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Pseudomonas aeruginosa is an Effective Indicator for Screening of Quorum Sensing Inhibition by Plant Extracts

Zahraa E Sadeq[1], Inam J Lafta[*2]

[1]Department of Biology, College of Medicine, Wasit University, Wasit, Iraq, [2]Department of Microbiology, College of Veterinary Medicine, University of Baghdad, Baghdad, Iraq

***Correspondence**:
inam.j@covm.uobaghdad.edu.iq
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A B S T R A C T

Quorum sensing is a well-known mechanism used by many bacteria to control important virulence factors through the production and subsequent response to *N*-acylhomoserine lactones (AHLs). Quorum sensing inhibition (QSI), targeting AHL-dependent signalling, has been reported as a strategy for the control of bacterial pathogenicity. Thus, this study aimed at investigating the capability of *Pseudomonas aeruginosa* as an indicator to rapidly screen for potential QSI caused by plant extracts based on pigment inhibition. Four ethanolic plant extracts including: dried flower buds of clove (*Syzygium aromaticum* L), bark of cinnamon (*Cinnamomum cassia* L), leaves of moringa (*Moringa oleifera* Lam), and Al-Gutub (*Tribulus terrestris* L) dried fruits were investigated for their QSI ability in comparison with furanone (the known QS inhibitor). Agar well diffusion assay was used, in which the indicator isolate, *P. aeruginosa*, was inoculated onto Pseudomonas special agar medium containing 8 mm wells to load different concentrations of the plant extracts along with furanone or phosphate buffered saline (negative control). The results showed that furanone was successful in inhibiting the pyocyanin pigment of *P. aeruginosa* without interfering with the bacterial growth, this verified the ability of this bacterium as a potent QS indicator organism. Importantly, clove extract at different concentrations caused complete inhibition of the pyocyanin pigment with little impact on the bacterial growth. On the use of cinnamon, only the high concentrations showed clear dye inhibition of the indicator organism. Moringa also caused certain degree of dye inhibition but only at high concentrations. Conversely, Al-Gutub neither affected on the pyocyanin production nor on the bacterial viability. In conclusion, *P. aeruginosa* isolate, pyocyanin producer, was successful in the screening of QS inhibitors. This simple study recommends the use of such isolate without relying on the import of certain strains of *Chromobacterium violaceum*, *Pseudomonas aureofaciens*, or *Agrobacterium tumefaciens*.

Keywords: *Pseudomonas aeruginosa*, quorum sensing, quorum sensing inhibition, indicator organism, plant extracts

INTRODUCTION

Several researchers have discussed the link between the quorum sensing (QS) signaling system and the synthesis of many virulence factors by pathogenic microbes (1-3). Bacterial cells communicate through a technique known as QS, which uses chemical signals called autoinducers (AIs) to alter the behavior of large populations of both Gram-positive and Gram-negative bacteria. This phenomenon, which encompasses the entire range of interspecies interactions as well as interactions between bacteria, arises when there is a high enough cell density in a particular bacterial environment (4). Li and Tian (5) explored the relationship between QS and biofilm development in Gram-negative and Gram-positive bacteria. Due to QS-mediated virulence and owing to development of

drug resistance by pathogens, as well as the fact that QS is not required for bacterial growth (6), there is an urgent need to produce powerful QS inhibitors, also known as quorum quenchers, as therapeutic agents (7).

Among the potent QS inhibitors is plant extracts. Plants generate a wide range of chemicals, including flavonoids, simple phenolics, terpenoids, and alkaloids (8). Utilizing plants, plant products, and their purified parts may pave the way for these chemicals to be used as innovative anti-QS agents (9). Plant extracts are complex combinations of active, partially active, and inactive ingredients (10). The development of novel, nontoxic, and broad-spectrum QS inhibitor medicines derived from plants has been extremely beneficial in recent years. For centuries, clove (*Syzygium aromaticum*) has been utilized for its therapeutic properties and as a food preservative (11). It has been linked to a number of medical benefits, and its extracts have been shown to exhibit strong antibacterial action against pathogenic *Klebsiella pneumoniae* and *Escherichia coli* (12). One of the main phytoconstituents of cinnamon is cinnamaldehyde, which has been shown to work as an anti-QS agent by inhibiting QS virulence factors and biofilm formation in *P. fluorescence* at sublethal concentrations (13). Because of its properties as antibacterial, antifungal, nematocidal, antipyretic, insecticidal, antioxidant, and antidiabetic, cinnamon is utilized in traditional medicine all over the world (14-17). Additionally, *Moringa oleifera* is a powerhouse of nutrition that has been used in traditional medicine for centuries. Its leaves, seeds, and bark contain a variety of compounds with antimicrobial activities (18). Significant reductions in violacein and pyocyanin synthesis, along with interference with swarming motility and biofilm formation, were seen in the extracts of moringa leaves, indicating a powerful suppression of QS (18). Finally, *Tribulus terrestris* (Al-Gutub) has been used for various ailments. Steroidal saponins, flavonoids, tannins, terpenoids, polyphenol carboxylic acids, and alkaloids have all been found in its extract as having a wide range of biological capabilities and chemical structures (19). Numerous *in vitro* investigations have demonstrated the antibacterial effectiveness of whole or fractionated *Tribulus terrestris* extracts against Gram-negative and Gram-positive bacteria (20-23). Its root's hydro-alcoholic extracts demonstrated positive quorum quenching effect by successfully suppressing QS-controlled processes, such as pigment synthesis and biofilm formation. Although they had no effect on organism development, they did divert Gram negative bacteria's signaling communication (24).

In light of the antibiotic resistance problem that currently impedes treatment of many bacterial infections, the prospect of targeting QS as a means of alleviating or preventing infection has been welcomed with high hopes (6). As a result, a variety of techniques have been employed; these techniques can be broadly categorized into two groups: biological screening and non-biological screening (7). Among the non-biological screening techniques are: 1) the combinatorial strategy, which primarily uses the solid-phase synthesis of peptide synthesizer to generate a library of compounds (25). It is recognized as one of the most effective ways for testing a large number of compounds against a variety of targets. 2) The in-silico approach is one of the most popular techniques employed in studies based on rational drug design. This is a low-cost method of leveraging high-performance computing to screen a lot of databases. The success rate of drug development programs is raised by the application of informatics approaches (26).

When it comes to the biological screening approach, different biomonitor species are used to screen for QS inhibitors (7). The increasing variety in QS signals produced by various organisms highlighted the need for biosensors capable of detecting QS inhibitors (7). Numerous bacterial reporter assays exhibiting QS-regulated phenotypes, such as bioluminescence and color pigments, have been devised to pinpoint chemical substances capable of disrupting or impeding various bacterial autoinducer QS systems (27-31). Three types of AIs are most frequently studied: peptide signals, which are used by Gram-positive bacteria; acylated homoserine lactones (AHLs), which are used by Gram-negative bacteria (also called autoinducer-1 [AI-1]); and autoinducer-2 (AI-2), which is used by both Gram-positive and Gram-negative bacteria. Beyond these classifications, there exist additional QS signals, such as the autoinducer-3 (AI-3), diffusible signal factor (DSF), and pseudomonas quinolone signal (PQS) (6).

Chromobacterium violaceum strains are widely utilized as reporter bacteria to investigate the potential anti-QS activities of bioactive essential oils and essential oil constituents, particularly in Gram-negative bacteria (32). The formation of the purple pigment violacein in *C. violaceum* strains ATCC 12472 and ATCC 31532 is triggered by distinct AHL autoinducers, but in strain CV026, which lacks AHL synthase, violacein production necessitates the exogenous addition of C6-AHL (33). Other reporter strains are also used for detection of anti-QS substances, e.g., specific strains of *Pseudomonas aureofaciens*, *Agrobacterium tumefaciens* and a few others. However, international trade of such microorganisms is prohibited, making developing countries, such as Iraq, deprived of such experiments for peaceful uses. Taken together, this study aimed at investigating the capability of *Pseudomonas aeruginosa* as an indicator to rapidly screen for potential QS inhibitors from plant extracts based on pigment inhibition in a simple protocol.

MATERIALS AND METHODS

Ethical Approval

This study was reviewed and approved by the local Ethics Committee of the Faculty of Veterinary Medicine of

University of Baghdad, with approval number 1665, dated 26/9/2023.

Bacterial Isolate

A confirmed isolate of *P. aeruginosa* obtained from a cat of two-years old infected with otitis externa was used in this study. The isolate was fully identified by the conventional and molecular assays described by Hamad and Abdulgafor (34). This clinical isolate was chosen to be used for the current study because it produced strong pyocyanin pigment on Pseudomonas agar medium (HiMedia, India). It was kept in Luria-Bertani (LB) agar (HiMedia, India) slant at 4 °C with proper sub-culturing in the Bacteriology Laboratory, Department of Microbiology, University of Baghdad, Baghdad, Iraq.

Preparation of Plant Extracts

Four medicinal plant extracts were used in this study, including dried flower buds of clove (*Syzygium aromaticum* L), bark of cinnamon (*Cinnamomum cassia* L), leaves of moringa (*Moringa oleifera* Lam), and Al-Gutub (*Tribulus terrestris* L) dried fruits. The herbs were purchased from a local market in Baghdad city, Iraq, and they were identified and authenticated by Directorate of Seed Testing and Certification, Ministry of Agriculture, Baghdad, Iraq.

Ethanolic plant extracts were made according to the procedure described by Abubakar and Haque (35). Briefly, 50 g of the ground plants were placed separately in a glass beaker (1 L volume) and mixed with 500 mL of 70% ethanol (BDH-limited poole, England). Then, the beaker was incubated onto a shaker incubator (Lab Companion, Korea) overnight at room temperature. Afterwards, the solution was filtered by filter paper (Whatman No.1) through the use of Buckner Funnel under negative pressure, and the filtrate was placed in a container and left at room temperature until the liquid evaporated and a powder was obtained (36). Finally, a stock from each plant extract was prepared by dissolving 1 mg of each extract into 1 mL of sterile phosphate buffered saline (PBS, pH 7.4). Different 2-fold serial dilutions were made from the stock extract (1000 µg/mL) using PBS to yield the following concentrations: 500 µg/mL, 250 µg/mL, 125 µg/mL, 62.5 µg/mL, 31.25 µg/mL, 15.63 µg/mL, 7.81 µg/mL, and 3.9 µg/mL. While PBS was used as a negative control, furanone (Tokyo Chemical Industry Co, China) was the positive control, in which a working concentration of 1 mg/mL was made from the stock (10 mg/mL) by mixing 100 µL of the stock with 100 µL of normal saline.

Plant Extraction Yield

The extraction yield percentage of the plants under study was determined according to the equation described by Magangana et al. (37).

$$\text{Yield Extract (\%)} = \frac{\text{Weight of plant extract}}{\text{Weight of crude plant powder}} \times 100$$

Assay of QS Detection

The QS detection assay was performed to confirm the suitability of *P. aeruginosa* isolate with pyocyanin pigment as an indicator for AHL (QS) whose signal had to be inhibited by the well-known QS inhibitor, furanone. The Agar well diffusion assay was applied as described by the Clinical and Laboratories Standards Institute (CLSI) (38) with some modifications. Briefly, overnight suspension of *P. aeruginosa* isolate containing 10^6 CFU/mL was spread by a sterile cotton swab on Pseudomonas agar base. Subsequently, wells of 8 mm diameter were punched into the agar medium and filled with 100 µL of furanone (the positive control), nutrient broth or PBS (negative controls), and allowed to diffuse at room temperature for 2 h. Then, the plates were incubated in the upside position at 37 °C for 24 h. After incubation, the plates were inspected for stain disappearance.

Assay for QSI Detection

The procedure of CLSI (38) described above was applied for the QSI detection. Briefly, after spreading the indicator *P. aeruginosa* onto two plates of the Pseudomonas agar, 8 mm wells were made (4 for each plate) and filled with 100 µL of different concentrations of the plants extracts mentioned above (500 µg/mL to 3.9 µg/mL). Furanone and PBS (as well as sterile nutrient broth in some cases) were used as positive and negative control, respectively. Following incubation, the plates were inspected for presence of growth inhibition zones or stain disappearance. A positive result was indicated by the presence of QSI (color disappearance) without or with few growths inhibition. The zones of pigment inhibition were measured using a ruler.

Statistical Analysis

Data were subjected to one-way analysis of variance (ANOVA) and the LSD test at each concentration separately ($P \leq 0.05$). Data were analyzed using JMP® Pro 16.0.0 software (SAS, Institute Inc., Cary, NC, USA).

RESULTS

Plant Extract Properties

Plants ethanolic extracts yielded products of different properties (Figure 1). For example, cinnamon extract was a powder of dark reddish brown color and had a pleasant pungent smell. The ethanolic extract of cinnamon had a yield of 18%. Clove extract, on the other hand, was a powder of brown to black color with a pleasant and pungent aroma and yielded 62%. Concerning moringa, its ethanolic extract yield was 32% and had a burnt brown to black colored powder with acceptable pungent odor. Finally, Al-Gutub had a yield of 18% with dark greenish-black color along with thick sticky texture with acceptable smell.

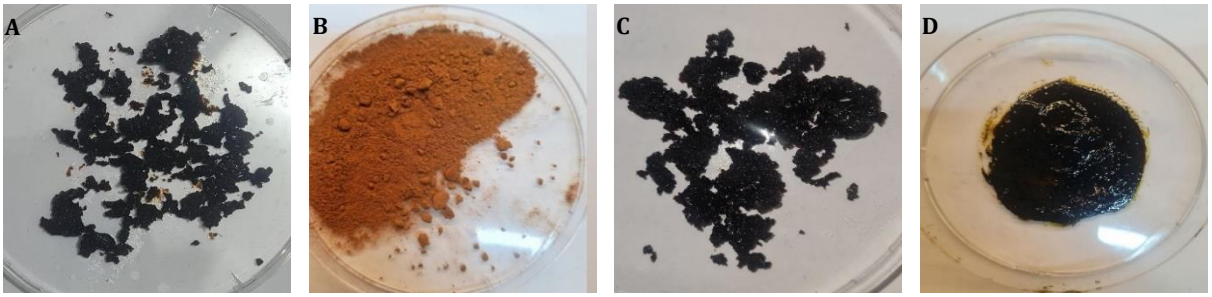

Figure 1. Ethanolic extracts of the plants used in this study. **(A)** Clove, **(B)** Cinnamon, **(C)** Moringa, **(D)** Al-Gutub

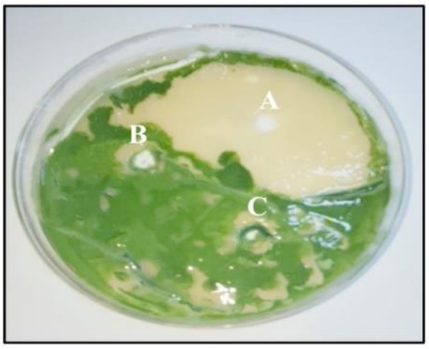

Figure 2. *Pseudomonas aeruginosa* grown on Pseudomonas special agar medium to be used as an indicator for QS. **(A)** 100 µL furanone (well-known QS inhibitor) as a positive control, **(B)** 100 µL PBS as a negative control, **(C)** 100 µL sterile nutrient broth as another negative control

Table 1. Approximate zones of pyocyanin inhibition of *Pseudomonas aeruginosa* treated with cinnamon, clove, moringa, and Al-Gutub extracts

	Inhibition zone diameter (mm)				
Concentration (µg/mL)	**Al-Gutub**	**Cinnamon**	**Clove**	**Moringa**	***P*-value**
3.9	11.7±0.33 ^{d}	40.0±0.58 ^{b}	50.3±0.33 ^{a}	18.7±0.67 ^{c}	<0.001
7.81	9.67±0.33 ^{d}	40.0±0.58 ^{b}	50.3±0.33 ^{a}	18.7±0.67 ^{c}	<0.001
15.63	12.0±0.58 ^{c}	50.0±0.58 ^{a}	50.3±0.33 ^{a}	19.7±0.33 ^{b}	<0.001
31.25	9.33±0.33 ^{c}	50.3±0.33 ^{a}	51.0±0.58 ^{a}	20.0±0.58 ^{b}	<0.001
62.5	10.0±0.58 ^{c}	50.3±0.33 ^{a}	51.0±0.58 ^{a}	39.0±0.58 ^{b}	<0.001
125	0.00±0.00 ^{c}	50.7±0.33 ^{a}	51.3±0.33 ^{a}	39.3±0.33 ^{b}	<0.001
250	0.00±0.00 ^{d}	50.7±0.33 ^{b}	51.7±0.33 ^{a}	40.3±0.33 ^{c}	<0.001

Values for each concentration are means ± SEM, with n = 3. $^{a-d}$Means in each row with a different superscript letter differ at $P \leq 0.01$, as examined by one-way ANOVA and the LSD test at each concentration individually

P. aeruginosa as QS indicator

Furanone was successful in inhibiting the pyocyanin pigment of *P. aeruginosa* with an inhibition zone of 40 mm, this verified the ability of this bacterium as a potent QS indicator organism (Figure 2). Pertaining to the growth inhibition, furanone showed no effect on the bacterial growth.

Screening for Plant Extracts with QSI Features

Table 1 illustrates the zones of pigment inhibition caused by each plant extract. Among the tested extracts, i.e. cinnamon, clove, moringa, and Al-Gutub, the first two extracts (i.e., cinnamon and clove) exhibited significant QSI, as observed by the absence of the *P. aeruginosa* pyocyanin pigment with very slight interference with the bacterial growth. Concerning cinnamon, the dye inhibition was clear at most of the concentrations (500 µg/mL, 250 µg/mL, 125 µg/mL, 62.5 µg/mL, 31.25 µg/mL, and 15.63 µg/mL) than the lower concentrations, particularly 7.81 µg/mL and 3.9 µg/mL (Figure 3).

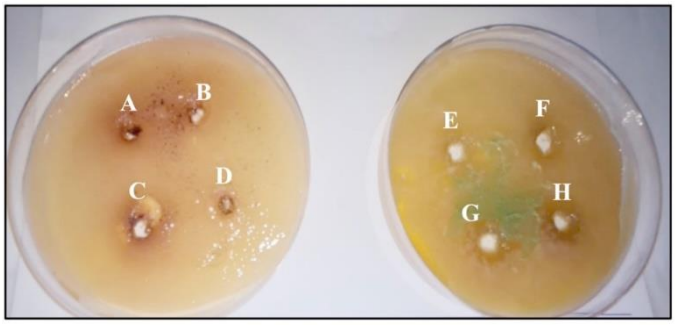

Figure 3. *Pseudomonas aeruginosa* grown on Pseudomonas special agar medium to detect inhibition of its pyocyanin dye by the QS inhibitor cinnamon extract. Each well contains 100 µL of **(A)** 500 µg/mL, **(B)** 250 µg/mL, **(C)** 125 µg/mL, **(D)** 62.5 µg/mL, **(E)** 31.25 µg/mL, **(F)** 15.63 µg/mL, **(G)** 7.81 µg/mL, and **(H)** 3.9 µg/mL of cinnamon ethanolic extract.

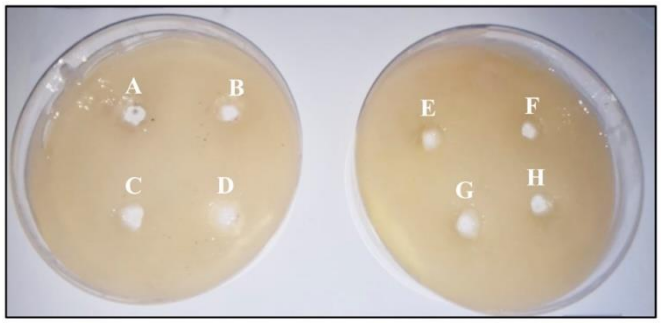

Figure 4. *Pseudomonas aeruginosa* grown on Pseudomonas special agar medium to detect inhibition of its pyocyanin dye by the QS inhibitor clove extract. Each well contains 100 µL of A- 500 µg/mL, (B) 250 µg/mL, C- 125 µg/mL, D- 62.5 µg/mL, E- 31.25 µg/mL, F- 15.63 µg/mL, G- 7.81 µg/mL, and H- 3.9 µg/mL of clove ethanolic extract

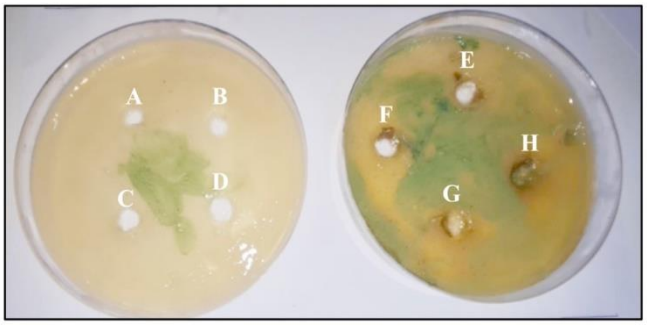

Figure 5. *Pseudomonas aeruginosa* grown on Pseudomonas special agar medium to detect inhibition of its pyocyanin dye by the QS inhibitor moringa extract. Each well contains 100 µL of **(A)** 500 µg/mL, **(B)** 250 µg/mL, C- 125 µg/mL, **(D)** 62.5 µg/mL, E- 31.25 µg/mL, **(F)** 15.63 µg/mL, G- 7.81 µg/mL, and **(H)** 3.9 µg/mL of moringa ethanolic extract

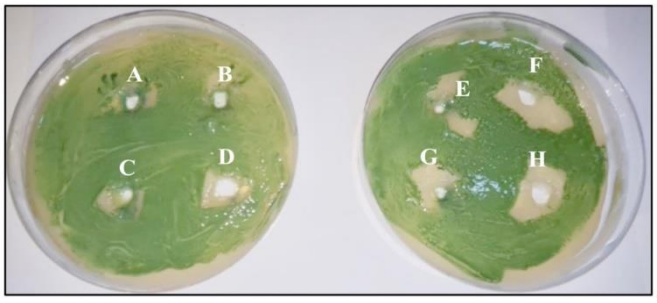

Figure 6. *Pseudomonas aeruginosa* grown on Pseudomonas special agar medium to detect inhibition of its pyocyanin dye by the QS inhibitor Al-Gutub extract. Each well contains 100 µL of **(A)** 500 µg/mL, **(B)** 250 µg/mL, **(C)** 125 µg/mL, D- 62.5 µg/mL, **(E)** 31.25 µg/mL, **(F)** 15.63 µg/mL, G- 7.81 µg/mL, and H- 3.9 µg/mL of Al-Gutub ethanolic extract

Figure 7. *Pseudomonas aeruginosa* grown on Pseudomonas special agar medium containing a well filled with PBS only without plants extracts as a control

Interestingly, clove extract caused complete inhibition for the pyocyanin pigment at all the concentrations tested (Figure 4) with little non-significant impact on the bacterial growth. On the other hand, moringa with high concentrations (500 µg/mL, 250 µg/mL, 125 µg/mL, and 62.5 µg/mL) reduced the dye to some extent compared to the low concentrations (31.25 µg/mL, 15.63 µg/mL, 7.81 µg/mL, and H- 3.9 µg/mL) (Figure 5). By contrast, Al-Gutub neither influenced the dye production nor inhibited the bacterial growth at high concentrations relative to the low ones, which showed small zones of dye disappearance; this was clearer at the concentrations of 15.63 µg/mL, 7.81 µg/mL, and 3.9 µg/mL (Figure 6). Figure 7 reveals strong pyocyanin dye of *P. aeruginosa* grown on Pseudomonas special agar medium containing a well of PBS only without plants extracts as a control.

DISCUSSION

A lot of work has gone into developing non-antimicrobial therapy alternatives for managing pathogenic bacterial activities. In the current study, furanone the well-known QS inhibitor was effective in inhibiting the pyocyanin pigment of *P. aeruginosa*, this verified the ability of this bacterium as a potent QS indicator organism that produces QS signal, which in turn dampened by the anti-QS furanone. This was clear through the large zone of dye disappearance formed around the well containing furanone relative to the negative controls, i.e. PBS. *Chromobacterium violacium* has long been the most popular reporter system for rapid evaluation of AHL-related inhibitory action. This bacterium is distinguished by its capacity to manufacture violacein, a water-insoluble purple pigment whose synthesis is positively regulated by AHLs (6).

Pseudomonas aeruginosa was chosen in this study as a QS indicator organism because among several bacterial species, Pseudomonas was reported to produce more AHL than other bacteria (39). It is known that its three QS genes (*lasI*, *rhlI*, and *qscI*) yield two AHL molecules: N-(3-Oxododecanoyl)-L-homoserine lactone and N-butanoyl homoserine lactone, which bind to the corresponding transcriptional receptors (LasR, RhlR, and QscR) (40–42). However, in the study of Mohammed and Zgair (43), it has been found that all the studied environmental and clinical *P. aeruginosa* isolated from different areas in Iraq contained seven genes (*rhlA*, *rhlR*, *rhlI*, *lasR*, *lasI*, *lasB*, and *phzA1*) associated with QS occurrence.

Plants ethanolic extracts used in this study yielded products of various properties and the extraction yield percentages differed among the used plants. For instance, the yield of cinnamon ethanolic extract was 18% compared to clove ethanolic extract, which had a yield of 62%. Compared with the study of Julianti et al. (44), the ethanolic extract of cinnamon bark showed yield percentage of 15.93% (w/w). Based on phytochemical screening,

cinnamon bark extract had alkaloids, flavonoid, steroid/ triterpenoid, tannin, and quinone without saponin (44). However, Waty *et al*. (45) revealed presence of alkaloid, flavonoid, saponin, and glycosides. In comparison with another study, cinnamon was found to contain tannins, carbohydrates, phenols, flavonoids, saponin, alkaloid, coumarins, terpenes, and steroids, but it was negative to three tests (glycosides, resins, and proteins) (paper under press). Pertaining to the ethanolic extract of clove, its yield was 10.40% w/w with yellowish color and pleasant smell (46). This yield percentage is much lower than that obtained in the present study, where the extract color was also different; i.e. brown to black color. Clove contained all of the components tested mentioned above except proteins (paper under press). On the other hand, Moringa extract had an extraction yield of 32% with a burnt brown to black colored powder of acceptable pungent odor. In comparison with the study of Husni *et al*., (47), Moringa leaf extract has a distinctive flavor, aroma, and blackish-brown appearance. However, maceration of 1 kg of dried *Moringa oleifera* Lam powder with 70% ethanol produced a viscous extract that weighed 217.3 g and contained 21.73% amendment (47). The ethanolic extract of Moringa leaves has been found to possess several groups of secondary metabolites, such as phenols, alkaloids, flavonoids, tannins, saponins, carbohydrates and quinones (48), glycosides, phytosterols, and proteins (47). This is partially consistent with another study where carbohydrate, alkaloids, proteins, terpenes, and steroids were absent, while the other components exist (paper under press). Finally, Al-Gutub had a sticky texture with dark greenish-black color along with a yield of 18% and acceptable smell. Limited number of studies focused on analyzing the active compounds present in this plant. In a study under press performed by us, Al-Gutub was demonstrated to contain tannins, carbohydrates, glycosides, phenols, flavonoids, saponin, and coumarins; however, it was deprived of resins, alkaloid, proteins, terpenes, as well as steroids. Its bacterial activity was found to vary depending on the origin and plant's part used, and the reported activity was due to presence of spirosaponins in this herb (49). The solvent extracts of Al-Gutub showed significant antibacterial activities against Gram-positive and Gram-negative bacteria (49).

When the plants extracts were employed as QS inhibitors, clove extract significantly caused complete inhibition of the pyocyanin pigment without interfering with the bacterial growth at the different concentrations used in the present study. This result is consistent with another investigation where clove oil (at 0.12%) inhibited violacein production in *C. violacium* CVO26 in sub-minimal inhibitory concentration (sub-MICs) without reducing bacterial cell growth (50, 51). Furthermore, Reichling (32) demonstrated that the essential oil that worked best in QSI was clove oil. In order to exclude the possibility that the observed reduction in violacein production was not caused by the antibacterial activity of clove oil, the inhibitory impact of clove oil was also examined using *C. violacium* ATCC 12472 in a quantitative test method. It was discovered that the violacein-inhibiting ability was dose-dependent and did not significantly reduce the viability of the bacteria (32). The last authors added that only in the higher concentration (0.16%), clove oil's antimicrobial capabilities, in addition to its anti-QS effect, contributed to the reduction in violacein production. Moreover, Adhikari *et al*. (52) found that clove methanolic extract has antimicrobial effect against multiple microbes, including *P. aeruginosa*, with a zone of inhibition increased with increasing the extract concentration. For instance, at a concentration of 40 mg/mL of clove extract, an inhibition zone of 11.61±0.22 mm was reported, while at 100 mg/mL, the zone of inhibition was 19.78±0.23 mm. Clove essential oils were found to possess MIC value of 2.5-5 mg/mL and minimum microbicidal concentration (MBC) value of 5–10 mg/mL, with the highest MIC and MBC were observed in *P. aeruginosa* (53). Compared with the present study, the highest concentration used was 500 µg/mL (0.5 mg/mL) that is much lower than what was used by other studies, and owing to the fact that the effect of the plants extract is a dose dependent; therefore, no noticeable growth inhibition occurred in the current study.

Likewise, on the use of cinnamon in the present study, clear pyocyanin inhibition was seen with almost all of the concentrations, despite no growth inhibition was observed. Cinnamon among other 21 plants essential oils exhibited significant inhibition of violacein production (32). Another study revealed that cinnamon methanolic extract had no influence on the growth of *P. aeruginosa* relative to other microorganisms tested (53). However, cinnamon oil showed an antibacterial activity against numerous microbes with MIC and MBC values of 2.5-5 mg/mL and 2.5-10 mg/mL, respectively. Noteworthy, these concentrations are considerably higher than those used in the present study (the highest concentration was 0.5 mg/mL).

It was concluded that essential oils of clove and cinnamon have an effective antibacterial activity against *E. coli* and *K. pneumoniae* isolates (54). In the same context, it has been shown that at all minimum and maximum concentrations, *P. aeruginosa* demonstrated the greatest zone of inhibition, followed by *E. coli* and *K. pneumoniae* (55). However, anti-virulence agents, i.e. cinnamon and clove, are distinguished by the fact that they suppress bacterial infection in sub-MIC levels without decreasing bacterial vitality or killing the bacteria. Because they suppress the QS system, these agents seem like interesting candidates for novel anti-virulence drugs (32). In the current investigation, moringa leaves extracts also caused certain degree of pyocyanin inhibition, but only at higher concentrations compared to the lower concentrations, with the sub-MIC of 62.5 µg/mL was able to inhibit the pyocyanin production without interfering with the

bacterial viability. This finding is consistent with that of Suhartono *et al*. (18) who demonstrated that the use of kelor (*M. oleifera*) ethanolic leaf extract has shown effective suppression of QS by drastically lowering violacein and pyocyanin production, and this extract had MIC value of 10 mg/mL for *P. aeruginosa* PAO1. However, another study used the indicator organism *C. violaceum* 12472 to compare the effects of various parts of the moringa plant (leaves, roots, seed, bark, fruit, and flowers) on the virulence factors of *P. aeruginosa* and QSI activity using the violacein inhibition assay (56). In that study, only the seed extract was able to reduce the virulence factors, including the production of pyocyanin, to 96%, by contrast to the leaf extract, which failed to do so (56).

In the same study, the QSI activity of moringa seed extract was higher than the other extracts examined, as evidenced by the observation of a wide turbid zone of inhibition of violacein pigment.

Conversely, the current data showed that Al-Gutub dried fruits extract had no effect on the growth and pyocyanin production in the high concentrations. This result might be explained by the fact that this part of the plant is dispensable compared to other important parts, such as the root. Vadakkan *et al*. (24) found that hydro-alcoholic extracts of *Tribulus terrestris* roots had positive QSI activity against *P. aeruginosa* by successfully downregulating QS-controlled processes, e.g., pigment synthesis. Nevertheless, at the low concentrations of Al-Gutub dried fruits extract used in the present study, mainly 15.63 µg/mL, there was a clear zone of pyocyanin disappearance. This is consistent with what was observed in another study that the root extract did not influence the growth of the organisms, albeit it distracted signaling communication of Gram-negative bacteria (57).

Taken together, the *in vitro* data show that clove and cinnamon extracts significantly inhibited the production of *Pseudomonas* pyocyanin dye. This was followed by the extract of moringa, possibly by interfering with the AHL signaling molecule and inhibiting the LasR/RhlR regulation system (anti-QS activity) (32). According to the literature, the LasR/RhIR transcriptional regulatory proteins govern the production of virulence factors, such as pyocynin (57, 58).

Biomonitor organisms are most typically used to check for QS inhibitors in natural products. Thus, it is essential to developing countries to apply other effective biosensor microorganisms as a surrogate to the commercial strains, such as *C. violaceum* and *A. tumefaciens*. In this study, *Pseudomonas aeruginosa* isolate, pyocyanin producer, was successful in the screening of QS inhibitors.

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Conflict of interest

The authors declare no conflict of interest.

Editorial Transparency

Inam J Lafta serves as a member of the editorial board for The Iraqi Journal of Veterinary Medicine. Despite this role, the peer review process and the final publication decision were made independently and impartially, ensuring no influence from the author's editorial position

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جرثومة الزائفة الزنجارية كاشف فعال للتحري عن المستخلصات النباتية المثبطة لاستشعار النصاب

زهراء عيسى صادق[1]، إنعام جاسم لفته[2]

[1]فرع الاحياء المجهرية، كلية الطب ، جامعة واسط، واسط، العراق، [2]فرع الاحياء المجهرية، كلية الطب البيطري، جامعة بغداد، بغداد، العراق

الخلاصة

استشعار النصاب (Quorum sensing, QS) هو آلية معروفة تستخدمها جراثيم عديدة للسيطرة على عوامل الضراوة الهامة من خلال الإنتاج والاستجابة اللاحقة ل .N-acylhomoserine (AHLs) جرى تسجيل تثبيط استشعار النصاب (QSI) ، الذي يستهدف الإشارات المعتمدة على AHL، كاستراتيجية للسيطرة على الأمراضية الجرثومية. وبالتالي، تهدف هذه الدراسة إلى البحث عن قدرة جرثومة الزائفة الزنجارية كمؤشر للفحص السريع عن QSI المحتمل المتسبب عن المستخلصات النباتية على أساس تثبيط الصبغة. استخدمت أربع مستخلصات نباتية كحولية بضمنها: براعم زهور القرنفل المجففة (Syzygium aromaticum L)، لحاء القرفة (Cinnamomum cassia L)، أوراق المورينجا (Moringa oleifera Lam)، والقطب (Tribulus terrestris L) وجرى مقارنة قابليتها على التثبيط QSI)) مع الفيورانون. استخدمت طريقة الانتشار في حفر الهلام، إذ جري تلقيح عزلة الزائفة الزنجارية على وسط الهلام الخاص بـها الذي احتوى على حفر بحجم ٨ ملم لتحميل تراكيز مختلفة من المستخلصات النباتية فضلا عن الفيورانون أو محلول الفوسفات الداريء (سيطرة سالبة). أظهرت النتائج نجاح الفيورانون في تثبيط صبغة البايوسيانين للزوائف الزنجارية دون التداخل مع النمو الجرثومي، وهذا يؤكد قدرة هذه العزلة الجرثومية كمؤشر فعال يستدل من خلاله على وجود QS. الأهم من ذلك، سبب مستخلص القرنفل بتراكيز مختلفة في تثبيط تام لصبغة البايوسيانين مع تأثير ضئيل على النمو الجرثومي. عند استخدام القرفة، أظهرت التراكيز العالية فقط تثبيطًا واضحًا لصبغة العزلة المستخدمة. تسببت المورينغا أيضًا في درجة معينة من تثبيط الصبغة ولكن بتراكيز عالية فقط. وعلى العكس من ذلك، لم يؤثر القطب على إنتاج البيوسيانين ولا على الحيوية الجرثومية. يستنتج من هذه الدراسة نجاح عزلة الزائفة الزنجارية المنتجة للبايوسيانين في فحص مثبطات QS. توصي هذه الدراسة البسيطة باستخدام مثل هذه العزلة ككاشف عن QSI دون الاعتماد على استيراد سلالات معينة من جراثيم Chromobacterium violaceum أو Pseudomonas aureofaciens أو Agrobacterium tumefaciens.

الكلمات المفاحية: الزائفة الزنجارية، استشعار النصاب، تثبيط استشعار النصاب، الكاشف الحي، المستخلصات النباتية