from the previous experiment (see sec. 2.4.) and inoculated in each well. DMSO was used as a negative control. The plates were incubated at 28°C for 48 hours. A white circle of bacterial growth, surrounded by a purple halo around the well (filled with the plant extract), suggested that the extracts exhibited anti-QS through inhibiting the violacein production around the well. When the diffused molecules of plant extracts became too diluted, then the purple color appeared again, which could be observed as an outer purple circle [26].

2.7. Anti-quorum sensing activity through QS signals Inhibition

Since the Quorum sensing is a vital system to regulate *Aeromonas* enterotoxin and *Pseudomonas* virulence factors and contributed on biofilm formation in both of them [38-40], *In-vitro* screening for anti-quorum sensing activity of herbal extracts were investigated against *A. veronii bv. Sobria* strain BC88 and *P. aeruginosa* isolated from hospitalized patient. *C. violaceum* CV026 biosensor strain violacein gene is also under control of QS. Meanwhile, this strain has a mutation in AHL producing gene, which results in lac of pigment production without the presence of external AHL signal. In this trial, *Aeromonas* and *pseudomonas* produce and diffuse the same AHL QS signals in LB-agar plate which is compatible with *C. violaceum* CV026 biosensor. At that point this Signals could be taken by the latter bacterium and let it produce purple violacein pigment. We hypothesis that the antibacterial activity of herbal extracts can be due to their interference with QS system of some certain bacteria and in other words blocking of bacterial cross talk. So, we expected, in the presence of plant extracts, these AHL

molecules might be blocked. Consequently, the biosensor bacteria cannot produce violacein pigment when they grow in the vicinity of *Aeromonas* and *Pseudomonas* on a plate containing herbal extracts. While, without the extracts the purple pigment will be clearly detectable. So, at the first place, we determined the MIC and sub-MIC value of the herbal extracts for these bacteria according to section (2.4). 200 µl of an overnight culture of CV026 was diluted to OD600=1 and then spread uniformly on the LB agar. A wells were punched on the center of plates. Then 90 µl of each *P. aeruginosa* and *A. veronii* suspension (OD600 = 1) separately plus sub-MIC concentration of herbal extracts (10 µl) was added to each well. In parallel, in control plates, *P. aeruginosa* and *A. veronii*, were not formerly mixed with any herbal extract. The plates were incubated for 48 h at 28 °C and diameter of the signals diffusion zone, during the bacterial growths in the presence of herbal extracts were evaluated through measuring the diameter of violacein production zone in (mm) by *C. violaceum* CV026 on the LB Agar

2.8. Statistical analysis

All experiments were performed in triplicates and statistical analyses were completed, using ANOVA to compare the differences between tests and controls using SPSS statistics 21.

3. Results

3.1. Antibacterial susceptibility screening and MICs

Before addressing the effects of Syzygium *aromaticum, Dionysia revolute* Boiss, and *Eucalyptus camaldulensis* extract on bacterial QS, their antimicrobial activities are listed in table 2. According to the results, all of the plant extracts from 50 to 0.39 mg/ml had antibacterial effects which was shown through inhibition of bacterial growth in comparison with negative (DMSO) and positive (Tetracycline) controls and these results were not significantly different ($P < 0.05$).

The MBC, MIC, and sub MIC concentration of these herbal extracts against *A. veronii* bv. Sobria BC88, *P. aeruginosa*, and *C. violaceum* are showed in (Table 3).

3.2. Inhibition of violacein production in *C. violaceum* CV026

According to the obtained results from 96 well-microplate titer, inhibition of violacein production was observed (OD585nm) following treatment with increasing sub-MIC concentration of herbal extracts in comparison with the controls (Fig 2). It is interesting to note that the growth of *C. violaceum* CV026 in the presence of plant extracts was not inhibited as the cell counts (OD600nm = 1), and showed no significant difference in comparison with the controls. Then, the violacein inhibition was compared with the control wells, and the analyzed data showed that *Eucalyptus camaldulensis* Dehnh at 0.78 & 0.39 mg/ml, *Syzygium aromaticum* at 0.39 & 0.195 mg/ml, and *Dionysia revolute* Boiss at 3.1 & 1.56 mg/ml concentration, have a maximum violacein inhibition (>50%) at the sub-MIC concentration (Fig 3). However, Sublethal minimal QS inhibitory concentration (MQSIC) through weak pigment inhibition was observed by *Dionysia revolute* Boiss at 0.39 mg/ml, *Eucalyptus camaldulensis Dehnh* at 0.097 mg/ml, and *Syzygium aromaticum* at 0.048 mg/ml, and no anti-QS activity was found at lower concentrations of plant extracts.

3.3. CV026 Agar assay

The sub-MIC concentration of each herbal extract, with maximum potential inhibition of violacein in 96 microplate results, showed an anti-QS effect on the LB agar plates. This QS-inhibitory effect was observed for *Eucalyptus camaldulensis* Dehnh at 0.78 mg/ml, *Syzygium aromaticum* at 0.39mg/ml, and *Dionysia revolute* Boiss at 3.1mg/ml with the formation of a

visible halo zone (growth without pigmentation) around the wells in comparison with the controls (DMSO) (Fig 4).

3.4. inhibition of QS signal molecules in *A. veronii* and *P. aeruginosa*

After evaluation of quorum-sensing inhibition in biosensor strain, we studied the anti-quorum sensing effect of herbal extracts on *A. veronii* bv. Sobria strain BC88 and *P. aeruginosa* isolated from a hospitalized patient with cystic fibrosis. According to results, *A. veronii* and *P. aeruginosa* populations produced and diffused QS signal molecules during the growth on the LB agar, and *C. violaceum* CV026 could produce violacein pigments in response to these exogenously provided signal molecules when they were cultured next to each other. Meanwhile in presence of a sub-MIC concentration of herbal extracts, *A. veronii*, and *P. aeruginosa* populations were constant (OD600=1) but signal diffusion was decreased in LB agar. These results showed by decreasing on the zone of violacein pigment productions in *C. violaceum* (Fig 5, 6). The results confirm the anti-quorum sensing activity of herbal extracts.

4. Discussion

Quorum sensing or bacterial talking, control the vital functions both in Gram-negative and-positive bacteria. Due to the clinical, environmental, and industrial application of the QS inhibitors, search for potential compounds with anti-QS activity has increased over the last few years [27]. Overall, the inhibition of QS pathways with natural and synthetic compounds is undoubtedly promising to combat multidrug-resistant bacteria, which makes pathogens more susceptible to host immune responses and antibiotics. Over the past few decades, it has become increasingly clear that herbal medicines are an abundant source of antibacterial and anti-QS compounds. In the Middle-Eastern countries, especially Iran, medicinal plants are used

extensively to treat many diseases [28, 29]. Even though *Syzygium aromaticum, Dionysia revolute* Boiss, and *Eucalyptus camaldulensis* have been used for a long time in Iran and different regions around the world for a wide range of medical treatment, and evidence show their antimicrobial activity and inhibiting growth of bacteria, the anti-quorum sensing properties remain unknown [18-22, 28-31]. In the present study, we investigated the anti-QS activity of this medicinal plant. In-vitro assays exhibited the potential of *Syzygium aromaticum, Dionysia revolute* Boiss, and *Eucalyptus camaldulensis* in inhibiting the production of QS-regulated virulence factors (violacein) in *C. violaceum* CV026. Furthermore, there are still other studies in Iran that reported the anti-QS effect of some herbal extracts, such as *Lepidium draba* and *Anethum graveolens, Raphanus sativus, Artemisia dracunculus, Althea officinalis,* which reportedly inhibit quorum sensing in *C. violaceum* CV026 [32, 33]. Hence, Mohabi et al. showed the activity of *Quercus infectoria* galls extract on virulence factor production and inhibition of quorum sensing (QS) in Pseudomonas aeruginosa [34]. Moreover, in other countries studies have shown the anti-QS and in-vitro anti-biofilm potential of *A. graveolens* against uropathogenic Serratia marcescens or pompia, and grapefruit essential oils potently inhibited biofilm formation, which could be used to control common polymicrobial infections [35, 36]. Since Quorum sensing disruption is an effective strategy to control infections caused by different bacteria that are pathogenic to plants, animals, and humans, it can provide new opportunities in a wide range of applications, such as therapeutic applications (humans and animals). For example, today's anti-QS drugs such as anti-QS based antiseptic ointments, mouthwash for oral infections, drops for ear infections have emerged. Similarly, functional foods such as plant products rich in compounds with anti-QS activity for controlling infections in immunocompromised patients [27]. The present study attempted to reconnoiter the anti-QS property of *Syzygium aromaticum,*

Dionysia revolute Boiss, and *Eucalyptus camaldulensis* against *C. violaceum* CV026, *A. veronii* bv. Sobria BC88 and *P. aeruginosa.* Further studies are necessary to identify the major compound responsible for anti-QS activity present in the tested plants. We hope that the present study can move from theoretical researchers to experimental.

5. Conclusions

In this study we conclude that some natural plants extracts, especially the *Syzygium aromaticum*, *Dionysia revolute* Boiss & *Eucalyptus camaldulensis* can be useful in the treatment of some kind of infections during the chronic disease by prevention and depletion of signaling communication between the bacterial community, and by controlling the bacterial population, leaving it to the immune system to eradicate the infection.

7. Conflicts of interest

the authors declare that there are no conflicts of interest regarding the publication of this article.

8. Funding

Shiraz University of Medical Sciences (**SUMS**) is highly acknowledged due to providing financial assistance to carry out this study with (grant number **94-01-01-10508** and **Ethics approval code: IR.SUMS.REC.1397.083**). This study is a part of the MSc thesis and performed in the Department of Bacteriology and Virology, School of Medicine, SUMS.

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10. Author contributions

NH Study concept and design, expanding, revision, and editing the article. FM Sample collection and laboratory examinations and interpretation of data and writing the first draft of the article. AB Critical revision of the manuscript.

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TABLES

Plant Species	***Dionysia revolute* Boiss.**	***Eucalyptus camaldulensis* Dehnh**	***Syzygium aromaticum***
***Voucher**	1093	780	1092
Plant Family	Primulaceae	Myrtaceae	Myrtaceae
Common Name	Stone Bride of Iran	Eucalyptus	Dianthus
Tested Part	Whole plant	Leaves	Stem
Weight (g)	88	60	90
Solvents (mixed)	n-hexane, methanol, 96%ethanol	n-hexane, methanol, 96%ethanol	n-hexane, methanol, 96%ethanol
Yield (%)	3.8%	11.5%	18.8%
Extract colors	brown	green	dark brown
Clinical Usage / Reference	Antimicrobial, anti-inflammatory, anti-cancer / [18,19]	Antibacterial, antifungal, Antioxidant / [20,22]	Antibacterial / [31]
Main Chemical components / Reference	2-Acetophenone, Benzaldehyde, 2- Acetyl phenol, β-Farnesyl alcohol, Eugenol, Rosifoliol, γ-Eudesmol, o-Hydroxychalcone & others / [42]	1,8-cineole, p-cymene, α-pinene, terpinen-4-ol, aromadendrin, α-terpineol / [43]	Eugenol, β-Caryophyllene, Vanillin, Crategolic acid, Kaempferol, Rhamnetin, Eugenitin, Ellagic acid, Gallic acid, Biflorin, Myricetin& others / [44]

Table 1. Information about the plant compound extracted in this study

*A voucher specimen was deposited in the Herbarium of Shiraz Faculty of Pharmacy, Shiraz, Iran

Plant concentration (mg/ml)	*k. pneumoniae*			*S. aureus*			*A. baumannii*			*S. marcescens*			*P. aeruginosa*		
	Eucalyptus camaldulensis	*Syzygium aromaticum*	*Dionysia revolute*	*Eucalyptus camaldulensis*	*Syzygium aromaticum*	*Dionysia revolute*	*Eucalyptus camaldulensis*	*Syzygium aromaticum*	*Dionysia revolute*	*Eucalyptus camaldulensis*	*Syzygium aromaticum*	*Dionysia revolute*	*Eucalyptus camaldulensis*	*Syzygium aromaticum*	*Dionysia revolute*
	Inhibition Zone (Means± SD (mm))														
50	18 ± 0.5	15 ± 0.7	15± 0.2	16± 0.1	14± 0.8	15± 0.1	15 ± 1.1	17.9± 0.7	14± 0.2	19± 1.2	17.1± 0.5	15± 1.1	14.5± 0.5	13.2± 0.6	13.5± 0.8
25	17.1± 0.3	14.3± 0.6	14.5 ± 0.3	15.2± 0.2	13.1± 0.5	14.1± 0.3	14.2± 1.4	17.1± 0.5	13.5± 0.3	18.6± 1.1	16.7± 0.5	14.2± 1.2	14 ± 0.6	12.5± 0.5	13.2± 0.5
12.5	16.2± 0.5	13 ± 0.4	13.4 ± 0.4	14.1± 0.3	12.4± 0.5	12.8± 0.1	13.2± 1.2	16.9± 0.2	12.6± 0.5	17.7± 1.3	16 ± 0.4	13.5± 1.3	13.2± 0.7	12.1± 0.5	12.8± 0.4
6.25	15.4± 0.6	12.1± 0.5	12.6 ± 0.4	13 ± 0.2	11.2± 0.6	11.9± 0.4	11.9± 1.5	15.3± 0.9	11.1± 0.4	17 ± 1.2	15.5± 0.9	12± 1.1	12.6± 0.3	11.6± 0.7	12.5± 0.5
3.12	14.5± 0.5	11.8± 0.3	10.9 ± 0.2	11.5± 0.2	10.5± 0.3	10.2± 0.2	10.6± 1.1	14.1± 0.3	10± 0.1	16.8± 1.1	14.4± 0.3	0	10.5± 0.5	11.1± 0.4	11.8± 0.6
1.56	13.2± 0.6	0	0	9.8 ± 0.3	9.6 ± 0.5	8.8± 0.3	9.5 ± 1.3	13.2± 0.5	8.4 ± 0.4	16.3± 1	13.1± 0.5	0	10 ± 0.7	10.5± 0.1	0
0.78	11.8± 0.6	0	0	7.5 ± 0.5	8.1 ± 0.3	5± 0.2	8.5 ± 1.4	11.8± 0.6	0	15.6± 1.1	0	0	0	9.7 ± 0.2	0
0.39	0	0	0	0	5.9 ± 0.4	0	0	10.1± 0.8	0	0	0	0	0	0	0
DMSO	0			0			0			0			0		
Tetracycline (30 µg)	22±0.2			29 ±0.3			18±0.5			19±0.3			14±0.2		

Table 2: Antibacterial effect of plant extracts on gram negative and gram-positive bacteria

Table 3: characteristics of plant extracts MICs *in-vitro*.

Plant Species	*C. violaceum* CV026			*P. aeruginosa*			*A.veronii* bv. Sobria BC88		
	MBC [a]	MIC [C]	Sub MIC [b]	MBC [a]	MIC [C]	Sub MIC [b]	MBC [a]	MIC [C]	Sub MIC [b]
D. revolute	25	6.25	3.1 to 0.39	6.25	1.56	0.78, 0.39	12.5	0.39	0.195, 0.097
E. camaldulensis	6.25	1.56	0.78 to 0.097	6.25	0.78	0.39, 0.195	3.1	0.195	0.097, 0.048
S. aromaticum	12.5	0.78	0.39 to 0.048	3.1	0.39	0.195, 0.97	6.25	0.97	0.048, 0.024

[a] MBC value: minimal bactericidal concentration (mg/ml)

[b] Sub-MIC value: sub minimal inhibitory concentration used for anti QS assay (mg/ml)

[c] MIC value: minimal inhibitory concentration (mg/ml)

The experiments were performed in triplicates and the results expressed as mean ±SD.

FIGURES

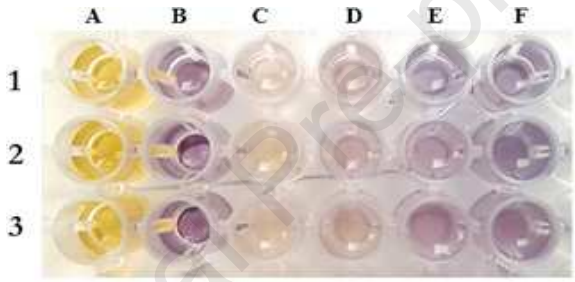

Fig 1. Violacein quantification assay in a 96 well-plate. (rows A1 to A3) *C. violaceum* CV026 culture without AHL and herbal extracts concentration (negative control), (rows B1 to B3) *C. violaceum* CV026 culture with AHL and without herbal extracts concentration as a positive control, (columns C1 to F1) *C. violaceum* CV026 culture with sub-MIC concentration of *Syzygium aromaticum* (0.39, 0.195, 0.097, 0.048mg/ml). (columns C2 to F2) *C. violaceum* CV026 culture with sub-MIC concentration of *Eucalyptus camaldulensis* (0.78, 0.39, 0.195, 0.097mg/ml). (columns C3 to F3*) C. violaceum* CV026 culture with sub-MIC concentration of *Dionysia revolute* Boiss (3.1, 1.56, 0.78, 0.39 mg/ml).

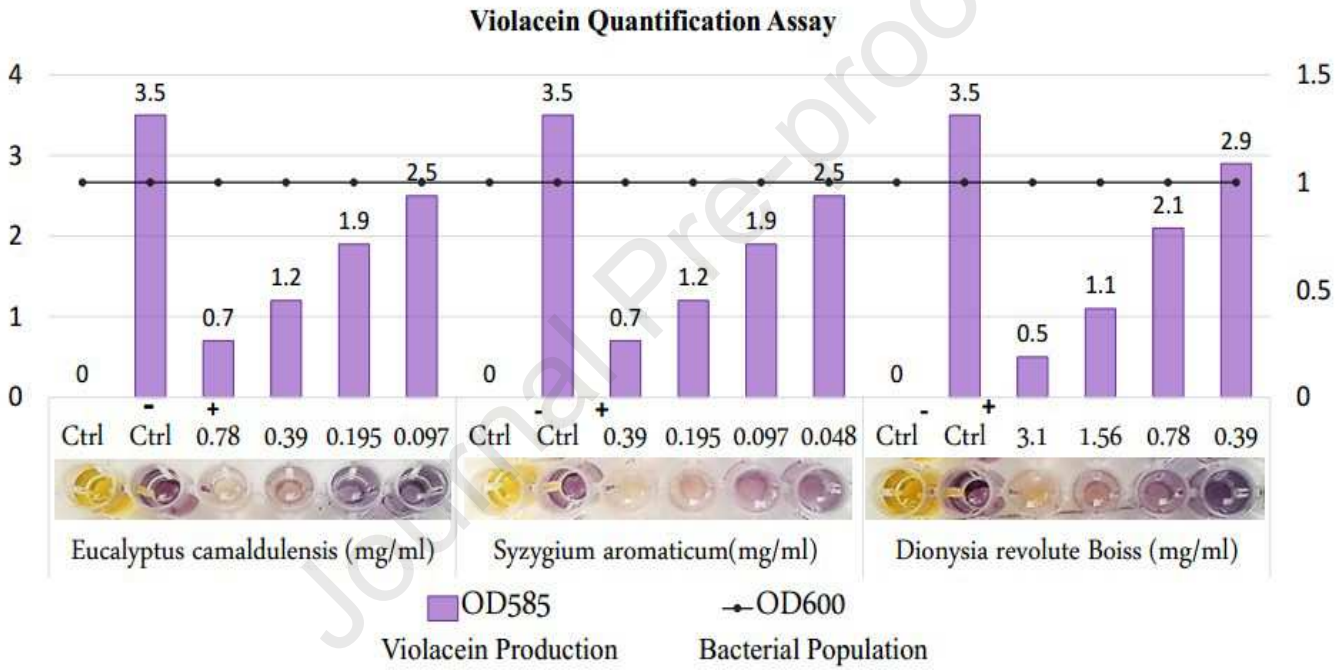

Fig 2. The result from Violacein quantification assay in a 96 well-plate. Bacterial population was stable in the presence of sub-MIC concentration of herbal extracts (OD600nm = 1); hence, the inhibition of Violacein production was observed following treatment by increasing sub-MIC concentration of herbal extracts in comparison with controls (OD585nm). The experiments were performed in triplicates and the results expressed as mean ±SD.

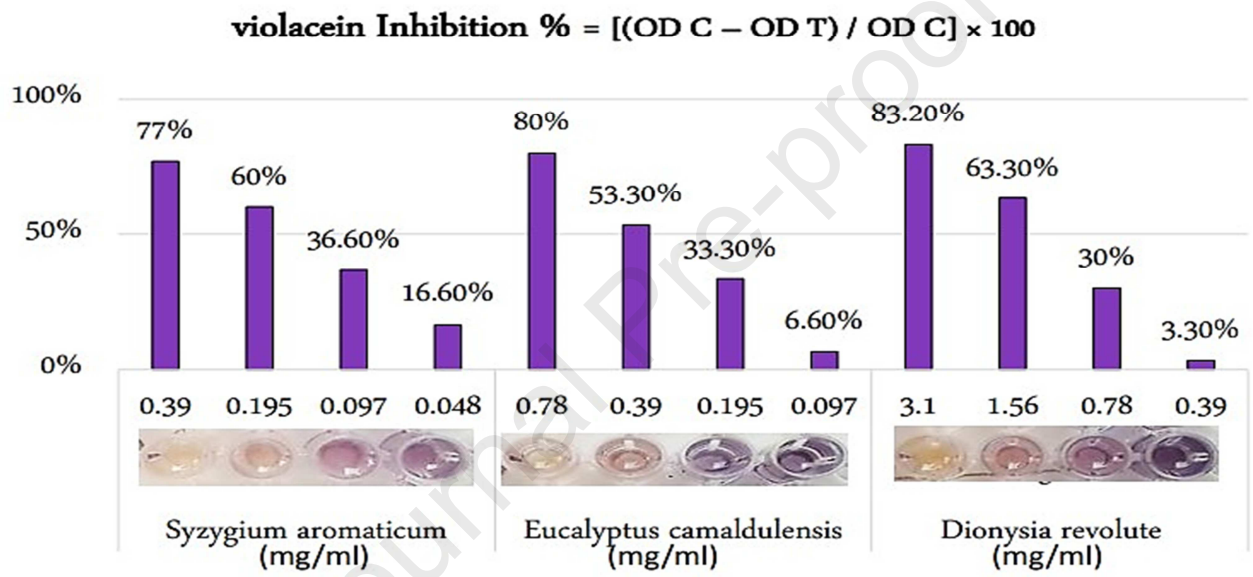

Fig 3. The results from the calculated of violacein inhibition percentage in a 96 well plate. OD C is OD585 for Violacein production in the control wells (without herbal extracts) and OD T is OD585 for Violacein production in the presence of herbal extracts. Analysis showed that *E. camaldulensis* in (0.78, 0.39 mg/ml), *S. aromaticum* in (0.39, 0.195 mg/ml), *D. revolute* (3.1, 1.56 mg/ml), showed maximum reduced Violacein production in *C. violaceum* CV026. The statistical significance of each test (n = 4) was evaluated by conducting a one-way ANOVA test and $P < 0.05$ as significant.

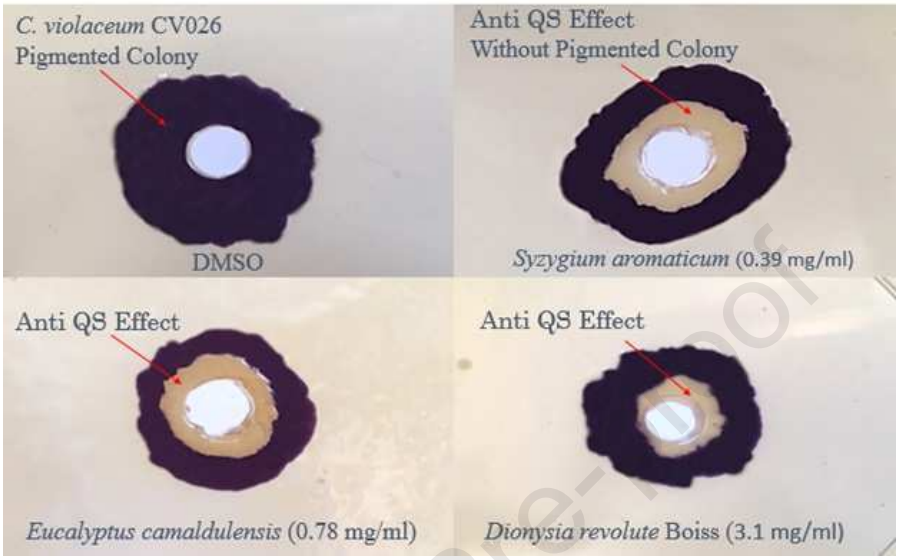

Fig 4. The results from QS inhibition assay on LB agar plates. Anti QS effect of *Syzygium aromaticum* (0.39 mg/ml), *Dionysia revolute* Boiss (3.1 mg/ml) and *Eucalyptus camaldulensis* (0.78 mg/ml) showed by without pigmented colony. DMSO served as the negative control and without anti QS effect.

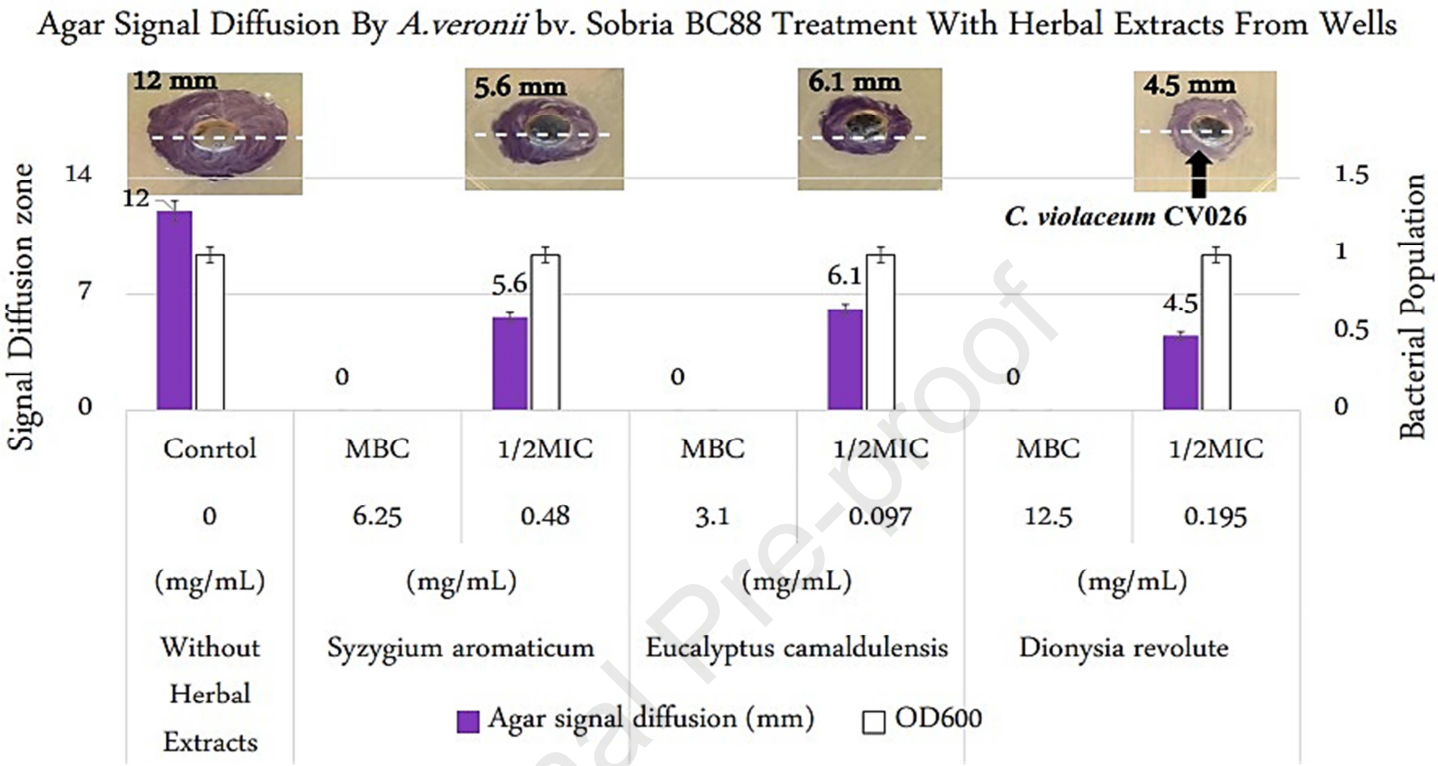

Fig 5. The results from anti QS activity through signal inhibition in *A. veronii* bv. Sobria BC88. In control well, exogenously QS signals produce and diffused through *A. veronii* and these molecules were tracked with purple pigments produced by *C. violaceum* CV026 in LB agar. In MBC concentration, *A. veronii* was killed and could not diffused signals but in presence of sub-MIC concentration of herbal extracts, bacterial population were fixed (OD600nm=1) and QS signals diffusion zone were decreased in compare with control (Means ± SD, $P < 0.05$).

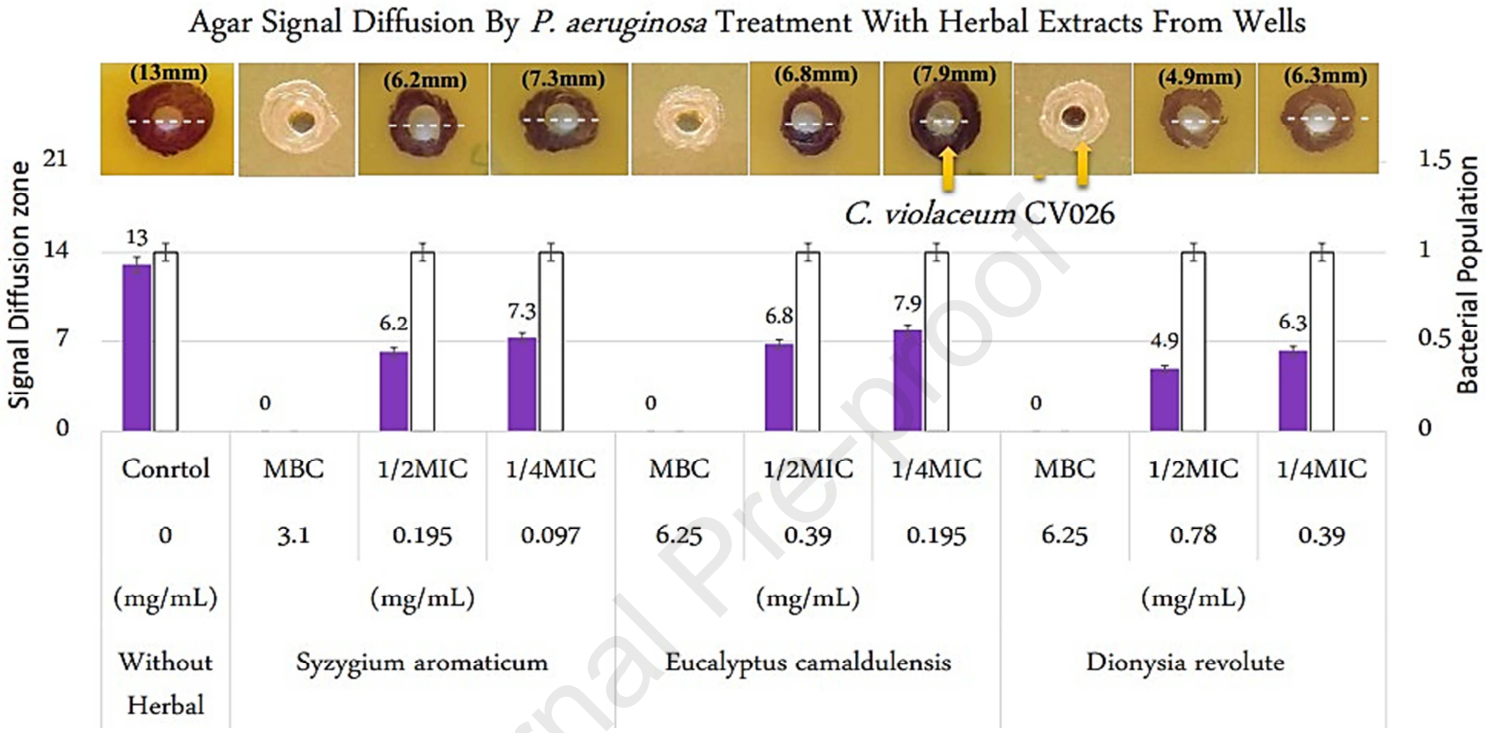

Fig 6. The results from anti QS activity through signal inhibition in *P. aeruginosa*. In control well, exogenously QS signals produce and diffused through *P. aeruginosa* and these molecules were tracked with purple pigments which produced by *C. violaceum* CV026 in LB agar. In MBC concentration, *P. aeruginosa* was killed and could not diffused signals but in presence of sub-MIC concentration of herbal extracts, bacterial population were fixed (OD600nm=1) and QS signals diffusion zone were decreased in compare with control (Means ± SD, $P < 0.05$).