



Original Article

Asian Pacific Journal of Tropical Biomedicine

journal homepage: www.apjtb.org



doi: 10.4103/2221–1691.303605

Impact Factor: 1.90

Corchorus olitorius aqueous extract attenuates quorum sensing–regulated virulence factor production and biofilm formation

Hanan M. Al–Yousef¹✉, Perwez Alam¹✉, Zakia Khanam², Musarat Amina¹, Wafaa H. B. Hassan³

¹Department of Pharmacognosy, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia

²University Malaysia Kelantan, Campus Jeli, 17600, Kelantan, Malaysia

³Department of Pharmacognosy, Faculty of Pharmacy, Zagazig University, 44519 Zagazig, Egypt

ABSTRACT

Objective: To investigate the effect of *Corchorus olitorius* aqueous fraction (COAF) on quorum sensing (QS)-regulated virulence factors and biofilm formation in *Pseudomonas aeruginosa* (PAO1).

Methods: The preliminary screening of the anti-QS effect of COAF was performed by evaluating the anti-pathogenic activity against *Chromobacterium violaceum* CV026 biosensor strain. Next, the inhibitory effects of COAF on QS-regulated pyocyanin production, proteolytic and elastolytic activities, swarming motility, and biofilm formation were evaluated in PAO1.

Results: The results showed that the treatment of COAF significantly decreased the biofilm biomass, attenuated virulence factors, and inhibited swarming motility of PAO1 without affecting the growth of the bacteria in a dose-dependent manner. COAF at 2000 µg/mL significantly decreased Las B elastase activity in PAO1 culture, exopolysaccharide production, swarming motility, pyocyanin level, and biomass of PAO1 by 55% ($P<0.05$), 60% ($P<0.01$), 61% ($P<0.01$), 65% ($P<0.01$) and 73% ($P<0.01$), respectively. In addition, the production of violacein was decreased by 62% ($P<0.01$) with the treatment of a high dose of COAF.

Conclusions: These findings indicate that COAF can be a potential source of anti-QS agents.

KEYWORDS: *Corchorus olitorius* L; Anti-quorum sensing activity; *Pseudomonas aeruginosa*; *Chromobacterium violaceum*; Virulence factors

1. Introduction

Corchorus olitorius (*C. olitorius*) L. (Family: Malvaceae), which is commonly known as “Saluyot,” is an edible leafy vegetable plant that

is distributed in the tropical and subtropical regions of Asia and Africa. *C. olitorius* is considered as a valuable food and a major source of fiber[1]. In English, *C. olitorius* is named as Jew’s mallow and Egyptian spinach as *C. olitorius* is a staple food among the Jewish and Egyptian communities since ancient times[2]. The leaves of *C. olitorius* are a rich source of iron, vitamins A, C, and E, calcium, protein, riboflavin, folate, niacin, thiamin, and dietary fibers[3]. Various studies have reported the pharmacological properties of *C. olitorius*, including anti-microbial, anti-fungal[4], anti-oxidant[5], anti-tumor[6], anti-inflammatory, analgesic[7], and anti-pathogenic properties. Additionally, *C. olitorius* can be used to treat gestational diabetes in pregnant women[8]. *Pseudomonas aeruginosa* (*P. aeruginosa*) is associated with many infectious diseases, such as pneumonia, bacteremia, endocarditis, meningitis, brain abscess, otitis media, keratitis, osteomyelitis, and diarrhea[9]. There are ongoing research efforts to develop strategies to control bacterial infection through anti-pathogenic agents, which exert anti-bacterial effects by inhibiting quorum sensing (QS), a bacterial communication process. The quorum communication system regulates the *Pseudomonas* virulence factors, such as protease, elastase, pyocyanin, alginate, biofilm formation, bacterial motility, and

✉To whom correspondence may be addressed. E-mail: aperwez@ksu.edu.sa (Perwez Alam), halyousef@ksu.edu.sa (Hanan M. Al-Yousef).

This is an open access journal, and articles are distributed under the terms of the Creative Commons Attribution-Non Commercial-ShareAlike 4.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.

For reprints contact: reprints@medknow.com

©2021 Asian Pacific Journal of Tropical Biomedicine Produced by Wolters Kluwer-Medknow. All rights reserved.

How to cite this article: Al-Yousef HM, Alam P, Khanam Z, Amina M, Hassan WHB. *Corchorus olitorius* aqueous extract attenuates quorum sensing-regulated virulence factor production and biofilm formation. Asian Pac J Trop Biomed 2021; 11(2): 66–73.

Article history: Received 27 February 2020; Revision 20 March 2020; Accepted 3 June 2020; Available online 29 December 2020

toxins[10]. QS in *P. aeruginosa* is regulated by *N*-acylated homoserine lactones (AHLs), which are signaling molecules. The concentration of these auto-inducers increases with the increase in bacterial population. These signaling molecules regulate bacterial pathogenicity[11]. Therefore, the inhibition of QS can be an alternative strategy to manage bacterial virulence and anti-microbial resistance[12]. This study aimed to investigate the quorum sensing inhibitory (QSI) effect of *C. olitorius* L. stem aqueous fraction (COAF) using the reporter *Chromobacterium violaceum* (*C. violaceum*). Additionally, the anti-pathogenic potential of COAF, which exhibited QSI activity, against the virulence factors of *P. aeruginosa* PAO1, including biofilm formation, pyocyanin, elastase production, and swarming motility was examined.

2. Materials and methods

2.1. Plant material

The fresh stem of *C. olitorius* was purchased from a supermarket in Riyadh, Saudi Arabia in June 2016. The plant was authenticated by Prof Mahmoud Abdul Aziz Mahmoud at the College of Food and Agricultural Sciences, King Saud University. A voucher specimen (HM37-2) was deposited at the department's herbarium. The plant material was air-dried and powdered to obtain a coarse powder (165 g).

2.2. Media, chemicals, and solvents

Luria-Bertani (LB) broth, LB agar, and tryptone were obtained from Lab M Limited (Lancashire, United Kingdom). Mueller-Hinton broth, Mueller-Hinton agar, Tryptone soya broth, and Tryptone soya agar were purchased from Oxoid (Hampshire, UK). Dimethyl sulfoxide (DMSO) was purchased from Sigma (St. Louis, USA). The analytical grade solvents, such as ethanol (95%), petroleum ether (boiling point 40–60 °C), chloroform, methanol, *n*-butanol, and ethyl acetate were purchased from BDH (UK), which were distilled before use.

2.3. Extraction and fractionation

The air-dried and powdered stem of *C. olitorius* was subjected to exhaustive extraction with 95% ethanol (2 L × 3 L, each) in a Soxhlet apparatus for 12 h at room temperature (18 °C). The pooled ethanol extracts were evaporated under vacuum to obtain 63.3 g of dark green residue. The residue was suspended in 1 L water/methanol (9:1) and subjected to successive extraction with petroleum ether (boiling point 40–60 °C) (3 L × 0.5 L), chloroform (3 L × 0.5 L), ethyl acetate (3 L × 0.5 L), *n*-butanol (4 L × 0.5 L). Each fraction was individually concentrated to obtain the petroleum ether (5.4 g), chloroform (6.2 g), ethyl acetate (9.5 mg), *n*-butanol (8.5

g), and aqueous (19.0 g) fractions. The COAF rich in quantity and chemical components were re-dissolved in 2.5% DMSO and used for evaluating the QSI activity. DMSO at a concentration of 1.5% does not inhibit the growth of microorganisms[13].

2.4. Qualitative phytochemical investigation

All prepared fractions were subjected to phytochemical screening in order to scrutinize their bioactive constituents. For this, 3–5 mg of each fraction was separately suspended in 1 mL of ethanol or distilled water in clean glass test tubes.

2.4.1. Detection of flavonoids

A total of 1 mL of stock solution was taken in a test tube and a few drops of 5% of ethanolic KOH was added. The appearance of intense yellow in the test tube which completely turned colorless on the addition of a few drops of 5% HCl indicated the presence of flavonoids[14].

2.4.2. Test for triterpenes/steroids

The stock solution of plant fractions was treated with acetic anhydride (1 mL) and five drops of concentrated H₂SO₄. The formation of a bluish green ring and conversion of violet color into blue indicated the presence of terpenoids and steroids[15,16], respectively in the plant fraction.

2.4.3. Detection of tannins

A total of 3 mL of sample solution of plant fraction was diluted with chloroform and 1 mL of acetic anhydride was added to this solution. Existence of tannins in the test samples was confirmed by the appearance of green color on addition of 1 mL of concentrated H₂SO₄[17].

2.4.4. Test for coumarins

A volume of 3 mL of 2 mol/L NaOH solution was mixed with 2 mL of the test sample and appearance of yellow color indicated the existence of coumarin. Confirmation test was carried out by adding 5 mol/L HCl (1 mL) and formation of a colorless upper layer in the test solution is considered positive[18].

2.4.5. Test for alkaloids

The stock solution of crude ethanolic extract of the plant was dissolved in a minimum solvent and applied to pre-coated TLC plates using toluene: ethyl acetate: diethylamine (70:20:10) as a solvent system. The detection of alkaloids was confirmed by the appearance of red or orange spots on TLC after spraying with Dragendorff's reagent[19].

2.4.6. Test for sesquiterpene lactones

Picric acid (1%) dissolved in 10% of NaOH solution (Baljet

reaction) was used for the detection of sesquiterpene lactones in the extracts. Equal volumes of reagents (1:1 ratio) were mixed and added to the 1 mL of stock solution. The color change of sodium picrate solution from yellow to orange-red indicated a positive reaction[20].

2.4.7. Test for quinones

Equal volumes of stock solution and concentrated H_2SO_4 (1:1 v/v) were mixed and the appearance of red color indicated the presence of quinones.

2.4.8. Test for carbohydrates

A total of 5 mg of plant extract was dissolved in distilled water treated with 0.2% of anthrone reagent and a few drops of concentrated H_2SO_4 . The appearance and dark green color indicated the presence of carbohydrate[21].

2.4.9. Test for carboxyl group

Ten drops of 10% $NaHCO_3$ were added in the solution and formation of CO_2 bubbles was considered as a positive reaction[22].

2.4.10. Test for phenols

A few drops of 1% $FeCl_3$ were added in the stock solution (1 mL) and the appearance of dark greenish-blue color indicated the presence of phenolic components[23].

2.5. Bacterial strains and growth conditions

The bacterial strains used in this study were *C. violaceum* (CV026; a mini-Tn5 mutant of *C. violaceum* 31532 that cannot endogenously synthesize AHLs but responds to exogenous C4 and C6 AHLs) and *P. aeruginosa* (PAO1; C4 and 3-Oxo-C12 HSL producer)[24]. All the bacterial strains were cultured in LB medium at 30 °C for 24 h. The culture medium used for CV026 was supplemented with hexanoyl homoserine lactone (C6-HSL; Sigma-Aldrich, St Louis, MO, USA)[25].

2.6. Determination of minimum inhibitory concentration (MIC)

The MIC of COAF was determined by the broth micro-dilution method (CLSI). The COAF (1.0 g) was serially diluted 2-fold in Mueller-Hinton broth (300, 150, 75, 37.5, 18.5, 9.25, 3.375, and 3.125 mg/mL). Next, each dilution of COAF was added to the wells (100 μ L/well) of a 96-well microtiter plate. Each dilution of COAF was incubated with 100 μ L of PAO1 (1×10^6 CFU/mL) suspension in Mueller-Hinton broth for 20 h at 37 °C. The MIC of COAF was defined as the lowest concentration of the extract that visibly inhibited bacterial growth. Additionally, the effect of sub-MICs of COAF on the growth of PAO1 was determined. The absorbance of untreated and COAF-treated cultures were measured at 600 nm using a Biotek Spectrofluorimeter (Biotek, USA)[26]. The sub-MICs

of COAF were selected for the assessment of anti-virulence and anti-biofilm activity in the test strain.

2.7. QSI effect of COAF on reporter strain

The QSI effect of COAF was estimated by agar well diffusion assay using CV026[24,25]. The bacteria were cultured in LB broth for 24 h at 28 °C. To prepare the LB agar plates (1.5% agar), 15 mL of medium was poured into each plate. Next, *C. violaceum* was inoculated (100 μ L/plate) into the LB soft agar (0.5% agar) and poured on the solidified LB agar plates. The wells with a diameter of 10 mm were bored in the LB agar medium. Next, sub-MICs (100 μ L) of COAF were added to individual wells. The assay plates were incubated at 28 °C for 48 h. Inhibition of pigment production by the indicator strain around disk was considered as positive for QS interference. The inhibition of QS was calculated as follows: $r_2 - r_1$ (in mm); where r_2 is the total growth inhibition (QSI) zone radius (TI) and r_1 is the clear zone radius (GI). QSI zone with a diameter <10 mm was considered moderate activity, whereas that with a diameter >10 mm was considered a potent effect[13].

2.8. Violacein inhibition assay

The effect of sub-MICs of COAF on violacein production was analyzed using CV026. Violacein was extracted from CV026 and quantified photometrically following the method of Blosser and Gray (2000)[27] with minor modifications proposed by Husain *et al.*[28].

Briefly, the COAF was subjected to qualitative analysis to evaluate its QSI potential against CV026. *C. violaceum* synthesizes the violet-colored pigment violacein in response to the QS signal molecule *N*-hexanoyl-*L*-homoserine lactone (HHL) produced by the autoinducer synthase CviI. HHL binds to its receptor CviR, which induces the production of violacein. The overnight culture (10 μ L) of *C. violaceum* [adjusted to an optical density (OD) at 600 nm to 0.4] was added into wells of sterile microtiter plates containing 2 mL of LB broth. The culture was incubated with various concentrations of COAF (0.25–2 mg/mL) at 30 °C for 16 h. The culture (1 mL) was centrifuged at 13000 rpm for 10 min to precipitate the insoluble violacein. The culture supernatant was discarded and 1 mL of DMSO was added to the pellet. The solution was vortexed vigorously for 30 s to completely solubilize violacein. The mixture was centrifuged at 13000 rpm for 10 min to remove the cells. The violacein-containing supernatant (200 μ L) was added to 96-well flat-bottom microplates (Polylab, India) in quadruplets. The absorbance of the solution was measured using a microplate reader (Thermo Scientific Multiskan Ex) at a wavelength of 585 nm. The inhibition of violacein production by COAF was measured as follows: % inhibition = $[(OD_{\text{control}} - OD_{\text{treatment}})/OD_{\text{control}}] \times 100$; where OD_{control} and $OD_{\text{treatment}}$ are the OD of control and treatment groups, respectively.

2.9. Effect of COAF on QS-regulated virulence factors

The effect of sub-MICs of COAF on the virulence factors of *P. aeruginosa*, such as LasB elastase, pyocyanin, swarming motility, and exopolysaccharide (EPS) production was assessed as described previously by Husain *et al.*[29,30]. In the LasB elastolytic activity assay, the bacterial culture was treated with COAF for 16 h at 37 °C. The treated and untreated culture supernatants (100 µL each) were incubated with 900 µL of elastin Congo red (ECR, Sigma, USA) buffer (100 mM Tris, 1 mM CaCl₂, pH 7.5) containing 20 mg of ECR at 37 °C in a shaker incubator for 3 h. The insoluble ECR was removed by centrifugation. The absorption of Congo red in the supernatant was measured at 495 nm. The LB medium without COAF was included as a negative control. In the pyocyanin production assay, the culture supernatant (5 mL) of PAO1 treated with or without sub-MICs of COAF was first extracted with chloroform (3 mL). Next, the mixture was extracted with 0.2 mol/L HCl (1 mL). The absorbance of the solution was measured at 520 nm. For assaying swarming motility, the test cultures (PAO1 and CV026) were point inoculated at the center of a medium (1% tryptone, 0.5% NaCl, and 0.3% agar) containing sub-MICs of COAF overnight. The diameter of the swarm area was measured. For estimating EPS production, PAO1 was cultured in the presence of sub-MICs of COAF. The culture was centrifuged to obtain the supernatant. The supernatant was filter sterilized and incubated with chilled absolute ethanol overnight to precipitate EPS at 4 °C.

2.10. Biofilm inhibition assay

The effect of COAF on biofilm formation was measured using the microtiter plate assay described by O'Toole and Kotler[31]. Briefly, 1% overnight culture (0.4 OD at 600 nm) of test pathogens was incubated with 1 mL of fresh LB medium containing sub-MICs of COAF. The bacteria were allowed to adhere and grow without agitation for 24 h at 30 °C. The medium containing the free-floating planktonic cells was removed. The biofilms were gently rinsed twice with sterile water. The surface-attached cells (biofilm) were stained

with 200 µL of 0.1% crystal violet (CV) (Hi-media, Mumbai, India) solution for 15 min. The CV solution was discarded completely and 200 µL of 95% ethanol was added to solubilize CV from the stained cells. The biofilm biomass was then quantified by measuring the absorbance at 470 nm in a microplate reader (Thermo Scientific Multiskan Ex, India).

2.11. Statistical analysis

Growth and biofilm-inhibition data were analyzed by SigmaPlot 12.0 and Minitab (Minitab, Inc.) software version 14.0 for Windows. All the experiments were performed in triplicates. Result data were presented as mean ± standard deviation (SD) and differences were considered as significant at $P < 0.05$. One-way ANOVA was used to compare the effects of different fractions. The difference between control and test groups was analyzed using LSD test.

3. Results

3.1. Phytochemical screening

Results of phytochemical investigation of different fractions of *C. olitorius* are summarized in Table 1 and it showed that polyphenolic, flavonoids, triterpenes, tannins, coumarins, quinones, steroids, carbohydrates, and amino acids were the main chemical components.

3.2. Assessment of MIC

Different fractions of *C. olitorius* were prepared in petroleum ether, chloroform, ethyl acetate, *n*-butanol, and aqueous and evaluated for QS modulatory activity against CV026 and PAO1. Out of all 5 fractions, aqueous fraction displayed dose-dependent anti-QS potential and was the most effective with inhibitory effect on the bacteria growth at 0.8 mg/mL. Pigment inhibition was also observed at higher concentrations, which is a determinant of anti-QS activity and accompanied by growth inhibition of CV026 and PAO1. Ethyl

Table 1. Phytochemical screening results of different fractions of *Corchorus olitorius*.

Compounds	Petroleum ether fraction	Chloroform fraction	Ethyl acetate fraction	<i>n</i> -Butanol fraction	Aqueous fraction
Flavonoids	-	++	++	+++	+++
Triterpenes	+	++	++	++	+++
Steroids	-	+	++	+++	+++
Polyphenolics	+	++	++	++	+++
Tannins	+	++	++	+	++
Quinones	-	+	+	++	++
Coumarins	+	+	+	++	+
Carbohydrates	-	+	+	+	++
Amino acid	-	+	++	++	+++
Carboxylic group	+	++	++	+	+
Unsaturated	-	-	-	+	++
Alkaloids	-	-	-	-	-

-: absent, +: low intensity, ++: medium intensity, and +++: strong intensity.

acetate fraction showed anti-QS activity only at 2000 mg/mL. The *n*-butanol fraction inhibited pigments at 500 and 700 mg/mL and it also inhibited the growth of bacteria at a similar concentration. Petroleum ether and chloroform fractions showed no inhibition of QS at 200–1800 mg/mL. Results of preliminary anti-QS activity screening by the agar well diffusion method showed that aqueous fraction had the highest activity and it was selected for further study. The MIC value of COAF against PAO1 was 2.4 mg/mL. The sub-MICs of COAF were selected for the assessment of anti-virulence and anti-biofilm activity in the test strain.

3.3. QSI activity of COAF

The COAF exhibited a potent QSI effect at high doses of 800 and 1000 µg/mL. In the violacein bioassay, treatment with COAF at doses of 800 and 1000 µg/mL generated a white halo with a diameter of 15 and 11 mm, respectively (Table 2). The higher inhibitions of violacein production by the supplementation of COAF were observed at doses of 2000 µg/mL and 1000 µg/mL (Figure 1). COAF displayed dose-dependent interference of QS activity by reducing violacein production in CV026. Growth curve analysis showed that sub-MICs of *C. olitorius* did not cause significant changes in the growth of *C. violaceum* (CV026). Thus, COAF inhibited the production of violacein without affecting the growth of CV026. The effect of COAF on PAO1 was evaluated and the results showed the dose-dependent effect of COAF of the virulence factors in PAO1.

3.4. Pyocyanin inhibition

The production of pyocyanin was observed in *P. aeruginosa* after 24–48 h of growth. The pyocyanin level in the COAF-treated PAO1 group was significantly lower than that in the untreated group in a concentration-dependent manner. The higher inhibition of pyocyanin production was observed at 2000 µg/mL ($P < 0.01$) and 1000 µg/mL ($P < 0.05$), followed by 500 and 250 µg/mL (Figure 2).

3.5. LasB elastase inhibition

Treatment with COAF at sub-MICs significantly and dose-dependently decreased the LasB elastase activity in the culture supernatant of PAO1 (Figure 2). The lowest inhibition of LasB elastase activity was observed in COAF at the dose of 250 µg/mL, whereas the maximum inhibition was observed in COAF at 2000 µg/mL ($P < 0.05$).

3.6. EPS and swarming motility inhibition

The supplementation of 2000 µg/mL of COAF reduced EPS production more significantly ($P < 0.01$) in the treated cultures of PAO1 compared to control. The COAF showed a reduction of 10%

and 40% in EPS at 250 and 1000 µg/mL, respectively. Similarly, COAF significantly reduced flagella-mediated swarming of PAO1 in a dose-dependent manner. The treatment of COAF significantly impaired swarming migration of PAO1 by 56% and 61% at 1000 and 2000 µg/mL, respectively (Figure 2).

Table 2. Anti-QS activity of aqueous fraction of *Corchorus olitorius* at different concentrations.

Concentration of fractions (µg/mL)	*Zone of inhibition (mm)			
	TI (r2)	GI (r1)	QSI (r2-r1)	Anti-QS potential
200	-	-	-	-
400	-	-	-	-
800	15	-	15	++
1000	22	11	11	++

*Data are the mean value of three experiments. TI, total inhibition, GI, growth inhibition, QSI, quorum sensing inhibition. -: moderate, ++: potent anti-quorum sensing activity. *Allium sativa* (positive control) has a potent anti-quorum sensing activity (++) 10 mm.

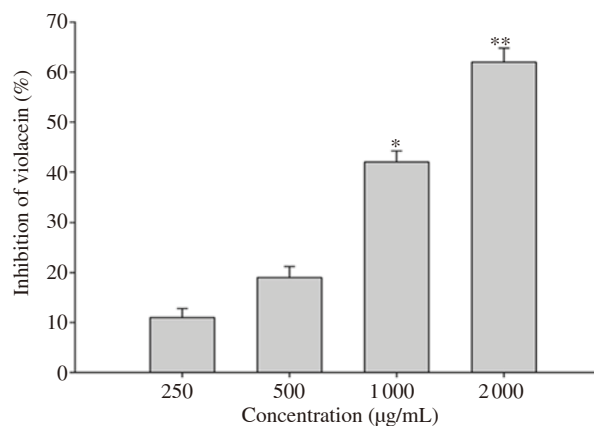


Figure 1. Effect of sub-minimum inhibitory concentrations of *Corchorus olitorius* aqueous fraction on the production of violacein. All data are presented as mean ± standard deviation. * $P < 0.05$, ** $P < 0.01$ compared with the untreated group.

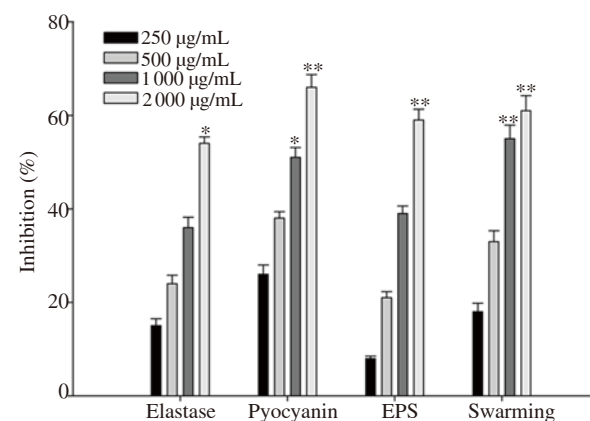


Figure 2. Effect of sub-minimum inhibitory concentrations of *Corchorus olitorius* aqueous fraction on quorum sensing-regulated virulence factors in *Pseudomonas aeruginosa* PAO1. All data are presented as mean ± standard deviation. * $P < 0.05$, ** $P < 0.01$, compared with the untreated group. EPS: Exopolysaccharide.

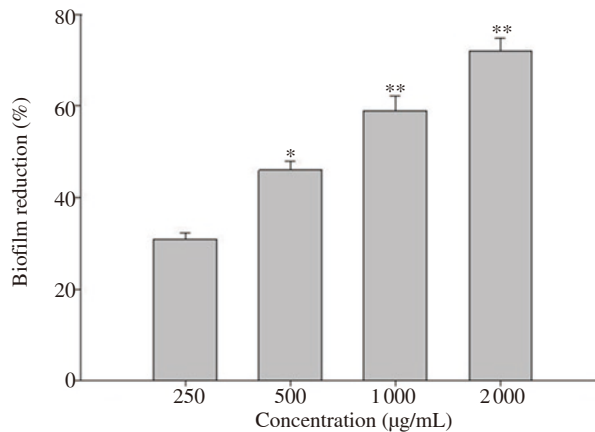


Figure 3. Effect of sub-minimum inhibitory concentrations of *Corchorus olitorius* aqueous fraction on biofilm formation by *Pseudomonas aeruginosa* PAO1. All data are presented as mean \pm standard deviation. * P <0.05, ** P <0.01, compared with the untreated group.

3.7. Effect of COAF on biofilm formation

The effect of COAF on *Pseudomonas* biofilm formation was evaluated using the tube assay method. Treatment with COAF at doses of 500 (P <0.05), 1000 (P <0.01), and 2000 (P <0.01) μ g/mL significantly decreased the biofilm biomass (Figure 3). This indicated that COAF could inhibit the biofilm formation of the target pathogens.

4. Discussion

In several bacteria, virulence factors are controlled through QS, a cell density-dependent gene regulatory mechanism. Interference of QS circuits is considered as a potential strategy to attenuate bacterial pathogenicity. In the present study, ethanol extract of *C. olitorius* was subjected to consequent fractionation with different organic solvents and evaluated for QS interference based on the reduction of violacein production in *C. violaceum* 31532. Among all the fractions, the aqueous fraction showed the highest anti-QS potential.

The aqueous fraction of *C. olitorius* showed apparent inhibition of AHL-dependent pigment production, suggesting the interference of QS-regulated function. Further, interference of QS by the aqueous fraction is also evident as it reduced the violacein production in CV026 without affecting the growth of *C. violaceum*. A study conducted on *Cuminum cyminum* and clove oil has shown the same effect on violacein production[32,33]. The QS inhibitor screening was carried out by using *C. violaceum* which showed the appearance of purple-colored pigmentation (violacein) in the indicator organism. The analysis of inhibition of pigment production in CV026 is a rapid and simple method for screening QSI activity. Thus, based on the reduction in violaceum production and lack of growth inhibition of indicator organism CV026, the aqueous fraction was found to be

the most active and was further tested against *P. aeruginosa* PAO1 to evaluate wide spectrum anti-QS potential because this organism involves a different QS system. *P. aeruginosa* produces a number of QS-regulated factors such as elastase, protease, and chitinase[34]. The hydrolytic enzymes (elastase and protease) of bacteria affect the proteins of host cells in the infected tissues and facilitate bacterial invasion and growth. In the current study, preincubation with the plant fraction resulted in dose-dependent inhibition of protease and elastase production. These results were in agreement with the literature where elastase, proteolytic, and chitinase activities of *P. aeruginosa* were lowered after treatment with the extracts, fractions, and essential oils of plant origin by 2%-80%[35].

A significant concentration-dependent decrease (P <0.01) was noticed with COAF in *C. violaceum* (CV026) and *P. aeruginosa* (PAO1) strains, being higher in CV026 (62%). The COAF potently inhibited violacein production in the dose range of 800-1000 μ g/mL. Treatment with COAF at doses of 2000 and 1000 μ g/mL inhibited violacein production by 62% and 42%, respectively. However, there was only 10%-20% of inhibition in violacein production by supplementations of COAF at 250 and 500 μ g/mL. Similar inhibition of violacein production was recorded in extracts of *Capparis spinosa* and *Cuminum cyminum*[32,36]. Another crucial virulence factor produced by QS-regulation is pyocyanin. It plays an important role in pathogenesis mainly in cystic fibrosis[37]. In the current study, we evaluated the effect of COAF on the production of pyocyanin in PAO1. Treatment with COAF significantly decreased pyocyanin level in the PAO1 culture when compared to that in the untreated cultures. Similar decreases in pyocyanin levels were recorded in different extracts of *Terminalia chebula*[38]. In addition, treatment with COAF concentration-dependently inhibited elastase activity in PAO1. A significant inhibition of 55% (P <0.05) in elastase activity was observed at 2000 μ g/mL.

QS mediated by AHLs plays an important role in the formation of biofilm. The results of our study showed that the COAF at sub-MICs significantly inhibited the biofilm biomass (P <0.01) without affecting the growth of PAO1. A study reported that surface conditioning promotes the surface adhesion and formation of microcolonies[39]. Our study revealed that treatment of COAF efficiently reduced the biofilm biomass formed by the bacterial strain. The possible interference in biofilm formation could be due to reducing surface adhesion or subsequent step of biofilm formation. The extract/fraction which has the ability to inhibit the QS is expected to affect the biofilm-forming capacity[40]. The results of our study are in accordance with activity of extracts of *Rhodomyrtus tomentosa*[41], *Lagerstroemia speciosa*[42], and *Sclerocarya birrea*[43].

Factors involved in biofilm formation of *P. aeruginosa* such as EPS production and swarming motility were assessed in this study. Production of EPS is an essential condition required for biofilm maturation[44], a QS-regulated feature[45]. Therefore, QS interference by plant extracts led to a reduction in EPS production[46]. Our study

also showed that treatment with 2000 µg/mL COAF reduced EPS production and impaired swarming migration more significantly ($P < 0.01$). It has shown that flagella-driven motility is needed for surface attachment initiation in biofilm development. Therefore, flagellar synthesis inhibition by the plant extracts/fraction would assist the reduced swarming migration. Thus, this COAF might have indirectly influenced biofilm formation by bacterial strains at least in part by interfering AHL-mediated QS-system. Our results are in support of earlier studies conducted on various plant extracts, which demonstrated that several plant extracts have the ability to inhibit the biofilm formation by PAO1[40].

Furthermore, this study indicated the presence of flavonoids, triterpenes, tannins, coumarins, quinones, carbohydrates, polyphenolic, and steroids in fraction that cause microorganism death by inhibiting DNA or RNA synthesis and flavonoids possibly involved in inhibition of extracellular microbial enzymes[47].

In conclusion, the irrational use of antibiotics has led to the emergence of antibiotic-resistant bacteria, which are a major threat to public health. The resistance to antibiotics is mediated by QS in the pathogenic bacteria. Thus, there is a need to develop novel inhibitors of QS, especially from natural sources. This study investigated the QSI activity of COAF. The effect of COAF on violacein pigment production in the *C. violaceum* biosensor system was evaluated. Treatment with COAF inhibited violacein production. This study demonstrated that the COAF inhibited biofilm formation, attenuated virulence factors, and inhibited swarming motility in *P. aeruginosa*. These results suggest that the COAF can be used to inhibit QS, biofilm formation, and virulence factors in pathogenic bacteria.

Conflict of interest statement

We declare that there is no conflict of interest.

Funding

This research project was supported by a grant from the “Research Center of the Female Scientific and Medical Colleges”, Deanship of Scientific Research, King Saud University.

Authors’ contributions

HMA and MA designed this research, carried out the extraction and fractionation of plant sample, WHBH and ZK performed the biological experiment, PA carried out statistical analysis. All authors revised and approved the paper.

References

- [1] Chaudhary SA. *Flora of the kingdom of Saudi Arabia*. Riyadh, KSA: Ministry of Agriculture and Water, National Agriculture and Water Research Center; 1999, p. 336-344.
- [2] Ganese AT, Shah SS, Swaminathan MS. Productivity for the good plant setting in the cross *Corchorus olitorius* and *C. capsularis*. *Curr Sci* 1957; **26**: 292-293.
- [3] Hasan HT, Kadhim EJ. Phytochemical investigation of leaves and seeds of *Corchorus olitorius* L. cultivated in Iraq. *Asian J Pharm Clin Res* 2018; **11**(11): 408-417.
- [4] Ilhan S, Savaroglu F, Colak F. Antibacterial and antifungal activity of *Corchorus olitorius* L. (Molokhia) extracts. *Int J Nat Eng Sci* 2007; **1**: 59-61.
- [5] Oboh G, Raddatz H, Henle T. Characterization of the antioxidant properties of hydrophilic and lipophilic extracts of jute (*Corchorus olitorius*) leaf. *Int J Food Sci Nutr* 2009; **60**: 124-134.
- [6] Furumoto T, Wang R, Okazaki K, Afm F, Ali MI, Kondo A, et al. Antitumor promoters in leaves of jute (*Corchorus capsularis* and *Corchorus olitorius*). *Food Sci Technol Res* 2002; **8**: 239-243.
- [7] Das AK, Sahu R, Dua TK, Bag S, Gangopadhyay M, Sinha MK, et al. Arsenic-induced myocardial injury: Protective role of *Corchorus olitorius* leaves. *Food Chem Toxicol* 2010; **48**: 1210-1217.
- [8] Al Batran R, Al-Bayaty F, Abdulla MA, Jamil Al-Obaidi MM, Hajrezaei M, Hassandarvish P, et al. Gastroprotective effects of *Corchorus olitorius* leaf extract against ethanol-induced gastric mucosal hemorrhagic lesions in rats. *J Gastroenterol Hepatol* 2013; **28**: 1321-1329.
- [9] Alhazmi A. *Pseudomonas aeruginosa* pathogenesis and pathogenic mechanisms. *Int J Biol* 2015; **7**: 44-67.
- [10] Zhang LH, Dong YH. Quorum sensing and signal interference: Diverse implications. *Mol Microbiol* 2004; **53**: 1563-1571.
- [11] Hong KW, Koh CL, Sam CK, Yin WF, Chan KG. Quorum quenching revisited--from signal decays to signalling confusion. *Sensors (Basel)* 2012; **12**: 4661-4696.
- [12] Fuqua C, Greenberg EP. Listening in on bacteria: Acyl-homoserine lactone signalling. *Nat Rev Mol Cell Biol* 2002; **3**: 685-695.
- [13] Zaki AA, Shaaban MI, Hashish NE, Amer MA, Lahloub MF. Assessment of anti-quorum sensing activity for some ornamental. *Sci Pharm* 2013; **81**(1): 251-258.
- [14] Sembiring EN, Elya B, Sauriasari R. Phytochemical screening, total flavonoid and total phenolic content and antioxidant activity of different parts of *Caesalpinia bonduc* (L.) Roxb. *Pharmacogn J* 2018; **10**(1): 123-127.
- [15] Fawehinmi AB, Lawal H, Etatuvi SO, Oyedeji FO. Preliminary phytochemical screening and antimicrobial evaluation of four medicinal plants traditionally used in Nigeria for skin infection. *Afr J Pure Appl Chem* 2013; **7**(2): 44-49.
- [16] Manas B, Rajesh Y, Kumar VR, Praveen B, Mangamma K. Extraction, phytochemical screening and *in-vitro* evaluation of anti-oxidant properties of *Commicarpus chinensis* (aqueous leaf extract). *Int J Pharma Bio Sci* 2010; **1**(4): 537-547.
- [17] Ali S, Khan MR, Sajid M, Zahra Z. Phytochemical investigation and

- antimicrobial appraisal of *Parrotiopsis jacquemontiana* (Decne) Rehder. *BMC Complement Altern Med* 2018; **18**(1): 43.
- [18] Jacob SJ, Shenbagaraman S. Evaluation of antioxidant and antimicrobial activities of the selected green leafy vegetables. *Int J Pharm Tech Res* 2011; **3**(1): 148-152.
- [19] AL-Sallami AS, Al-Labban ZS, Ali SM. Cytotoxic activity of alkaloids extracted from *Trigonella foenum graecum* (Fenugreek) against breast cancer cell line. *J Pharm Sci Res* 2019; **11**(4): 1662-1666.
- [20] Ivanescu B, Miron A, Corciova A. Sesquiterpene lactones from *Artemisia* genus: Biological activities and methods of analysis. *J Anal Methods Chem* 2015; **2015**: 247685. Doi: 10.1155/2015/247685.
- [21] Khlif I, Jellali K, Michel T, Halabalaki M, Skaltsounis AL, Allouche N. Characteristics, phytochemical analysis and biological activities of extracts from Tunisian Chetoui *Olea europaea* Variety. *J Chem* 2015; **2015**. Doi: 10.1155/2015/418731.
- [22] Yogeshwari C, Kalaichelvi K. Comparative phytochemical screening of *Acmella calva* (dc.) rk jansen and *Crotalaria ovalifolia* wall: Potential medicinal herbs. *J Med Plant* 2017; **5**: 277-279.
- [23] Archana P, Samatha T, Mahitha B, Chamundeswari NR. Preliminary phytochemical screening from leaf and seed extracts of *Senna alata* L. Roxb-an ethno medicinal plant. *Int J Pharm Biol Res* 2012; **3**: 82-89.
- [24] McLean RJ, Pierson LS, Fuqua C. A simple screening protocol for the identification of quorum signal antagonists. *J Microbial Methods* 2004; **58**: 351-360.
- [25] McClean KH, Winson MK, Fish L, Taylor A, Chhabra SR, Camara M, et al. Quorum sensing and *Chromobacterium violaceum*: Exploitation of violacein production and inhibition for the detection of *N*-acylhomoserine lactones. *Microbiol (Reading Engl)* 1997; **143**: 3703-3711.
- [26] Clinical and Laboratory Standards Institute. *Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically*. Approved standard, CLSI Document M07-A9, 32, No. 3. Wayne, PA, USA; 2012.
- [27] Blosser RS, Gray KM. Extraction of violacein from *Chromobacterium violaceum* provides a new quantitative bioassay for *N*-acyl homoserine lactone autoinducers. *J Microbiol Methods* 2000; **40**: 47-55.
- [28] Husain FM, Ahmad I, Khan MS, Ahmad E, Tahseen Q, Khan MS, et al. Sub-MICs of *Mentha piperita* essential oil and menthol inhibits AHL mediated quorum sensing and biofilm of Gram-negative bacteria. *Front Microbiol* 2015; **6**: 420.
- [29] Husain FM, Ahmad I, Asif M, Tahseen Q. Influence of clove oil on certain quorum-sensing-regulated functions and biofilm of *Pseudomonas aeruginosa* and *Aeromonas hydrophila*. *J Biosci* 2013; **38**: 835-844.
- [30] Husain FM, Ahmad I, Al-thubiani AS, Abulreesh HH, AlHazza IM, Aqil F. Leaf extracts of *Mangifera indica* L. inhibit quorum sensing-regulated production of virulence factors and biofilm in test bacteria. *Front Microbiol* 2017; **8**: 727.
- [31] O'Toole GA, Kolter R. Initiation of biofilm formation in *Pseudomonas fluorescens* WCS365 proceeds via multiple, convergent signalling pathways: A genetic analysis. *Mol Microbiol* 1998; **28**: 449-461.
- [32] Packiavathy IASV, Agilandeswari P, Musthafa KS, Pandian SK, Ravi AV. Antibiofilm and quorum sensing inhibitory potential of *Cuminum cyminum* and its secondary metabolite methyl eugenol against Gram negative bacterial pathogens. *Food Res Int* 2012; **45**: 85-92.
- [33] Sankar Ganesh P, Vittal R. Evaluation of anti-bacterial and anti-quorum sensing potential of essential oils extracted by supercritical CO₂ method against *Pseudomonas aeruginosa*. *J Essent Oil Bear Pl* 2015; **18**: 264-275.
- [34] Shukla V, Bhatena Z. Broad spectrum anti-quorum sensing activity of tannin-rich crude extracts of indian medicinal plants. *Scientifica* 2016; **2016**: 5823013. Doi: 10.1155/2016/5823013.
- [35] Sánchez E, Rivas Morales C, Castillo S, Leos-Rivas C, García-Becerra L, Ortiz Martínez DM. Antibacterial and antibiofilm activity of methanolic plant extracts against nosocomial microorganisms. *Evid Based Complement Alternat Med* 2016; **2016**: 1572697. Doi: 10.1155/2016/1572697.
- [36] Saeidi S, Javadian E, Javadian F. Anti-microbial and anti-biofilm activity of leaf fruit and stem *Capparis spinose* extract against *Acinetobacter baumannii*. *Biomed Res* 2019; **30**(6): 501-506.
- [37] Das T, Manos J. *Pseudomonas aeruginosa* extracellular secreted molecules have a dominant role in biofilm development and bacterial virulence in cystic fibrosis lung infections. In: Sriramulu D (ed.) *Progress in understanding cystic fibrosis*. IntechOpen; 2017, p. 101.
- [38] Sarabhai S, Sharma P, Capalash N. Ellagic acid derivatives from *Terminalia chebula* Retz. downregulate the expression of quorum sensing genes to attenuate *Pseudomonas aeruginosa* PAO1 virulence. *PLoS One* 2013; **8**(1): e53441.
- [39] Armbruster CR, Parsek MR. New insight into the early stages of biofilm formation. *Proc Natl Acad Sci* 2018; **115**(17): 4317-4319.
- [40] Sasirekha B, Megha DM, Chandra MS, Soujanya R. Study on effect of different plant extracts on microbial biofilms. *Asian J Biotechnol* 2015; **7**(1): 1-12.
- [41] Saising J, Ongsakul M, Voravuthikunchai SP. *Rhodomyrtos tomentosa* (Aiton) Hassk. ethanol extract and rhodomertone: A potential strategy for the treatment of biofilm-forming staphylococci. *J Med Microbiol* 2011; **60**(12): 1793-1800.
- [42] Singh BN, Singh HB, Singh A, Singh BR, Mishra A, Nautiyal CS. *Lagerstroemia speciosa* fruit extract modulates quorum sensing controlled virulence factor production and biofilm formation in *Pseudomonas aeruginosa*. *Microbiology* 2012; **158**: 529-538.
- [43] Sarkar R, Chaudhary SK, Sharma A, Yadav KK, Nema NK, Sekhoacha M, et al. Anti-biofilm activity of Marula—A study with the standardized bark extract. *J Ethnopharmacol* 2014; **154**(1): 170-175.
- [44] Limoli DH, Jones CJ, Wozniak DJ. Bacterial extracellular polysaccharides in biofilm formation and function. *Microbial Biofilms* 2015; **7**: 223-247.
- [45] Asfour HZ. Anti-quorum sensing natural compounds. *J Microsc Ultrastruct* 2018; **6**(1): 1-10.
- [46] Bouyahya A, Dakka N, Et-Touys A, Abrini J, Bakri Y. Medicinal plant products targeting quorum sensing for combating bacterial infections. *Asian Pac J Trop Med* 2017; **10**(8): 729-743.
- [47] Tagousop CN, Ekom SE, Ngnokam D, Voutquenne-Nazabadioko L. Antimicrobial activities of flavonoid glycosides from *Graptophyllum grandulosum* and their mechanism of antibacterial action. *BMC Complement Altern Med* 2018; **18**(1): 252.