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Inhibitory Action of Essential Oils on Quorum Sensing Activity of *Pseudomonas aeruginosa* by Effecting on Pyocyanin Production

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Abstract

Background: Quorum sensing (QS) is considered an important behavior of bacterial cells that communicate with each other. Pyocyanin produced by Pseudomonas aeruginosa is one QS activity. Its synthesis can affect in the presence of essential oils.

Methods: Four types of essential oils of hermal seeds, black seeds, radish, and linseeds were used against the growth and pyocyanin production of *P. aeruginosa*.

Results: All four essential oils showed antibacterial activity against *P. aeruginosa* with a variable MIC value. In comparison with hermal oil, pyocyanin production was more inhibited by the other types of essential oils.

Conclusions: Essential oils of radish and linseeds have more antibacterial action against *P. aeruginosa* and its production of pyocyanin. Hermal oil has no effect on the QS pathway as indicated by pyocyanin production with less effect on bacterial growth.

Keywords

Pyocyanin; Radish; Linseeds; Hermal; Black seeds; Qs

Introduction

Pyocyanin is one of extracellular pigments produced mainly by clinical and environmental isolates of *P. aeruginosa* [1,2]. It plays an important role as a virulence factor during pathogenicity of this bacteria [3] and as an antimicrobial agent against several types of other bacteria and fungi [4,5]. Regulation of pyocyanin production is usually under the control of quorum sensing (QS) activity that has an important role in bacterial communication through several signal molecules [6]. Synthesis of pyocyanin is mainly encoded by *phzM* and *phzS* genes of phz operons through regulation of transcriptional activity of *MvfR* gene [3,7].

Essential oils of different plants are recorded to have an ability to inhibit pyocyanin synthesis through an effect on QS. Ferula oil prevents QS of *P. aeruginosa* from producing pyocyanin and other products at 25 µg/ml [3,8]. Lower concentration of other oils could also perform such type of inhibition as with cinnamon oil at 0.1-0.2 µl/ml [9]. Meanwhile, reduction of pyocyanin expression needs a higher concentration (15 mg/ml) of *Ocimum gratissimum* leaf oil [10].

QS activity of *P. aeruginosa* represented by pyocyanin production was investigated after treatment with different types of essential oils.

Materials and Methods

Bacteria isolate

Pseudomonas aeruginosa was isolated from male (44 years) with urinary tract infection while admitted in Al-Ammam Al-Hussein Medical city of Karbala in January 2017. The isolate was cultured on Mueller Hinton agar (MHA) (Himedia, India) for 24 h at 37°C. Pyocyanin with blue color was clearly observed in bacterial culture media. *P. aeruginosa* was diagnosed based on biochemical characters of API 20 E (BioMérieux, France).

Essential oils

Essential oils of hermal seeds (*Peganum harmala* L.), black seeds (*Nigella sativa* L.), radish (*Raphanus sativus* L.), and linseeds or flax (*Linum usitatissimum* L.) were purchased from Hemeni-Karachi, Pakistan.

Antibacterial assay

The isolate was cultured in Luria-Bertani (LB) medium for 24 h at 37°C. Cell density of bacteria was adjusted to 1.5×10^8 cells in each ml of sterilized normal saline after matching with the 0.5 McFarland standard [11]. Different concentrations of essential oils (1, 3, 5, and 10 µl/ml) were prepared by mixing with ethanol. Well-diffusion method was used for antibacterial assay [12]. A 0.1 ml of standard bacteria was spread on MHA plate and 6 mm wells were made by sterile cork borer. Four wells were made in a single inoculated plate. In each well, 100 µl of essential oil concentration was added. Media with either of an essential oil or distilled water were used as a negative control. Gentamicin dissolved in sterile D.W (10 µg/ml) was used as a positive control. Cultures were incubated for 24 h at 37°C and inhibition zones were measured.

Determination of minimum inhibitory concentration (MIC)

Determination of MIC was performed according to NCCLS method, M7-A5 [13]. A 100 µl of standard bacterial suspension was inoculated into each single well of microtiter plate (96 wells). Serial two-fold concentrations of essential oil were prepared and 100 µl of each one was added into inoculated wells. For each test plate, two essential oil-free controls were included, one with medium alone and the other with medium inoculated with bacteria. Plates were incubated at 37°C and read visually after 24 h of incubation.

Pyocyanin determination

A vial with 2 ml of LB medium and 100 µl of essential oil concentration was inoculated with 100 µl of standardized bacteria. Cultures were incubated for 48 h at 37°C. Pyocyanin concentration was measured according to protocol mentioned by Kalia et al. [9]. Briefly, supernatants from *P. aeruginosa* culture grown in the presence or absence of essential oil were collected. Pyocyanin was extracted using chloroform, followed by 0.2 M HCl. The absorption was measured by a UV-spectrophotometer (ABEL, Japan) at 520 nm.

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Statistical analysis

All of the experiments were triplicated. The data were analyzed statistically by analysis of variance (ANOVA) test. The minimum level of (p) value was <0.01 which was considered significant.

Results

The effect of essential oils on the growth and QS activity of *P. aeruginosa* as indicated by pyocyanin production was investigated. Bacterial growth was inhibited at concentration up to 3 µl/ml of three tested essential oils. Meanwhile, hermal oil showed no activity against *P. aeruginosa* up to 10 µl/ml (Table 1). The MIC value of essential oils against *P. aeruginosa* were 7.5, 4.5, 4, and 3.5 µl/ml for hermal, black seeds, radish, and linseed oils, respectively (Table 2).

Pyocyanin production was completely inhibited at 1 µl/ml of radish and linseed oils, while hermal oil exhibited no effect on production of pyocyanin at 1 and 3 µl/ml. Black seeds oil showed initial reduction of pyocyanin production at 1 µl/ml until complete inhibition of pigment production at higher concentration (3 µl/ml) (Table 3 and Figure 1).

Table 1: Zone of inhibition of essential oils on *Pseudomonas aeruginosa* growth.

Essential oils	Zone of inhibition (mm)			
	Concentrations (µl/ml)			
	1	3	5	10
Hermal oil	-	-	-	12
Black seeds oil	21	18	12	11
Radish oil	20	19	12	11
Linseed oil	14	12	10	10
Gentamicin (10 µg/ml)	19			

Table 2: MIC value of essential oils on *Pseudomonas aeruginosa* growth.

Essential oils	MIC (µl/ml)
Hermal oil	7.5
Black seeds oil	4.5
Radish oil	4
Linseed oil	3.5

Table 3: Production of pyocyanin in media with essential oils.

Essential oils	Concentrations (µl/ml)	
	1	3
Hermal oil	+++	+++
Black seeds oil	+	-
Radish oil	-	-
Linseed oil	-	-

+: Low presence of pyocyanin; ++: High presence of pyocyanin; +++: Very high presence of pyocyanin

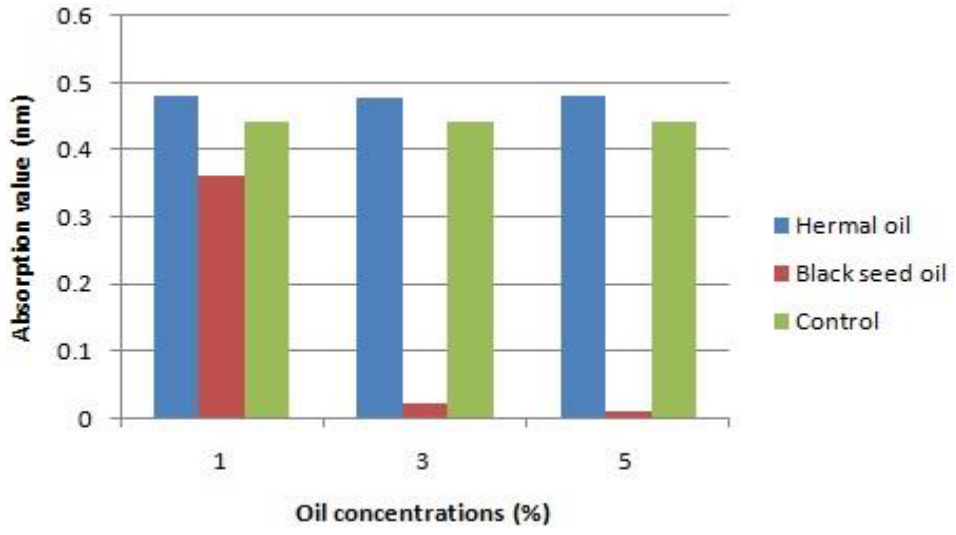

Figure 1: Effect of hermal and black seeds oils on pyocyanin production.

Discussion

Quorum sensing (QS) is a communication path connecting microbial cells with each other by special signals in order to coordinate motility, virulence factor production, and biofilm formation [6]. In *P. aeruginosa*, QS stimulates production of several types of pigments as virulence factors such as pyocyanin and pyovardine [8]. Pyocyanin is a blue extracellular-pigment that is synthesised under the control of phz operons and *MvfR* gene through the synthesis of QS quinolone molecules [3,7]. About 25% of clinical and 38.2% of environmental isolates of *P. aeruginosa* have the ability to produce pyocyanin, while 3.2% of these are non-pigmented [2]. Antimicrobial activity is the first function of pyocyanin against a wide range of microorganisms giving *P. aeruginosa* a competitive advantage over other organisms [4,5]. The second function of pyocyanin is represented by its role as a virulence factor through increased intracellular levels of reactive oxygen species (ROS) [14].

QS was found to be affected by many natural essential oils. Pyocyanin production as one of QS activities is also reduced by some of these essential oils such as with the oils of Ferula, Cinnamon, pungent (6-gingerol), *Ocimum gratissimum*, peppermint and clove [3,8-10,15-17].

In the present study, radish, linseeds, and black seeds oils exhibited an inhibitory action on pyocyanin production. Antibacterial activity of different parts of the radish plant against *P. aeruginosa* was recorded by several studies [18-20]. This inhibitory activity may relate to the plant contents of alkaloids, flavonoids, glycosides, phenols, tannins, saponin, sterols and proteins [19,20]. Essential oil of radish that blocked QS activity as indicated by inhibition of pyocyanin production is mainly composed of pentyl hexyl, 4-methylpentyl isothiocyanate, dimethyl disulfide, methyl methanethiosulfinate and 1-methylthio-3-pentanone [18].

Linseed oil showed antibacterial effect on *P. aeruginosa* with prevention of QS activity through inhibition of pyocyanin production. Antibacterial activity of linseed oil recorded by previous studies was variable [21]. Gawad et al. found a negative effect of linseed oil on bacteria of lumen, while other studies found a good activity of linseed oil against some bacteria [22,23]. *P. aeruginosa* was less affected by linseed oil proteins [24]. However, linseed oil contains several compounds that have a potential activity against microorganisms such as fats, flavonoids, glycosides, phenols and tannins [25]. Semicarbazide fatty acid and phenylpropanoids of linseed oil exhibited an inhibitory effect against many types of bacteria [26,27].

Black seeds oil was found to have antibacterial activity against different types of bacteria and fungi [28-33]. *P. aeruginosa* and its multi-drug resistant isolates revealed more sensitivity to black seeds oil which has a bacteriostatic action [28,30-31,33-36]. Essential oil of black seeds is composed of large amounts of fixed oil and less of volatile oil [32]. Generally, the main chemical components of black seed oil, which are related to its antibacterial activity are thymoquinone, thymohydroquinone, dithymoquinone, thymol, carvacrol, nigelline-N-oxide, nigellicine, nigellidine and alpha-hederin, oleic acid, monoterpenes, sesquiterpenes, polyphenols and aromatic aldehydes [30].

Hermal oil revealed no activity against the growth of *P. aeruginosa* or its production of pyocyanin, even at high concentrations. These results were also proved by another study [37]. Extraction of *Peganum harmala* seeds exhibited antibacterial effects on various bacteria, including *P. aeruginosa* [38-40]. The MIC value of seeds extract against *P. aeruginosa* ranged from a low value (15-50 μg/ml) to a high value (50-400 mg/ml) [38,39,41]. However, hermal oil contains many components with a potential antibacterial activity such as oxygenated monoterpenes and sesquiterpenes [41].

Conclusion

Essential oils of radish and linseeds have antibacterial action against the growth of *P. aeruginosa* and its production of pyocyanin. Hermal oil has no effect on pyocyanin production with less effect on bacterial growth.

Funding and Conflict of Interesting

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