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ORIGINAL ARTICLE

Anti-quorum sensing activity of *Psidium guajava* L. flavonoids against *Chromobacterium violaceum* and *Pseudomonas aeruginosa* PAO1

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ABSTRACT

***Psidium guajava* L., which has been used traditionally as a medicinal plant, was explored for anti-quorum sensing (QS) activity. The anti-QS activity of the flavonoid (FL) fraction of *P. guajava* leaves was determined using a biosensor bioassay with *Chromobacterium violaceum* CV026. Detailed investigation of the effects of the FL-fraction on QS-regulated violacein production in *C. violaceum* ATCC12472 and pyocyanin production, proteolytic, elastolytic activities, swarming motility and biofilm formation in *Pseudomonas aeruginosa* PAO1 was performed using standard methods. Possible mechanisms of QS-inhibition were studied by assessing violacein production in response to *N*-acyl homoserine lactone (AHL) synthesis in the presence of the FL-fraction in *C. violaceum* ATCC31532 and by evaluating the induction of violacein in the mutant *C. violaceum* CV026 by AHL extracted from the culture supernatants of *C. violaceum* 31532. Active compounds in the FL-fraction were identified by liquid chromatography–mass spectrometry (LC–MS). Inhibition of violacein production by the FL-fraction in a *C. violaceum* CV026 biosensor bioassay indicated possible anti-QS activity. The FL-fraction showed concentration-dependent decreases in violacein production in *C. violaceum* 12472 and inhibited pyocyanin production, proteolytic and elastolytic activities, swarming motility and biofilm formation in *P. aeruginosa* PAO1. Interestingly, the FL-fraction did not inhibit AHL synthesis; AHL extracted from cultures of *C. violaceum* 31532 grown in the presence of the FL-fraction induced violacein in the mutant *C. violaceum* CV026. LC–MS analysis revealed the presence of quercetin and quercetin-3-*O*-arabinoside in the FL-fraction. Both quercetin and quercetin-3-*O*-arabinoside inhibited violacein production in *C. violaceum* 12472, at 50 and 100 μg/mL, respectively. Results of this study provide scope for further research to exploit these active molecules as anti-QS agents.**

Key words flavonoids, *Pseudomonas aeruginosa*, *Psidium guajava*, quorum sensing.

Traditional treatment of diseases generally relies on natural sources for remedies; many plant species have been used for treating microbial infections or as antiseptics. *Psidium guajava* L. (Myrtaceae), commonly called guava, is used in traditional medicine worldwide to treat human ailments such as diarrhea, dysentery, wounds, ulcers, cholera, and dental diseases (1–3). A number of metabolites from *P. guajava* that belong mainly to the FL, carotenoid, phenolic and terpenoid groups have been shown to possess useful biological

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List of Abbreviations: AHL, *N*-acyl homoserine lactone; C_6-HSL, *N*-hexanoyl-L-homoserine lactone; DMSO, dimethyl sulfoxide; ECR, elastin Congo red; FL, flavonoid fraction; LB, Luria–Bertani medium; LC–MS, liquid chromatography–mass spectrometry; MIC, minimum inhibitory concentration; QS, quorum sensing; TLC, thin layer chromatography.

activities. *Psidium guajava* leaves contain high concentrations of FLs such as quercetin, myricetin, kaempferol and luteolin (4).

Studies on antibacterial activity of different solvent extracts of guava plant have revealed relatively high MIC values for some gram-negative bacteria, for example, methanol extracts of guava plant leaves showed an MIC of 250 mg/mL against *Pseudomonas aeruginosa* (5). Similarly, these guava extracts showed high MIC values against *Staphylococcus aureus* and *Escherichia coli* (6). Antibacterial activity of guava has been attributed to the FL-fraction, which contains quercetin, quercetin-3-O-arabinoside, morin and morin-3-O-arabinoside (7). Subsequent studies have shown that guava FLs have bacteriostatic effects against eight different pathogenic bacteria (8). Phytocompounds produced by these plants have specific modes of action against different microorganisms. Studies have shown that some FLs inhibit a cell-to-cell signaling mechanism, namely QS, in bacteria. Glycosylated flavanones such as naringenin, naringin and hesperidin have been studied for their ability to interfere with QS in *P. aeruginosa* PAO1 (9, 10).

Quorum sensing is a population density-dependent mechanism mediated through small signaling molecules called autoinducers by which bacteria regulate gene expression; in gram-negative bacteria the autoinducers are AHLs (11, 12). In some pathogenic bacteria, the QS system controls expression of genes responsible for virulence factor production (13). Targeting QS has emerged as an alternative strategy for controlling bacterial virulence and scientific investigations have proved that QS can be disrupted by synthetic or natural compounds, including some phytocompounds (14, 15).

Considering the traditional use of guava as an effective anti-infective agent and the meager information on the mechanism of guava's antibacterial activity, we used *Chromobacterium violaceum* and *P. aeruginosa* PAO1 to investigate the possibility of QS inhibition by the FL-fraction of *P. guajava*. In *C. violaceum*, the LuxR homolog, CviR regulates the production of a purple pigment, violacein (16). *P. aeruginosa* PAO1, an opportunistic pathogen, utilizes two inter-related QS systems, LasI/R and RhlI/R, which regulate pyocyanin production, proteolytic and elastolytic activity, swarming motility and biofilm formation (17).

MATERIALS AND METHODS

Bacterial strains, media and culture conditions

Bacterial strains used to detect QS inhibition were *C. violaceum* ATCC12472, *C. violaceum* ATCC31532, a mini-Tn5 mutant *C. violaceum* CV026 and *P. aeruginosa* PAO1. All bacteriological media were purchased from Hi-Media (Mumbai, India). The bacteria were grown in LB medium at 32 °C for 24 hr unless otherwise specified. When required, the medium for *C. violaceum* CV026 was supplemented with C_6-HSL (Sigma–Aldrich, St Louis, MO, USA). For all the experiments, inocula were prepared by growing the bacteria in 10 mL LB broth with shaking (130 rpm) for 24 hr at 32 °C. The cell density was measured by recording OD_{600} spectrophotometrically (UV-1800, Shimadzu, Kyoto, Japan).

Plant materials and extract preparation

Fresh leaves of *P. guajava* were collected from the local region of Mangalore, India. The plants were authenticated by an expert taxonomist and the voucher specimen deposited in the institutional repository of the Yenepoya University, Mangalore, India (accession number: YU-myr/001). The leaves were washed in sterile water, shade dried and pulverized to a fine powder in an analytical mill (IKA, Staufen, Germany). Powder (100 g) was extracted in methanol and the FL-fraction separated as described by Arima and Danno (7). Concentrated FL-fraction (yield: 400 mg) was redissolved in DMSO before use to the required concentrations.

Biosensor bioassay

Detection of QS inhibition by FL-fraction of *P. guajava* was carried out by biosensor bioassay using *C. violaceum* CV026 reporter strain (18). Different concentrations of FL-fraction of *P. guajava* (10–100 μg/disc) were loaded onto 6 mm sterile discs (Hi-Media) and placed on the surfaces of *C. violaceum* CV026 inoculated LB agar plates supplemented with C_6-HSL (final concentration of 10 μmol/mL). Discs loaded with solvent (DMSO) were included as vehicle-only controls. After incubation at 32 °C for 24 hr, inhibition of QS was detected by the presence of a zone of colorless but viable cells around the discs that was clearly distinguishable from the zone of growth inhibition.

Quantification of inhibition of violacein production in *C. violaceum* 12472

Based on the bioassay results, detailed investigation of inhibition of QS-controlled violacein production in *C. violaceum* 12472 by FL-fraction was carried out according to a previously described method (19). For this, FL-fractions at concentrations ranging from 25 to 400 μg/mL in 5 mL LB broth were inoculated with 100 μL (10^6 CFU/mL) of *C. violaceum* 12472. Solvent controls were prepared similarly and all the tubes were

incubated for 24 hr with shaking (130 rpm). Violacein was extracted using water-saturated *n*-butanol (1 mL) from the cells collected by centrifuging (8000 rpm, 10 min) 1 mL culture broth. Violacein was quantified by recording OD_{585} spectrophotometrically (UV-1800). Inhibition of violacein production was calculated with respect to the controls. Bacterial viability in the presence of tested concentrations of FL-fraction in the culture broth was measured after serial dilution by a standard plate count method.

Effect of FL-fraction on production of QS-controlled virulence factors in *P. aeruginosa* PAO1

Inhibition by FL-fraction in concentrations ranging from 25 to 400 μg/mL of pyocyanin production, proteolytic and elastolytic activities, swarming motility and biofilm formation, all of which are regulated by QS in *P. aeruginosa* PAO1, were investigated. In all experiments, cell viability was measured by a standard plate count method.

To determine the effect on pyocyanin production, *P. aeruginosa* PAO1 (10^6 CFU/mL) was grown in glycerol alanine minimal medium supplemented with FL-fraction and incubated for 24 hr. Pyocyanin from the cell-free supernatant (5 mL) was extracted with chloroform (3 mL) after which the chloroform layer was acidified with 0.2 M HCl (1 mL). The pyocyanin-containing acid layer was separated and quantified by recording OD_{520} spectrophotometrically (20). For studying the inhibition of elastolytic and proteolytic activities, *P. aeruginosa* PAO1 (10^6 CFU/mL) was grown in LB medium containing FL-fraction and incubated for 16 hr. For elastase activity, culture supernatant (100 μL) was added to 900 μL of ECR buffer (100 mM Tris, 1 mM $CaCl_2$, pH 7.5) containing 20 mg ECR (Sigma–Aldrich) and incubated for 3 hr at 37 °C. Insoluble ECR was removed by centrifugation and absorbance of the supernatant measured spectrophotometrically at OD_{495} (21). To quantify proteolytic activity, culture supernatant (100 μL) was mixed with 900 μL of ECR buffer containing 3 mg of azocasein (Sigma–Aldrich) and incubated at 37 °C for 30 min. Next, 100 μL of trichloroacetic acid (10%) was added and 30 min later the tubes were centrifuged (8000 rpm, 10 min) and the OD_{440} measured spectrophotometrically (22). For swarming assays, LB semisolid agar plates (0.5% agar) supplemented with FL-fraction were point inoculated with *P. aeruginosa* PAO1 (10^6 CFU/mL) and incubated for 24 hr. The extent of swarming was determined by measuring the swarming diameter (23).

Inhibition of biofilm formation was studied as described by Vandeputte *et al.* (24). Briefly, 50 μL of overnight-grown *P. aeruginosa* PAO1 culture (10^6 CFU/mL) in LB medium was diluted with 2950 μL of fresh tryptone broth medium containing the FL-fraction and incubated statically for 18 hr. After incubation, the medium was removed, washed thrice with PBS and the biofilm fixed in methanol for 15 min. The solutions were discarded, dried at room temperature, and stained with crystal violet (0.1% in water) for 15 min. Unbound stain was removed by two washes with water, dried, solubilized with acetic acid (33%), and the OD_{590} was recorded.

Effect of FL-fraction on AHL production

The effect of the FL-fraction on AHL production was determined by using an AHL overproducing strain, *C. violaceum* 31532 and its mutant *C. violaceum* CV026, which does not produce AHL (23). LB medium (10 mL) containing FL-fraction (0, 50, 100, 200, 300 and 400 μg/mL) was inoculated with *C. violaceum* 31532 and incubated for 24 hr. Violacein produced was quantified spectrophotometrically as described earlier. Simultaneously, AHL was extracted from the cell-free supernatant (5 mL) using dichloromethane (3:1 v/v) (18). The collected AHL fractions were dried under a thin stream of nitrogen, resuspended in 20 μL methanol (70%) and added to 5 mL fresh LB medium inoculated with *C. violaceum* CV026 (10^6 CFU/mL). Induction of violacein production by the exogenous AHL in the mutant was measured after 24 hr as described earlier. Synthetic C_6-HSL (10 μmol/mL) was used as a positive control.

Identification of the major active compounds and their anti-QS activities

The FL-fraction was subjected to silica gel TLC by eluting with a chloroform:ethyl acetate (80:20) solvent system. The developed plates were overlaid with *C. violaceum* 12472-seeded LB medium and incubated for 24 hr. Spots corresponding to violacein inhibition zones recovered from preparative TLC plates were further tested for the presence of glycoside and aglycone as described previously (25). Broad anti-QS spots from the preparative TLC were also subjected to LC–MS analysis on BDS HYPERSIL RP-C_{18} columns (4.6 mm internal diameter × 250 mm, Thermo Finnigan Surveyor; Thermo Fisher Scientific, Pittsburgh, PA, USA) with CH_3CN:H_2O (40:60) solvent system at a flow rate of 0.5 mL/min. The molecular weights of the compounds were determined using a Thermo LCQ Deca XP MAX electrospray ionization mass spectrometer (Thermo Fisher Scientific).

The major compounds identified, namely quercetin and quercetein-3-*O*-arabinoside, were purchased from Sigma–Aldrich and tested for their anti-QS activity by

evaluating the inhibition of violacein production in *C. violaceum* 12472, as described earlier.

Data analysis

All experiments were conducted in quadruplicates at least twice. ANOVA was used to analyze the differences between the treatments using STATISTICA. $P \leq 0.01$ was considered as significant unless otherwise specified.

RESULTS

Inhibition of QS by FL-fraction of *P. guajava*

The FL-fraction of *P. guajava* showed anti-QS activity in a *C. violaceum* CV026 biosensor bioassay. At a concentration of 20 μg/disc, a colorless turbid zone of violacein inhibition (6 mm) was evident and at higher concentrations (50 and 100 μg/disc), conspicuous zones of QS inhibition (18 and 22 mm) were observed (Table 1). However, at a lower concentration (10 μg/disc), the FL-fraction did not inhibit QS activity.

Inhibition of violacein production in *C. violaceum* 12472

Concentration-dependent inhibition of violacein production in *C. violaceum* 12472 by FL-fraction was observed. At 50 μg/mL, FL-fraction inhibited violacein production by 50%; at 300 μg/mL, complete inhibition of violacein production was observed (Fig. 1). For all tested concentrations, viable cell counts did not differ significantly from those of controls (Table 2).

Effect of FL-fraction on virulence factor production in *P. aeruginosa* PAO1

Pyocyanin production in *P. aeruginosa* PAO1 was reduced by 50% at 25 μg/mL and completely inhibited at 200 μg/mL when grown in the presence of FL-fraction (Fig. 2a). Similarly, significant decreases in elastolytic and proteolytic activities were observed, with complete inhibition at 200 μg/mL (Fig. 2b,c). Swarming motility was completely inhibited at a minimum concentration of 25 μg/ml of FL-fraction (Table 3). In the presence of FL-fraction at all the tested concentrations, bacteria were able to grow and form colonies at the inoculated sites; however, tendril formation and other features indicative of swarming motility were not observed (Fig. S1). Similarly, biofilm formation was significantly reduced (80%) at a concentration of 200 μg/mL compared with controls (Table 3), whereas planktonic cell density was not affected.

Table 1. The effect of flavonoid fraction (FL-fraction) of *Psidium guajava* on QS-regulated violacein production in *Chromobacterium violaceum* CV026 in biosensor bioassay

Concentration (μg/disc)	Diameter of violacein inhibition zone (mm)
10	NA
20	06 ± 0.5
50	18 ± 1.0
100	22 ± 0.5

Values are presented as mean ± SD, $n = 4$.
NA, no activity.

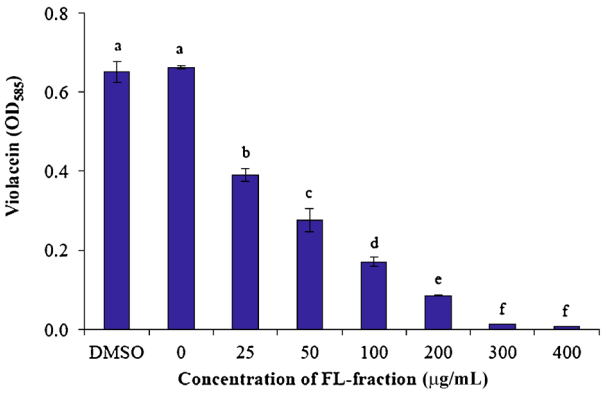

Fig. 1. Inhibition of violacein production in *Chromobacterium violaceum* ATCC12472 by different concentrations of *Psidium guajava* FL-fraction. Values are presented as mean ± SD, $n = 4$. Matching letters on the columns indicate no significant difference ($P < 0.05$).

Effect of FL-fraction on AHL synthesis

The effects of FL-fraction on AHL synthesis were determined using a C_6-HSL overproducing wild type strain, *C. violaceum* 31532 and its mutant *C. violaceum* CV026, which responds to exogenous AHL. Figure 3a shows inhibition of violacein production in *C. violaceum* 31532 by FL-fraction. However, the AHL extracted from these culture supernatants was able to induce violacein production in the mutant *C. violaceum* CV026 (Fig. 3b). This indicates that AHL synthesis is not affected by the FL-fraction. Interestingly, at all the tested concentrations of FL-fraction, the viable counts in the cultures of *C. violaceum* 31532 were not significantly different from those of the controls (Table 2).

Identification of the major active compounds in the FL-fraction and their anti-QS activities

To further identify the compounds responsible for anti-QS activity, TLC separation of the FL-fraction and

Table 2. The effects of increasing concentrations of FL-fraction of *Psidium guajava* in LB medium on growth of *Chromobacterium violaceum* ATCC12472, *C. violaceum* ATCC31532 and *Pseudomonas aeruginosa* PAO1

Concentration of FL-fraction (μg/mL)	*C. violaceum* 12472 (10^8 CFU/mL)	*C. violaceum* 31532 (10^8 CFU/mL)	*P. aeruginosa* PAO1 (10^8 CFU/mL)
Control	2.18 ± 0.03	2.32 ± 0.04	2.23 ± 0.03
Solvent control	2.17 ± 0.05	2.30 ± 0.03	2.24 ± 0.04
25	2.12 ± 0.02	2.28 ± 0.03	2.25 ± 0.02
50	2.15 ± 0.03	2.31 ± 0.05	2.21 ± 0.02
100	2.09 ± 0.04	2.27 ± 0.04	2.19 ± 0.01
200	2.13 ± 0.03	2.29 ± 0.02	2.25 ± 0.03
300	2.11 ± 0.01	2.28 ± 0.02	2.21 ± 0.04
400	2.12 ± 0.02	2.25 ± 0.01	2.22 ± 0.03

Values are presented as mean ± SD, $n = 4$, $P < 0.05$.

biosensor overlay revealed a violacein inhibition broad spot (R_f value ~0.30), indicating the possibility that more than one compound was present (data not shown). Acid hydrolysis and further separation revealed the presence of a glycoside with R_f value similar to that of standard arabinose. In a separate TLC system, the R_f value of aglycone from the hydrolysate matched with standard quercetin. However, LC–MS analysis exhibited two major peaks with m/z 433 $(M\text{-}H)^-$ and m/z 301 $(M\text{-}H)^-$, corresponding to quercetin-3-*O*-arabinoside and quercetin, respectively (Fig. S2). Interestingly, both authentic standards, quercetin and quercetin-3-*O*-arabinoside, showed complete inhibition of violacein production in *C. violaceum* 12472 at concentrations of 50 and 100 μg/mL, respectively, without any bactericidal effect (Fig. 4).

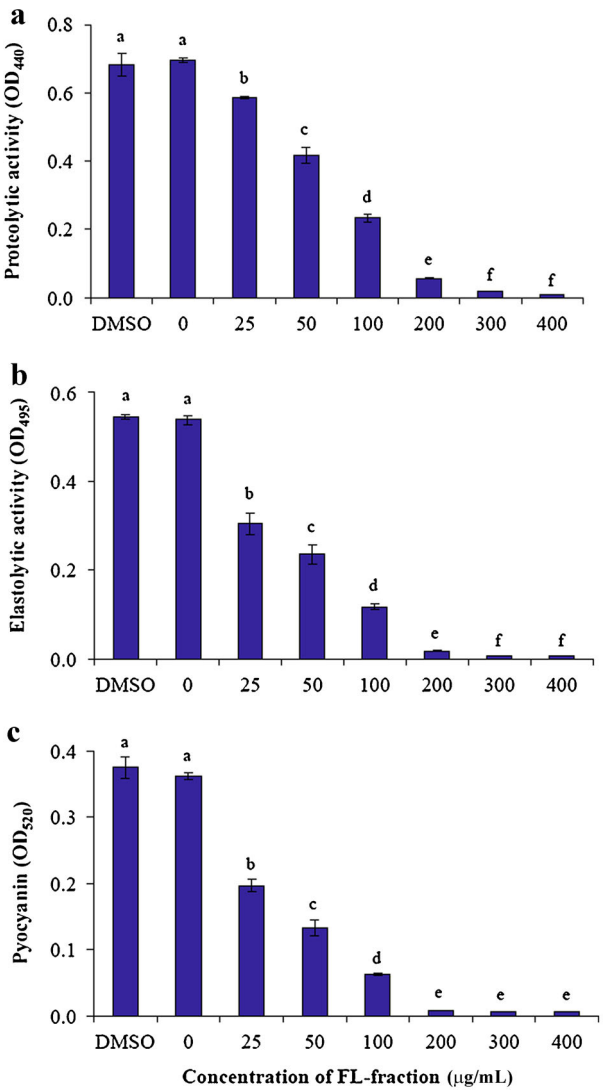

Fig. 2. Effect of *Psidium guajava* FL-fraction on QS-regulated virulence factor production in *Pseudomonas aeruginosa* PAO1. (a) Proteolytic activity; (b) elastolytic activity; (c) pyocyanin production. Values are presented as mean ± SD, $n = 4$. Matching letters on the columns indicate no significant difference ($P < 0.05$).

DISCUSSION

The findings of this study explain the anti-QS activity of the FL-fraction of *P. guajava* in *C. violaceum* and *P. aeruginosa* PAO1. The FL-fraction studied completely inhibited QS-controlled violacein production at 300 μg/mL. However, even at 400 μg/mL, no inhibition of bacterial growth was observed, strongly indicating that inhibition of violacein production is not attributable to bactericidal activity. Interfering with QS pathways inhibits the bacterial population's ability to monitor the number of cells within that population and thereby interferes with QS activation of virulence factor expression (26).

Because infections with *P. aeruginosa* PAO1 are potentially fatal, QS in these organisms has been studied extensively and is often used in anti-QS screening assays. They have a well-established QS mechanism that controls the expression of a number of genes involved in the production of virulence factors and biofilm formation (27). At 200 μg/mL, the FL-fraction of *P. guajava* completely inhibited QS-controlled pyocyanin production and elastolytic activity in *P. aeruginosa* PAO1. However, complete inhibition of proteolytic activity required a higher concentration of FL-fraction (300 μg/mL). Pyocyanin production is under the control of the RhlI/R QS system and plays a significant role in cystic fibrosis (28). Over 75% of clinical isolates of

Table 3. Inhibition of swarming motility and biofilm formation in *Pseudomonas aeruginosa* PAO1 by different concentrations of *Psidium guajava*

Concentration of FL-fraction (μg/mL)	Swarming diameter (mm)	Biofilm (OD_{590})
Control	65.75 ± 0.95	0.454 ± 0.008
25	15.25 ± 1.50[a]	0.220 ± 0.012[a]
50	08.50 ± 1.29[b]	0.119 ± 0.005[b]
100	05.00 ± 1.08[c]	0.086 ± 0.016[c]
200	05.00 ± 0.83[c]	0.041 ± 0.012[d]
300	05.00 ± 1.30[c]	0.019 ± 0.014[de]
400	04.00 ± 0.74[c]	0.009 ± 0.012[e]

Matching letters in the columns indicate no significant difference ($P < 0.05$).

Bacteria were grown in LB semisolid medium and in tryptone broth for swarming and biofilm assay, respectively. Values are mean presented as mean ± SD. $n = 4$.

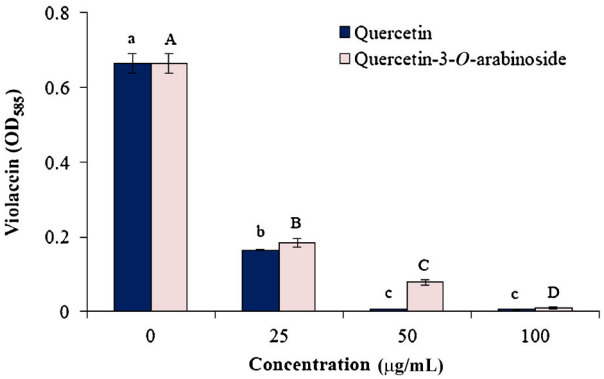

Fig. 4. Inhibition of violacein production in *Chromobacterium violaceum* ATCC12472 by different concentrations of standard quercetin and quercetin-3-*O*-arabinoside. Values are presented as mean ± SD, $n = 4$. Matching letters on the columns indicate no significant difference ($P < 0.05$).

P. aeruginosa secrete elastase B, an elastolytic metalloproteinase that is encoded by the lasB gene under QS regulation. *In vitro* studies have demonstrated that LasB elastases are able to degrade a number of components of both the innate and adaptive immune systems (29). Because of the apparent role of QS in biofilm formation in *P. aeruginosa* (30), QS inhibitors have been proposed as promising antibiofilm agents (31). Swarming motility has also been implicated in the early stages of *P. aeruginosa* biofilm establishment (32). In this study, *P. guajava* extract inhibited both swarming motility and biofilm formation in *P. aeruginosa* PAO1.

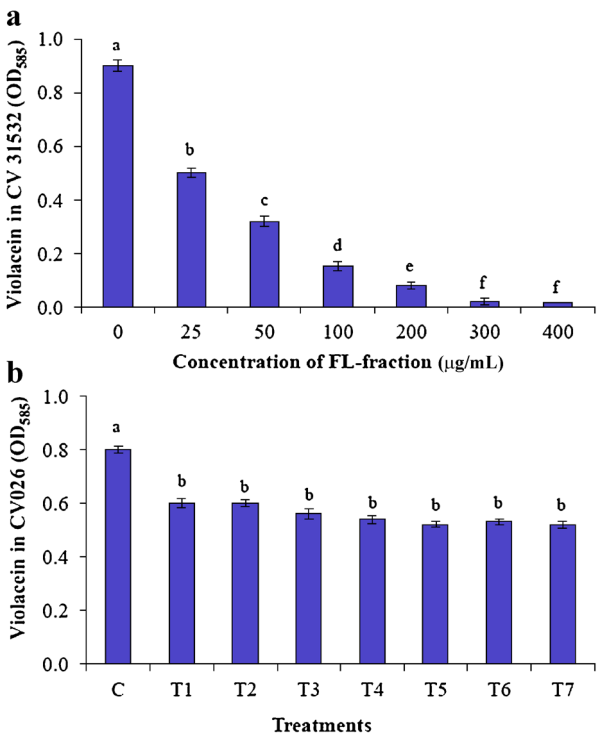

Fig. 3. Effect of FL-fraction of *Psidium guajava* on (a) Violacein production in *Chromobacterium violaceum* ATCC31532 and (b) violacein production in the mutant *C. violaceum* CV026 by AHL extracted from the culture supernatants of *C. violaceum* 31532 grown in the presence of FL-fraction. Values are presented as mean ± SD, $n = 4$. Matching letters on the columns indicate no significant difference ($P < 0.05$).

Analytical investigations of FL-fraction of *P. guajava* showed that quercetin and quercetin-3-*O*-arabinoside are responsible for anti-QS activity. Both these compounds individually showed anti-QS activity against *C. violaceum* 12472 at lower concentrations than the crude FL-fraction. Advances in natural product extraction technology offer better separation processes for more efficient extraction of the active fractions in purer forms. Recent research highlights the benefits of complementary and synergetic effects of multiple phytocompounds in the disease-healing process (33). At a high concentration of 4 mM (~1.2 mg/mL), quercetin was earlier shown to inhibit violacein production in *C. violaceum* CV026, along with some reduction in bacterial growth (9). Though quercetin-3-*O*-arabinoside was earlier reported to have antibacterial activity (MIC 300 μg/mL) against *Salmonella enteritidis*, its anti-QS activity is poorly understood. Quercetin-3-*O*-arabinoside shows a high MIC (4 mg/mL) against *Streptococcus mutans* and, at sub-MIC levels (up to 2 mg/mL), it reportedly inhibits sucrose-dependent adherence and aggregate formation (34). This indicates that quercetin-3-*O*-arabinoside has different modes of action on different bacteria.

Anti-QS compounds, namely, furonones, catechin, iberin and epigallocatechin gallate, show a similar mode of action, that of targeting the QS system without

affecting bacterial growth (35, 36). At higher concentrations, most anti-QS compounds have bactericidal effects, which has often led to them being mistakenly identified as antibacterials. Alternatively, a few antibacterial compounds of natural origin with higher MIC values have been shown to have potential activity against the QS signaling mechanism at sub-MIC concentrations. Compounds such as malabaricone C from *Myristica cinnamomea* and standard catechin inhibit pyocyanin production in *P. aeruginosa* by 50% at a concentration of 1 mg/mL (37). Similarly, Vandeputte *et al.* reported a 50% decrease in pyocyanin and 30% decrease in elastase activity in *P. aeruginosa* at 16 and 4 mM concentrations, respectively, of catechin from *Combretum albiflorum* (24).

Inhibition of QS can be achieved either by interruption of AHL signal generation, inhibition of AHL signal dissemination or inhibition of AHL signal reception (38). Given that the AHL extracted from cultures of *C. violaceum* 31532 induces violacein production in the mutant *C. violaceum* CV026, it can be assumed that the FL-fraction interferes with the QS by the inhibition of AHL signal response rather than production. Cell viability and AHL production in *C. violaceum* 31532 was not affected significantly in presence of FL-fraction. This indicates the target-specific activity of the phytocompounds in the FL-fraction.

The medicinal use of *P. guajava* has been reported in indigenous system of medicines and its use continues today in topical home remedies and personal care products. In clinical trials, standardized leaf extract of *P. guajava* significantly reduced infectious gastroenteritis without any adverse effects on patients (39). Acute toxicity tests in rats and mice have proven the LD_{50} of *P. guajava* leaf extracts is more than 5 g/kg body weight (40).

The quorum sensing system is considered a promising and novel target for the development of anti-pathogenic drugs, especially for reducing the selection pressure caused by conventional antibiotics. Over the last few years, inhibition of QS has become a very intense area of research because of its applications in medicine, industry and agriculture. *Psidium guajava* is a potential source of anti-QS compounds for the development of effective alternative therapeutics. The findings from this study warrant further research at the molecular level to explore the exact mechanisms of action of these phytocompounds.

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DISCLOSURE

There are no conflicts of interest.

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Supporting Information

Additional supporting information may be found in the online version of this article at the publisher's web-site.

Fig S1. Inhibition of swarming motility in *Pseudomonas aeruginosa* PAO1 by different concentrations of *Psidium guajava* FL-fraction. (a) Control, (b) 25 μg/mL, (c) 50 μg/mL, (d) 100 μg/mL.

Fig S2. Identification of anti-QS compounds present in FL-fraction of *Psidium guajava*. (a) LC/MS analysis of FL-fraction showing two major peaks and their respective MS analysis. (b) Quercetin-3-O-arabinoside (RT 8.58, m/z 433 [M-H]).