ORIGINAL ARTICLE

Evaluation of quorum-sensing inhibitory effects of extracts of three traditional medicine plants with known antibacterial properties

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Abstract

Today an alternative approach to control bacterial infections is the use 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 revoluta* Boiss. and *Eucalyptus camaldulensis* Dehnh. were evaluated. These plants are local to the Middle East region and have since ancient times been used for their antibacterial activity. Plant compounds were extracted with *n*-hexane, methanol and 96% ethanol mixed solvent. Antibacterial activity of this herbal extracts against five Gram-negative and Gram-positive bacteria were assessed. The effective sub−minimum inhibitory concentration (MIC) of this extract on bacterial QS systems were investigated by a violacein quantification assay in the *Chromobacterium violaceum* CV026 biosensor strain, inhibition of exogenously QS signal molecules in *Aeromonas veronii* bv. Sobria strain BC88 and *Pseudomonas aeruginosa* isolated from a patient with cystic fibrosis *in vitro*. Results found that *Syzygium aromaticum* 0.39 to 0.048 mg/mL, *Dionysia revoluta* Boiss. 3.1 to 0.39 mg/mL and *E. camaldulensis* 0.78 to 0.097 mg/mL showed anti-QS activities by reducing the violacein formation depletion of QS signals produced in *A. veronii* and *P. aeruginosa* at sub-MICs. Regarding the anti-QS effects of these herbal extracts, their effective components may be candidates for use in combating bacterial infections at sub-MICs.

Keywords: Anti−quorum sensing, *Dionysia revoluta*, *Eucalyptus camaldulensis*, plant extracts, *Syzygium aromaticum*
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Introduction

Controlling bacterial infections has recently become difficult because of the excessive use of antibiotics in human medicine; such therapy is considered to be a major factor in widespread bacterial resistance, and the formation of biofilm greatly 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 of, diffusing and/or destroying of QS signals, or by inhibiting their receptors by imitating signal structures [4]. In bacterial populations, QS regulates biological behaviour including biofilm formation, bioluminescence and enzyme 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 in cases of low-density bacteria [7,8]. Many eukaryotes, fungi, medicinal plants and fruits and vegetables secrete compounds which 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 use of natural antimicrobial agents such as natural plant compounds. Medicinal plant extracts can play an important role in combating bacterial infection by producing a diverse antimicrobial compound, such as phenolic, terpenoids, flavanones and quinones, as a result of the similarity of their chemical structure to those of QS signals (acyl-

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homoserine lactone, AHL) and their ability to degrade signal receptors [4,14,15]. Antimicrobial activities of herbal medicine against some bacterial species have been reported, especially from extracts and fractions of *Syzygium aromaticum*, *Dionysia revoluta* and *Eucalyptus camaldulensis* [16–19]. Furthermore, some researchers have reported antifungal, anti-inflammatory, anticancer and antioxidant effects of these herbal medicines [18,20–22].

We evaluated the antibacterial effects and the role of sub–minimum inhibitory concentrations (MICs) 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* isolated from a hospitalized patient as clinical samples.

Materials and methods

Collection of plant material and extract preparation

The study was approved by the Shiraz University of Medical Sciences (SUMS) ethics committee (approval IR.SUMS.REC.1397.083). Plant materials were obtained from a local market in Shiraz, Iran. A voucher specimen and the scientific identity of the plant compounds were confirmed at the SUMS herbarium department. A voucher specimen was deposited in the herbarium of the Shiraz Faculty of Pharmacy, Shiraz, Iran. The plant samples were washed twice with distilled water and dried in the dark 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 following formula (Table 1):

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.

Bacterial strain and culture conditions

For the antibacterial assays, five clinical isolates—*Klebsiella pneumoniae, Staphylococcus aureus, Acinetobacter baumannii, Serratia marcescens* and *Pseudomonas aeruginosa* (isolated from a patient with cystic fibrosis)—were provided from the clinical bacteriology laboratory of Shahid Faghihi Hospital, Shiraz, Iran. *Aeromonas veronii* bv. Sobria BC88, *Chromobacterium violaceum* CV026 (mini-Tn5 mutant of the wild-type strain; NCTC 13278), −80°C stock in the Bacteriology and Virology Department of SUMS, were used for the QS inhibition assay. We used C_6-AHL (*N*-hexanoyl-L-homoserine lactone) in this study as a signal molecule (56395-10MG; Sigma-Aldrich, St Louis, MO, USA). *C. violaceum* CV026 was cultured in Luria-Bertani (LB) 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 at 28°C.

Antibacterial properties

In this study, we used the disc-diffusion method based on Murray et al. [27] to determine the antibacterial activity of herbal extracts on bacterial strains. Herbal extracts were dissolved in DMSO (50–0.39 mg/mL), and Müller-Hinton agar plates (Merck, Darmstadt, Germany) were uniformly inoculated with 1 mL of each bacterial suspension (10^8 CFU/mL). Therefore, herbal extract dilutions (50 μL) were placed onto sterile paper discs (6 mm), set on the bacterial culture plate and incubated at 37°C for 48 hours. Tetracycline (30 μg) and DMSO were used as positive and negative controls respectively. The antibacterial activity of herbal extracts was determined by measuring the diameter of the inhibition zone in millimetres.

TABLE 1. Plant compounds extracted

Characteristic	Species		
	***Dionysia revoluta* Boiss.**	***Eucalyptus camaldulensis* Dehnh.**	**Syzygium aromaticum**
Voucher[a]	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 colours	Brown	Green	Dark brown
Clinical use [reference]	Antimicrobial, anti-inflammatory, anticancer [18,19]	Antibacterial, antifungal, antioxidant [20,22]	Antibacterial [23,39]
Main chemical components [reference]	2-Acetophenone, benzaldehyde, 2- acetyl phenol, β-farnesyl alcohol, eugenol, rosifoliol, γ-eudesmol, o-hydroxychalcone, other [24]	1,8-Cineole, *p*-cymene, α-pinene, terpinen-4-ol, aromadendrin, α-terpineol [25]	Eugenol, β-caryophyllene, vanillin, crategolic acid, kaempferol, rhamnetin, eugenitin, ellagic acid, gallic acid, biflorin, myricetin, other [26]

[a]Voucher specimen was deposited in herbarium of Shiraz Faculty of Pharmacy, Shiraz, Iran.

Minimum inhibitory concentrations

Before the anti-QS assay, The minimal bactericidal concentration (MBC), MIC and sub-MIC values of herbal extracts were determined using serial macrodilution assays in control and test tubes according to 2015 Clinical and Laboratory Standards Institute guidelines [28]. 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 twofold (control and test tube) to 0.048 mg/mL. Each test tube contained 0.5 mL of each concentration and was inoculated with equal volumes of the microbial suspension (10^6 CFU/mL). The control tubes contained herbal extract concentration and broth medium without bacterial inoculum; this was done to eliminate plant extracts and broth media turbidity so we could more easily follow bacterial growth in the test tube during the spectrophotometric assay at 600 nm. An extract-free tube containing a microbial suspension was used as a positive control. All of the tubes were incubated at 37°C for 24 hours. The tubes without bacterial growth showed bactericidal effects; the lowest concentration of plant extracts that could at least inhibit 50% of the microorganism's growth was considered to be the MIC, which was calculated as follows [29]:

Inhibition % = [(OD C − OD T)/OD C] × 100,where OD C is OD_{600nm} to track bacterial growth in the positive control tube and OD T is OD_{600nm} 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 3 mm Müller-Hinton agar and incubated overnight at 37°C.

Violacein quantification assay

To explore the anti-QS potential of sub-MICs 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 was prepared of *C. violaceum* CV026 diluted 1:50 in 4 mL of fresh LB broth, supplemented with 0.25 μg/mL C_6-AHL and different sub-MICs 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, OD_{600nm} = 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 at 585 and 600 nm for violacein production and bacterial population growth respectively with a Polar Star Omega Microplate reader (BMG Labtech, Ortenberg, Germany) (Fig. 1). The experiment was repeated in triplicate, and the percentage of violacein pigment inhibition

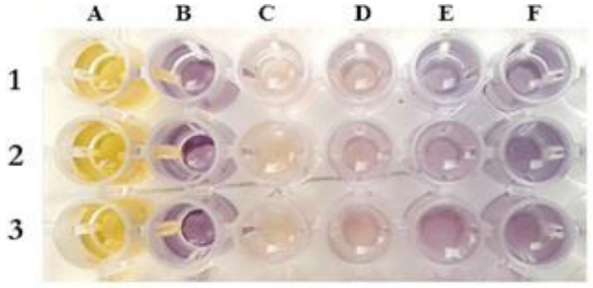

FIG. 1. Violacein quantification assay in 96-well plate. (A1–A3) *Chromobacterium violaceum* CV026 culture without AHL and herbal extract concentration (negative control). (B1–B3) *C. violaceum* CV026 culture with AHL and without herbal extract concentration as positive control. (C1–F1) *C. violaceum* CV026 culture with sub−minimum inhibitory concentration (MIC) of *Syzygium aromaticum* (0.39, 0.195, 0.097, 0.048 mg/mL). (C2–F2) *C. violaceum* CV026 culture with sub-MIC of *Eucalyptus camaldulensis* Dehnh. (0.78, 0.39, 0.195, 0.097 mg/mL). (C3–F3) *C. violaceum* CV026 culture with sub-MIC of *Dionysia revoluta* Boiss. (3.1, 1.56, 0.78, 0.39 mg/mL). AHL, acyl-homoserine lactone.

compared to control wells was calculated using the following equation [30,31]:

Inhibition (%) = [(OD_{585nm} C − OD_{585nm} T) / OD_{585nm} C] × 100where OD C is OD_{585nm} for violacein production in control wells (without herbal extracts) and OD T is OD_{585nm} 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 comparing the bacterial population in all of the wells with the controls (without herbal extracts) at OD_{600nm} = 1. The constant of bacterial population in all wells indicated that the inhibition of violacein production was due to anti-QS effects.

C. violaceum CV026 assay

For *C. violaceum* CV026 assay, an overnight culture of CV026 was diluted to OD_{600nm} = 1, and 200 μL was spread circularly in the middle of LB agar plates that were supplemented with C_6-AHL (0.25 μg/mL). Wells were punched in the centre of the culture plate after incubating it for 30 minutes. The sub-MIC of plant extracts (50 μL) with maximum anti-QS effects was obtained from the previous experiment 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 by inhibiting violacein production around the well. When the diffused molecules of plant extracts became too diluted, the purple colour reappeared, which could be observed as an outer purple circle [32].

Anti-QS activity through QS signal inhibition

Because QS is a vital system to regulate *Aeromonas* enterotoxin and *Pseudomonas* virulence factors, and because it contributes to biofilm formation in both [33–35], *in vitro* screening for the anti-QS activity of herbal extracts was performed against *A. veronii* bv. Sobria strain BC88 and *P. aeruginosa* isolated from a hospitalized patient. The *C. violaceum* CV026 biosensor strain violacein gene is also under the control of QS. This strain has a mutation in the AHL-producing gene, which results in a lack of pigment production without the presence of an external AHL signal. In this trial, *Aeromonas* and *Pseudomonas* produce and diffuse the same AHL QS signals in the LB agar plate which are compatible with the *C. violaceum* CV026 biosensor. These signals could then be taken by the latter bacterium, letting it produce purple violacein pigment. We hypothesize that the antibacterial activity of herbal extracts can be due to their interference with the QS system of certain bacteria, blocking bacterial cross-talk. We thus expected that 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, whereas without the extracts, the purple pigment will be clearly detectable.

We therefore first determined the MIC and sub-MIC values of the herbal extracts for these bacteria. A total of 200 µL of an overnight culture of CV026 was diluted to OD_{600nm} = 1 and then spread uniformly on the LB agar. Wells were punched in the centres of plates. Then 90 µL of each *P. aeruginosa* and *A. veronii* suspension (OD_{600nm} = 1) separately plus a sub-MIC of herbal extracts (10 µL) were 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 hours at 28°C, and we evaluated the diameter of the signal diffusion zone during bacterial growth in the presence of herbal extracts by measuring the diameter of the violacein production zone by *C. violaceum* CV026 on LB agar.

Statistical analysis

All experiments were performed in triplicate. Statistical analyses were performed using ANOVA to compare the differences between tests and controls using SPSS Statistics 21 software (IBM, Armonk, NY, USA).

Results

Antibacterial susceptibility screening and MICs

The antimicrobial effects of *Syzygium aromaticum*, *Dionysia revoluta* Boiss. and *Eucalyptus camaldulensis* extract on bacterial QS are listed in Table 2. According to the results, all of the plant

TABLE 2. Antibacterial effect of plant extracts on Gram-negative and Gram-positive bacteria

Characteristic	*Klebsiella pneumoniae*			*Staphylococcus aureus*			*Acinetobacter baumannii*			*Serratia marcescens*			*Pseudomonas aeruginosa*		
	EC	SA	DR	EC	SA	DR	EC	SA	DR	EC	SA	DR	EC	SA	DR
Plant concentration															
50 mg/mL	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 mg/mL	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 mg/mL	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 mg/mL	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 mg/mL	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 mg/mL	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 mg/mL	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 mg/mL	0	0	0	0	5.9 ± 0.4	0	0	10.1 ± 0.8	0	0	0	0	0	0	0
DMSO (negative control)	0			0			0			0			0		
Tetracycline (30 µg) (positive control)	22 ± 0.2			29 ± 0.3			18 ± 0.5			19 ± 0.3			14 ± 0.2		

Plant extract genus and species abbreviations are as follows: EC, *Eucalyptus camaldulensis* Dehnh.; SA, *Syzygium aromaticum*; DR, *Dionysia revoluta* Boiss. Data are shown as mean ± SD (mm) inhibition zone.
DMSO, dimethyl sulfoxide.

extracts from 50 to 0.39 mg/mL had antibacterial effects, as shown through inhibition of bacterial growth compared to negative (DMSO) and positive (tetracycline) controls; these results were not significantly different ($p < 0.05$). The MBC, MIC and sub-MIC of these herbal extracts against *A. veronii* bv. Sobria BC88, *P. aeruginosa* and *C. violaceum* are shown in Table 3.

Inhibition of violacein production in *C. violaceum* CV026

According to the obtained results from 96-well microplate titres, inhibition of violacein production was observed (OD_{585nm}) after treatment with increasing sub-MICs of herbal extracts compared to control (Fig. 2). Interestingly, the growth of *C. violaceum* CV026 in the presence of plant extracts was not inhibited in the cell counts ($OD_{600nm} = 1$) and showed no significant difference compared to control. Violacein inhibition was then compared to the control wells. The analysed data showed that *Eucalyptus camaldulensis* Dehnh. at 0.78 and 0.39 mg/mL, *Syzygium aromaticum* at 0.39 and 0.195 mg/mL and *Dionysia revoluta* Boiss. at 3.1 and 1.56 mg/mL concentration have a maximum violacein inhibition (>50%) at sub-MICs (Fig. 3). However, sublethal minimal QS inhibitory concentration through weak pigment inhibition was observed by *Dionysia revoluta* 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.

CV026 agar assay

The sub-MIC of each herbal extract, with maximum potential inhibition of violacein in 96-well microplate results, showed an anti-QS effect on LB agar plates. This QS-inhibitory effect was observed for *Eucalyptus camaldulensis* Dehnh. at 0.78 mg/mL, *Syzygium aromaticum* at 0.39 mg/mL and *Dionysia revoluta* Boiss. at 3.1 mg/mL with the formation of a visible halo zone (growth without pigmentation) around the wells compared to the DMSO control (Fig. 4).

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

After evaluation of QS inhibition in a biosensor strain, we studied the anti-QS effect of herbal extracts on *A. veronii* bv. Sobria strain BC88 and *P. aeruginosa* isolated from a hospitalized patient with cystic fibrosis. Results indicated that *A. veronii* and *P. aeruginosa* populations produced and diffused QS signal molecules during growth on 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 the presence of sub-MICs of herbal extracts, *A. veronii* and *P. aeruginosa* populations were constant ($OD_{600nm} = 1$) but signal diffusion was decreased in LB agar. These results were demonstrated by a decrease in the zone of violacein pigment productions in *C. violaceum* (Figs. 5 and 6). The results confirm the anti-QS activity of the herbal extracts we assessed.

Discussion

QS, or bacterial talking, controls vital functions in both Gram-negative and-positive bacteria. As a result of the clinical, environmental and industrial applications of QS inhibitors, the search for potential compounds with anti-QS activity has recently intensified [36]. Overall, the inhibition of QS pathways with natural and synthetic compounds is undoubtedly a promising path to combating 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 a promising and abundant source of antibacterial and anti-QS compounds. In Middle Eastern countries, especially Iran, medicinal plants are used extensively to treat many diseases [37,38]. Even though *Syzygium aromaticum*, *Dionysia revoluta* Boiss. and *Eucalyptus camaldulensis* Dehnh. have been used for a long time in Iran and elsewhere around the world for a wide range of medical treatment, and even though evidence has demonstrated their antimicrobial activity and their ability to inhibit bacteria growth, the nature of their anti-QS properties remains unknown [18–23,37,38].

In the present study, we investigated the anti-QS activity of several medicinal plants. *In vitro* assays demonstrated the potential of *Syzygium aromaticum*, *Dionysia revoluta* Boiss. and

TABLE 3. Characteristics of plant extract MICs *in vitro*

	Chromobacterium violaceum CV026			*Pseudomonas aeruginosa*			*Aeromonas veronii* bv. Sobria BC88		
Plant species	**MBC**	**MIC**	**Sub-MIC**	**MBC**	**MIC**	**Sub-MIC**	**MBC**	**MIC**	**Sub-MIC**
Dionysia revoluta Boiss.	25	6.25	3.1 to 0.39	6.25	1.56	0.78, 0.39	12.5	0.39	0.195, 0.097
Eucalyptus camaldulensis Dehnh.	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
Syzygium 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

MBC, minimal bactericidal concentration (mg/mL); MIC, minimum inhibitory concentration (mg/mL); sub-MIC, sub–minimum inhibitory concentration used for anti–quorum-sensing assay (mg/mL).

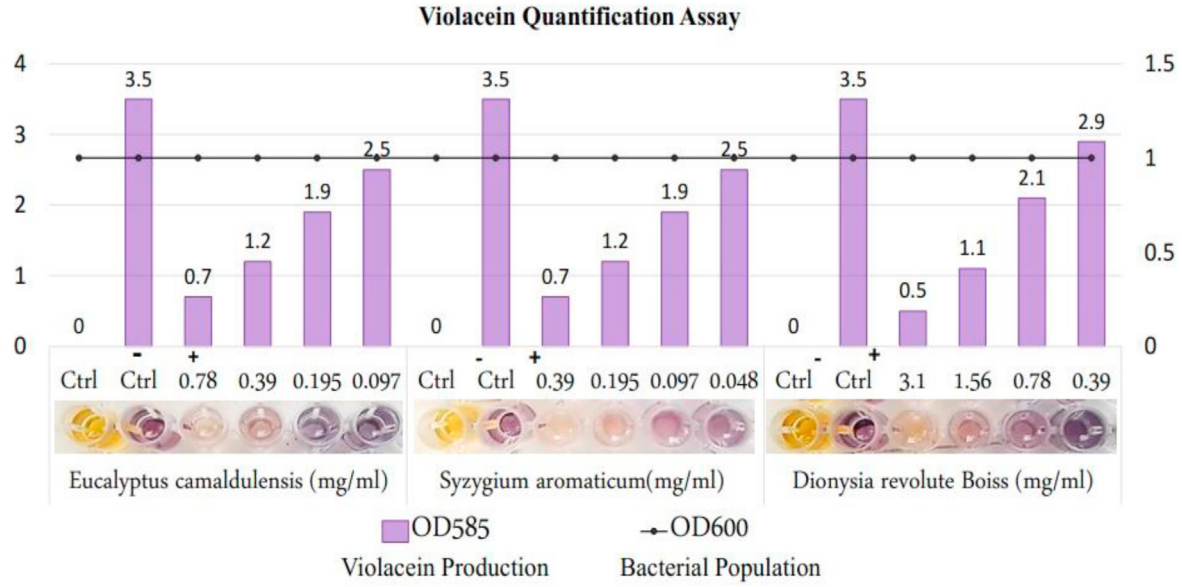

FIG. 2. Results of violacein quantification assay in 96-well plate. Bacterial population was stable in presence of sub−minimum inhibitory concentration (MIC) of herbal extracts (OD_{600nm} = 1); inhibition of violacein production was thus observed after treatment by increasing sub-MIC of herbal extracts compared to controls (OD_{585nm}). Experiments were performed in triplicate and results expressed as mean ± SD.

Eucalyptus camaldulensis Dehnh. in inhibiting the production of QS-regulated virulence factors (violacein) in *C. violaceum* CV026. Furthermore, other studies in Iran have reported the anti-QS effects of some herbal extracts, such as *Lepidium draba, Anethum graveolens, Raphanus sativus, Artemisia dracunculus* and *Althea officinalis*, which reportedly inhibit QS in *C. violaceum* CV026 [40,41]. Hence, Mohabi et al. [42] showed the activity of *Quercus infectoria* gall extract on virulence factor production and inhibition of QS in *Pseudomonas aeruginosa*. Moreover, in other countries, studies have shown that the anti-QS and *in vitro* antibiofilm potentials 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 [43,44]. Because QS 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, including therapeutic applications for both humans

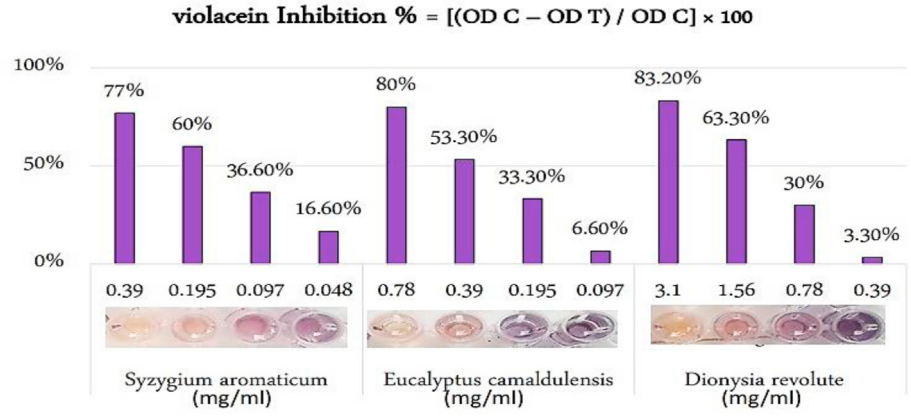

FIG. 3. Results from calculated violacein inhibition percentage in 96-well plate. OD C is OD_{585nm} for violacein production in control wells (without herbal extracts), and OD T is OD_{585nm} for violacein production in presence of herbal extracts. Analysis showed that *Eucalyptus camaldulensis* Dehnh. in 0.78, 0.39 mg/mL, *S. aromaticum* in 0.39, 0.195 mg/mL and *Dionysia revoluta* Boiss. in 3.1, 1.56 mg/mL showed maximum reduced violacein production in *Chromobacterium violaceum* CV026. Statistical significance of each test (n = 4) was evaluated by conducting one-way ANOVA, with $p < 0.05$ considered statistically significant.

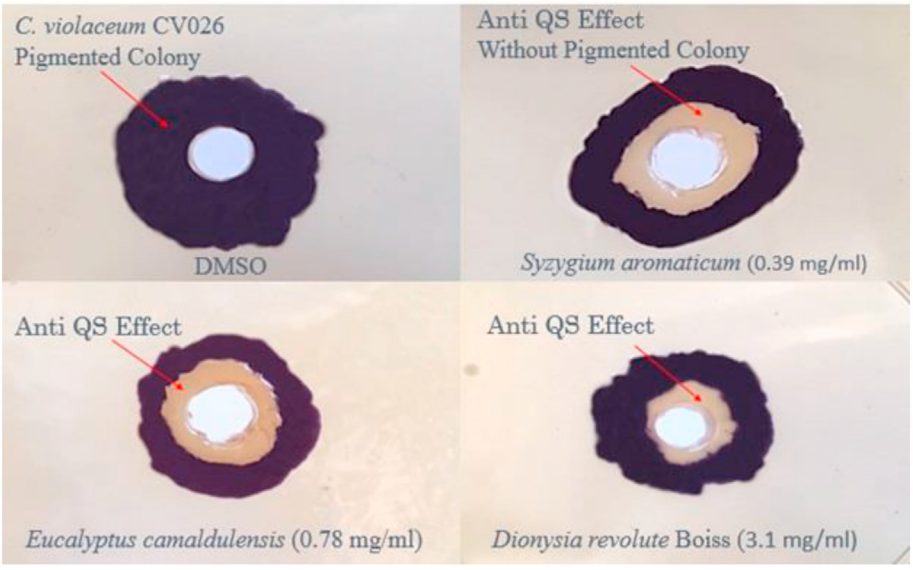

FIG. 4. Results from quorum-sensing (QS) inhibition assay on Luria-Bertani agar plates. Anti-QS effect of *Syzygium aromaticum* (0.39 mg/mL), *Dionysia revoluta* Boiss. (3.1 mg/mL) and *Eucalyptus camaldulensis* Dehnh. (0.78 mg/mL) shown without pigmented colony. Dimethyl sulfoxide (DMSO) served as negative control and had no anti-QS effect.

and animals. For example, today, anti-QS drugs, such as anti-QS–based antiseptic ointments, mouthwash for oral infections and drops for ear infections, have emerged. Similarly, functional foods such as plant products are rich in compounds with anti-QS activity for controlling infections in immunocompromised patients [36].

The present study assessed the anti-QS properties of *Syzygium aromaticum*, *Dionysia revoluta* Boiss. and *Eucalyptus camaldulensis* Dehnh. against *C. violaceum* CV026, *A. veronii* bv. Sobria BC88 and *P. aeruginosa*. Further studies are necessary to identify the major compounds responsible for the anti-QS activity present in the tested plants. We hope that the

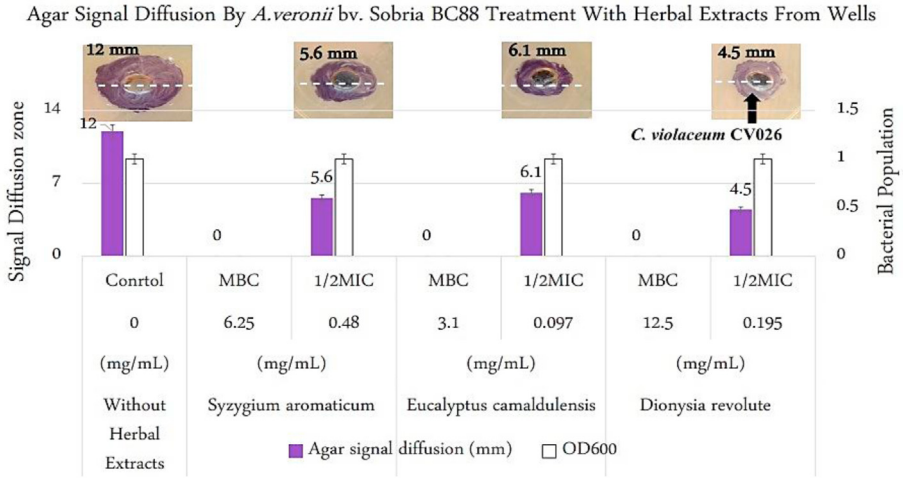

FIG. 5. Results from anti–quorum-sensing (QS) activity through signal inhibition in *Aeromonas veronii* bv. Sobria BC88. In control well, exogenous QS signals were produced and diffused through *A. veronii;* these molecules were tracked with purple pigments produced by *Chromobacterium violaceum* CV026 in Luria-Bertani agar. At minimal bactericidal concentration, *A. veronii* was killed and could not diffuse signals, but in presence of sub–minimum inhibitory concentration (MIC) of herbal extracts, bacterial populations were fixed (OD_{600nm} = 1), and QS signal diffusion zone was decreased compared to control (mean ± SD, $p < 0.05$).

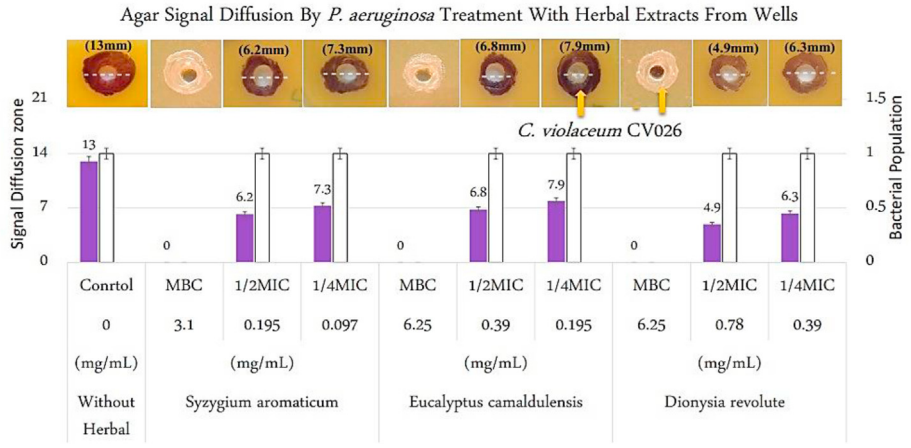

FIG. 6. Results from anti–quorum-sensing (QS) activity through signal inhibition in *Pseudomonas aeruginosa*. In control well, exogenously QS signals are produced and diffused through *P. aeruginosa;* these molecules were tracked with purple pigments produced by *Chromobacterium violaceum* CV026 in Luria-Bertani agar. In minimal bactericidal concentrations, *P. aeruginosa* was killed and could not diffuse signals, but in presence of sub–minimum inhibitory concentration (MIC) of herbal extracts, bacterial populations were fixed (OD_{600nm} = 1), and QS signal diffusion zone was decreased compared to control (mean ± SD, $p < 0.05$).

present study can move research from the theoretical to the experimental.

Conclusions

Some natural plant extracts, especially *Syzygium aromaticum*, *Dionysia revoluta* Boiss. and *Eucalyptus camaldulensis* Dehnh., can be useful in the treatment of some kinds of infections during chronic disease by preventing and depleting the signaling communication between members of the bacterial community and by controlling the bacterial population, thus permitting the immune system to eradicate the infection.

Conflict of interest

None declared.

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