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RESEARCH ARTICLE OPEN ACCESS

Inhibition of *Pseudomonas aeruginosa* Quorum Sensing by *Curcuma xanthorrhiza* Roxb. Extract

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Abstract

Microorganisms such as *Pseudomonas aeruginosa* have always been adaptable in surviving the harsh environment such as antimicrobial agents via the quorum sensing (QS) mechanism. Studies have shown that quorum sensing mechanism cases have been highly associated with foodborne illnesses. Since synthetic compounds such as azithromycin (AZM) are reported to have detrimental effects on human, using medicinal local plants have been gaining attention as an anti-quorum agent. The aim of this study was to determine the anti-quorum sensing activity of the *Curcuma xanthorrhiza* Roxb. extract against *P. aeruginosa* ATCC35554 quorum sensing system including swarming motility, pyocyanin production and biofilm formation. The results indicated that the extract required a high concentration to inhibit and kill the *P. aeruginosa* with minimum inhibition concentration (MIC) and minimum bactericidal concentration (MBC) values of 200 and >700 mg/mL, respectively. Thus, anti-quorum sensing assays were done in concentration up to 200 mg/mL. The inhibition of quorum sensing activity of *C. xanthorrhiza* Roxb. extract on *P. aeruginosa* quorum sensing was concentration dependent manner. At 200 mg/mL of the extract exhibited 72.12% reduction of swarming motility, 84.30% inhibition of the pyocyanin production and 78.35% reduction in the biofilm formation. In conclusion the crude extract of *C. xanthorrhiza* Roxb. extract has ability to reduce the virulence factors; swarming motility, pyocyanin production and biofilm formation regulated by quorum sensing. Thus, the extract *C. xanthorrhiza* Roxb. extract has anti-quorum sensing or quorum quenching activity.

Keywords: Anti-quorum sensing, *Curcuma xanthorrhiza* Roxb., *Pseudomonas aeruginosa,* swarming motility, pyocyanin production, biofilm formation.

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INTRODUCTION

The global food safety and the economy have been reported to be seriously affected by the increasing foodborne illnesses and food spoilage[1,2]. Contamination by the foodborne pathogens have led to numerous cases of diarrhea, vomiting, abdominal pain and even deaths[3-5]. While urbanized countries such as the United States and the United Kingdom have a good statistical reports on the phenomenon[6], less developed countries such as Malaysia are not as able to efficiently tackle the issue due to the lack of incidents reported[7,8].

In order to combat and prevent the foodborne contaminations, antimicrobial agents used often possess a selective toxicity and targets the difference between the microorganism metabolism and the human cell's' structures and features[9,10]. However, the frequent usages of these products such as antibiotics have led to an emergence of antibiotic resistant bacteria strain[11]. Therefore, to reduce the antibiotic dependencies, alternative stratagems such as quorum sensing have been researched[12,13].

Quorum sensing (QS) is a bacteria cell-to-cell communication mechanism used to determine the bacterial physiology including the local population density as well as adapting to the harsh and ever-changing environment such as antibacterial agents innate immune responses[14-16]. QS is a type of bacterial communication systems that allow determination of the bacterial physiology via the production of diffusible signaling molecules known as autoinducers (AI) such as oligopeptides and N-acyl homoserine lactones in Gram-positive and Gram-negative bacteria, respectively[15]. QS system is greatly associated with bacterial pathogenicity and spoilage contamination on food products[17]. Through the production of diffusible signaling molecules known as autoinducers (AI) in Gram-positive (oligopeptides) and Gram-negative (N-acyl homoserine lactone (AHL)) bacteria[15], these microorganisms are able to regulate the QS mechanism. *P. aeruginosa* QS systems have been reported to be highly adaptable in responding towards the external biological stresses by producing virulence factors such as pyocyanin, swarming and biofilm formation[19].

Since synthetic quorum quenching compounds such as azithromycin (AZM) are reported to have detrimental effects on human, usage of natural products from plant extracts have been gaining popularity in eliminating microbial contamination on the food products[20]. Javanese turmeric (*Curcuma xanthorrhiza* Roxb.) or locally known as "temu lawak" can be found in tropical countries such as Malaysia and Indonesia[21]. *C. xanthorrhiza* Roxb. has been traditionally used for food and medicinal purposes[22,23]. The Javanese turmeric has been reported to have bioactive compounds including curcuminoids, camphor, geranyl acetate, zerumbone, β-curcumene, zingiberene, ar-curcumene and xanthorrhizol[24]. Therefore, the aim of this study is to evaluate the antimicrobial and quorum quenching activity of the ethanolic extract against *P. aeruginosa in vitro*.

MATERIALS AND METHODS

Bacterial Strain

P. aeruginosa strain used in this research was ATCC35554 obtained from the American Type Culture Collection and cultured in *Pseudomonas* agar. During the study, bacteria was grown at least over 12 hours in Luria Bertani (LB) broth and streaked onto a LB agar media to obtain a single *P. aeruginosa* colony. The culture was preserved in a sterile universal bottle at 4°C for short term holding and at -20°C for long term holding in sterile universal bottles and agar plates[25,26].

Curcuma xanthorrhiza Roxb. Rhizome Sample and Extraction

A 10 month old of *C. xanthorrhiza* Roxb. or Javanese turmeric rhizomes were obtained from Kebun Percobaan, Cikabayan, Damaga, Bogor, Bogor Agricultural University (IPB). The rhizomes were sorted to remove all of the soil and dirt. The rhizome was then chopped manually approximately 5-7 mm. The sliced rhizomes were dried using oven drying (55°C) and powdered into 60 meshes with a grinder. *C. xanthorrhiza* Roxb. rhizome extraction was done as per method by Ab Halim *et al.*[27]. Briefly, 100 g of the powdered rhizome was soaked in 400 mL of ethanol for 48 hours at room temperature. The mixture was then filtered using Whatman filter No.2 and evaporated with a vacuum evaporator at 50°C to obtain a concentrated ethanolic crude extract. The ethanolic extract of *C. xanthorrhiza* Roxb. was preserved in an universal bottle at 4°C for further use[28].

Determination of Minimum Inhibitory Concentration (MIC) and minimum Bactericidal Concentration (MBC)

Determination of MIC and MBC of *C. xanthorrhiza* Roxb. extract on *P. aeruginosa* culture were done using the standard method of Clinical and Laboratory Standards Institute (CLSI)[30]. Through MIC and MBC assay, the concentrations of the extract to inhibit and kill the *P. aeruginosa* culture, respectively, were conducted. Briefly, in the study, 10 mg/mL chlorhexidine and dimethyl sulfoxide (DMSO) were used as positive and negative controls, respectively. Since chlorhexidine have been known to for its effectiveness against vast spectrum of bacteria while DMSO is commonly used as the negative control due to the absence of antimicrobial activity. As per the CLSI[30], the first two wells of the 96 well plate, were allocated for the positive and negative control for MIC assay. Meanwhile, the rest of the wells were aliquoted with the extract diluted with MH broth to obtain concentration of 10-800 mg/mL. In MBC test, 10 µL aliquot from each well was inoculated onto a sterile MH agar and incubated for 24 hours at 37°C. After the incubation, wells that shows no bacterial growth represents the minimum concentration needed to kill the *P. aeruginosa.*

Growth Assay

To further study the antimicrobial activity of the ethanolic extract against *P. aeruginosa* growth, Log_{10} Colony Forming Units (CFU) was done on Nutrient agar plates. The method was chosen as the bacterial count capacity can be adjusted via serial dilutions and the method allow only viable bacterial colony to be counted[31]. A culture (OD600 ~0.1) incubated at 37°C with shaking at 200 RPM for four hours was inoculated into sterile universal bottles containing 9 mL of LB broth. 1 mL of the extract (0-200 mg/mL) was added to the respective bottles and incubated at 37°C for 24 hours. A 100 µL of culture was aseptically spread onto Nutrient agars using a glass hockey stick before 24 hours incubation. The colonies formed were counted and the CFU/mL was calculated.

Swarming Activity Assay

Based from the antimicrobial assays, the extract concentration needed to observe the *C. xanthorrhiza* Roxb. quorum quenching activity against *P. aeruginosa* was 0-200 mg/mL. In swarming inhibition assay, approximately 0.1 OD_{600} *P. aeruginosa* culture was inoculated into universal bottles containing the extract (0-200 mg/mL) and incubated overnight at 37°C with 180 rpm shaking. The culture was then inoculated onto a swarming agar media consisting of 0.5% (w/v) agar and 8 g/L nutrient broth using a sterile toothpick[32]. The plates were then incubated at 37°C for 24 hours and the mean length of the swarming distance between the central of the inoculation site was determined.

Pyocyanin Production Assay

Inhibition on pyocyanin pigment production was done according to the method by King *et al.*[33]. From the swarming assay, the swarming colony was scooped from the media, cut into small pieces and added with 5 mL of saline (0.85% NaCl) in centrifuge tubes. The tubes were centrifuged at 10,000 RPM for 10 minute twice[34]. A 5 mL of the supernatant was mixed vigorously with 3 mL chloroform before discarding the aqueous phase[34-37]. The pigment was then re-extracted with 1 mL of 0.2 N HCl and the mixture was mixed vigorously to elicit a pink red solution[35,38]. The red pink solution was centrifuged at 8,000 RPM for 10 minutes and the relative pyocyanin concentration was measured using a spectrophotometer at OD_{520} with 0.2 N as blank[36,38,39]. The pigment concentration was then calculated by multiplying the optical density value by 17.072[49,41].

Biofilm Formation Assay

The effect of the ethanolic extract of *C. xanthorrhiza* Roxb. against *P. aeruginosa* biofilm formation was done according to Varposhti *et al.*[42]. Each well of the 96-microtiter flat-well plate consist of 50 µL of the LB broth and 50 µL of the extract (0-200 mg/mL). A100 µL of the bacterial culture at 10^8 CFU/mL was added into each well, mixed thoroughly and incubated for 24 hours at 37°C. Post incubation, the wells were washed with pre-warmed physiological saline and let dry for 10 minutes. Then, 100 µL of the tetrazolium salt (XTT)/menadione solution was added into each well and incubated in the dark at 37°C for 4 hours. After the incubation period, the content of each well was transferred into a new 96 wells microtiter plate and the absorbance at OD_{490} was measured. The anti-biofilm activities of the plant extracts was calculated using the percentage mean of the optical density at 490 nm wavelength against the untreated biofilm. The decrease in biofilm

formation of treated samples was compared to the untreated biofilm were determined.

Statistical Analysis

The tests were done by 2 x 2 and the results were analyzed using the MINITAB17 software. In the data analysis, the one-way variance analysis (ANOVA) and the Tukey's test were used to determine the significant difference ($P<0.05$) between different concentration of plant extract used (0-200 mg/mL).

RESULTS AND DISCUSSIONS

Yield of *Curcuma xanthorrhiza* Roxb. extract

The average crude extract yield of *C. xanthorrhiza* Roxb. rhizome was 4.01 ± 0.89% (Table 1). This result is comparable with the extraction yield of *C. xanthorrhiza* Roxb. rhizome (5.9%) obtain from Ab Halim *et al.*[27]. This difference in yield could be from variety of reasons such as the method of extraction. The method chosen for this test was chosen for its simplicity, cheap and convenience[43]. Soaking the powdered material in the solvent allow for the plant's cell walls to soften and broken down, releasing the soluble phytochemicals into the solvent[43].

Table 1. Extraction yield of the *Cucurma xanthorrhiza* Roxb. rhizome

Dried powder (g)	Extraction	Yield (g)	Yield (9%)
100	1st	4.98	4.98
100	2nd	3.22	3.22
100	3rd	3.82	3.82
Average ± SD		4.01 ± 0.89	4.01± 0.98

SD, standard deviation

Ethanol was chosen as the solvent due to its safer and less toxic nature than acetone, methanol and other organic solvents[44-46]. Human liver naturally produce an enzyme known as alcohol dehydrogenase which functions to convert alcohol into acetylaldehyde as its source of energy[47]. However, when methanol reacts with alcohol dehydrogenase, it leads to the production of formaldehyde that is very reactive and may interact with a host molecule in the body to shut down the enzymatic pathways[47]. Since ethanol is a versatile solvent with a universal characteristic, it can attract both non-polar and polar compounds such as alkaloid, curcuminoid and terpenoid[48,49]. Ab Halim *et al.*[27] have reported that ethanol solvent have been reported to elicit high phytochemical compounds such as phenols and tannins compared to other solvents.

Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

The MIC and MBC result of the *C. xanthorrhiza* Roxb. ethanolic extract against *P. aeruginosa* was 200 and >700 mg/mL (Table 2). The results indicated that a high concentration of the extract was needed to inhibit and kill the *P. aeruginosa* culture (200 and 800 mg/mL, respectively). In the study done by Diastuti *et al.*[50], the MIC and MBC values of the *C. xanthorrhiza* Roxb. extracts on the *P. aeruginosa* were much lower than in this study. The difference in the concentration needed for MIC and MBC was due to the difference in the extraction methods and solvents used when isolating the phytochemical compounds from *C. xanthorrhiza*

Table 2. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of *Curcuma xanthorrhiza* Roxb. extract against *Pseudomonas aeruginosa*

	C. xanthorrhiza Roxb. extract (mg/mL)
MIC	200
MBC	>700

Roxb. rhizome. Different extraction method and solvents could have certain affinity on several phytochemicals over the other. For example, in the study by Ab Halim *et al.*[27], ethanol extract of the *C. xanthorrhiza* Roxb. possesses more phytochemical compounds such as terpenes, phenols, flavonoids among others compared to the aqueous solvent. Difference of affinity in phytochemical extractions could be due to the polarity attraction from the ethanol solvent due to its universal characteristics and versatile solvent[51].

Furthermore, the high extract concentration needed for MIC and MBC could be due difference between cell wall characteristic of Gram-negative of *P. aeruginosa.* Mangunwardoyo *et al.*[52] has reported that an extraction of *C.*

xanthorrhiza Roxb. extract with aquadest, 70% ethanol and dichloromethane have also failed to exhibit zones of inhibition on the Gram-negative bacteria such as *P. aeruginosa, E. coli, P. gingivalis* as well as fungi *C. albicans*. Selim *et al.*[53] and Mangunwardoyo *et al.*[52] have explained that gram-negative bacteria possess a higher resistance towards antimicrobial agents as compared to the Gram-positive bacteria. Such resistance could be due to higher concentration of lipid in the cell wall[54]. Bacterial cell walls are commonly composed of lipopolysaccharides, lipoproteins and periplasms that are bonded to the peptidoglycans[55-57]. These lipopoly-saccharides in the cell wall serves as the bacteria defense system that only selectively allows foreign objects to pass through the cell wall[52].

Since Gram-negative bacterial cell walls are reported to possess a non-polar characteristic, this makes polar and semi-polar derived extraction methods have a higher difficulty to permeate the cell wall[58]. Therefore, due to its semi-permeable outer membrane, Gram-negative bacteria are able to reduce its susceptibility against antimicrobial agents by minimizing intake of dangerous foreign substance such as antimicrobial agents[59]. Furthermore, *P. aeruginosa* outer membrane permeability is lower than other Gram-negative bacteria by 12-100 fold[60]. This serves as a crucial barrier for the bacteria against penetration by the antimicrobial agents as these agents will have to take a longer time to pass through *P. aeruginosa* cell wall[58]. During this time, the bacteria has ample time to intrinsically gain resistance against the agents by synergizing via its internal mechanisms such as the efflux pumps and periplasmic β-lactamases to actively pump out and/or degrade the compounds[58].

Growth assay

Growth assay at $\log_{10}$ was done to further confirm the effect of MIC extract concentration (0-200 mg/mL) on the bacterial growth. The result showed that the *C. xanthorrhiza* Roxb. extract does not or have little insignificant effect on the growth of *P. aeruginosa* (Fig. 1). The figure shows that apart from the positive control, *P. aeruginosa* growth was barely affected by the *C. xanthorrhiza* Roxb. extract whereby the negative control (DMSO) and extract at concentration of 0-200 mg/mL showed an insignificant difference of bacterial growth ranging from 8.99 ± 0.18 to 9.73 ± 0.05 $\log_{10}$ CFU/mL. Similarly, Ugurlu *et al.*[35] have also reported that concentrations up to 4 mmol/L had no effect on the growth of *P. aeruginosa* has shown an inhibiting effect on the bacterium quorum sensing mechanisms. Since compounds or concentration that do not kill or inhibit the microbial growth is less likely to promote selective pressure to develop antibacterial resistance[35], *C. xanthorhiza* Roxb. ethanolic extract at 0-200 mg/mL were used to study the quorum quenching activity against *P. aeruginosa.*

Swarming assay

Inhibition on swarming motility was done by measuring the diameter of the swarming

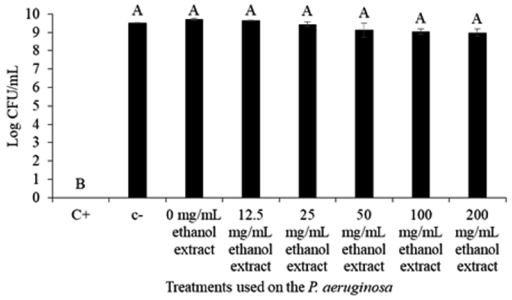

Fig. 1. Log_{10} CFU/mL of the *P. aeruginosa* ATCC 35554 growth against treatment with positive control (C+) 10 mg/mL Chlorhexidine, negative control (C-) DMSO, and *C. xanthorrhiza* Roxb. extract concentration (0-200 mg/mL). The alphabetical grouping represent the significance different between extract concentration (Those that share similar letter is not significant different).

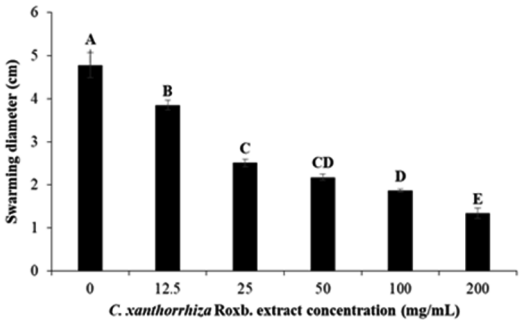

Fig. 2. Effect of the *Curcuma xanthorrhiza* Roxb. extract (0 - 200 mg/mL) on the *P. aeruginosa* swarming motility. The reduction of swarming diameter (cm) when added with *C. xanthorrhiza* Roxb. extract. The alphabetical grouping represent the significance different between extract concentration (Those that share similar letter is not significant different).

colonies in the presence of 0-200 mg/mL of *C. xanthorrhiza* Roxb. ethanolic extract. Fig. 2 display the decrement of *P. aeruginosa* swarming colony diameter. At 0 mg/mL, highest swarming colony was observed at 4.78 ± 0.29 cm and at 200 mg/mL, the extract managed to inhibit 72.12% of *P. aeruginosa* swarming with a diameter of 1.33 ± 0.12 cm. Crude extract of the spice clove (*Syzygium aromaticum*) has also been reported to exhibit reduction of *P. aeruginosa* PA01[37,61]. Since the swarming activity in *P. aeruginosa* is induced and regulated by the *rhl* system, presence of *rhl* inhibitor in the *C. xanthorrhiza* Roxb. ethanolic extact is associated with the reduction of *P. aeruginosa* swarming activity[37].

Progression of swarming motility is hugely affected by the surfactant productions such as rhamnolipids (RLs) and 3-hydroxyalkanoic acids (HAAs)[62-64]. Such molecules affect swarming motility via inhibiting and promoting the tendril formation by displaying different diffusion kinetics on the agar[64]. Surfactant such as rhamnolipid depends on the activation of rhlA, rhlB and rhlC genes which are governed by the RhlR QS system and stimulated by the N-butyryl homoserine lactone induction[65,66]. Caiazza *et al.*[67] have reported that mutation or inactivation on the rhlC gene that encodes the rhamnosyltransferases to initate the formation of monorhamnolipids can affect the inhibition on swarming motility.

In another study by Kim and Park[68], ginger (*Zingiber officinale*) extract, instead of inhibiting the swarming activity of *P. aeruginosa*, the extract promoted its motility. Despite previous study by Rasmussen *et al.*[69] have reported the extract quorum quenching ability such as on biofilm formation on *P. aeruginosa* PA14, no such inhibition was observed in Kim and Park's study[68]. This phenomenon can be explained in the study by Caiazza *et al.*[70] whereby the inverse regulation of *P. aeruginosa* swarming and biofilm activities is regulated by the flagella reversal and formation of Pel polysaccharides. Such mechanisms are crucial in the transition from swarming to biofilm formation by affecting the initial attachment between the bacterial and the substratum[71].

O'may and Tufenkji[72] reported that cranberry products which contains a condensed A-type of proanthocyanidins (PACs), hydrolysable tannin in pomegranate, catechins containing B-type PACs in the green tea extracts all managed to inhibit *P. aeruginosa* swarming, suggesting the quorum quenching of tannins. As swarming and biofilm require the QS system to effectively work, tannin compounds have been reported to be able to impede the mechanism[73,74] as they are able to bind and precipitate various types of proteins[75-77]. Apart from tannins, phenols and phenolic compounds have also been reported to inhibit *P. aeruginosa* swarming motility[35,78]. Therefore, as the ethanol solvent of *C. xanthorrhiza* Roxb. exhibited the highest recovery of tannins[45] and phenols and phenolic compounds[27], the swarming inhibition of *P. aeruginosa* by the *C. xanthhorhiza* Roxb. extract could be due to its anti-QS properties.

Pyocyanin assay

In the pyocyanin inhibition assay, the extract was able to inhibit 84.30% of the pigment production by *P. aeruginosa.* The inhibition of pyocyanin pigment production was dependent on the extract concentration whereby at 0 and 200 mg/mL of the extract, 13.01 ± 1.37 mg/mL and 2.04 ± 0.59 mg/mL of pyocyanin concentration were produced (Fig. 3). During the extraction of pyocyanin, chloroform and 0.2 N HCl gave a blue and red colour, respectively. Pyoycanin colour and absorption spectrum have been reported to be pH sensitive and changes according to the exchange of electrons in the pigment[79]. For example, at a neutral pH, pycoyanin produces a strong blue colour, greenish blue at alkaline and red at an acidic pH[79]. Since of the three, the reduced form

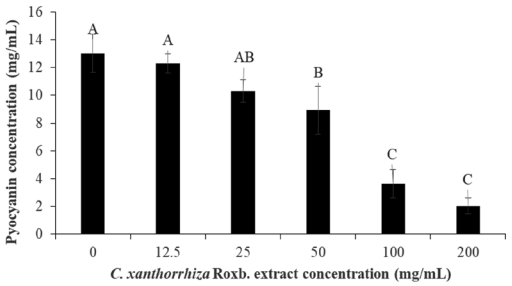

Fig. 3. Pyocyanin inhibition by the *Curcuma xanthorrhiza* Roxb. extract concentration (0-200 mg/mL). The pyoycanin concentration (mg/mL) after treated with *Curcuma xanthorrhiza* Roxb. extract concentration (0-200 mg/mL). The alphabetical grouping represent the significance different between extract concentration (Those that share similar letter is not significant different).

of pyocyanin have been reported to be unstable and reactive with molecular oxygen rapidly[80,81], after extraction with chloroform, the pigment was re-extracted with 0.2 N HCl.

Production of the pyocyanin pigment is commonly regulated by the Las, Rhl and PQS systems in which suggesting that *P. aeruginosa* pigment inhibition by *C. xanthorrhiza* Roxb. affect such systems by binding the autoinducers to its protein receptors nor interfering with pyocynin biosynthesis. In the study by Krishnan *et al.*[37] suggested that the hexane, chloroform and methanol extract of *S. aromatic* or clove can affect *P. aeruginosa* production of swarming motility and pyocyanin pigment. Apart from chloroform, both hexane and methanolic clove extract exhibited reduction in pyocyanin production and this suggest that the inhibitions are done by the las and rhl inhibitors present in the extract[37]. Therefore, this could also suggest that the phytochemicals present in the *C. xanthorrhiza* Roxb. ethanolic extract might also consist of such inhibitors.

P. aeruginosa pyocyanin pigment production has also been reported to be affected by the concentration of phenols and phenolic compounds. For example, phenolic compounds duch as vanilic acid, caffeic acid, cinnamic acid and ferulic acid prepared in ethanol/water mixtures were able to reduce approximately 9-21% pyoycanin production[35]. Such compounds were also reported to inhibit 50% of the biofilm formation and swarming motility by *P. aeruginosa*. Inhibitions of quorum sensing virulence activity and biofilm formation by phenolic compounds are also supported by various studies such as in the study by Borges *et al.*[81] and the phenolic compounds present in *Moringa oleifera*[82].

Meanwhile, Ab Halim *et al.*[27] have made a study on the qualitative phytochemical screening of *C. xanthorrhiza* Roxb. extract to show presence of terpenoids, phenols, flavonoids, saponins, cardiac glycosids, alkaloids and coumarins. Futhermore, through the total phenol content (TPC) test on *C. xanthorrhiza* Roxb. ethanolic extract yielded a higher TPC value compared to its methanolic extract[83,84]. Phenols and its compounds have been known for its medicinal properties such as for skin infections and wound treatments[85]. Therefore, apart from its antibacterial, antifungal[86] and other medicinal benefits, phenols and its compounds present in the *C. xanthorrhiza* Roxb. ethanolic extract might serves as a quorum quenching agent.

Biofilm assay

P. aeruginosa biofilm formation was done colorimetrically to measure the inhibition via the absorbance at 495 nm when the XTT dye was added to the culture. Pyocyanin is involved in the formation of biofilm, therefore, in *P. aeruginosa* biofilm formation, green colour can be observed. Post incubation, the culture is washed with saline to remove the unattached culture to the wells and XTT dye was added. XTT dye is used as it contains tetrazolium salt that changes the colour to orange formazan in the presence of metabolic active cells in the biofilm[87]. In this assay, wells treated with 200 mg/mL of the extract showed the lightest orange colour, while well with 0 mg/mL extract showed the darkest orange colour. Fig. 4 shows the inhibition of the biofilm formation to be concentration dependent. Culture with no extract (0 mg/mL) exhibited the highest absorbance at 1.50 ± 0.048 but gradually decreases as the concentration of the extract increases. This is portrayed when the addition of 12.5 mg/mL of the extract managed to inhibit 24.07% of the biofilm formation while 200 mg/mL of the extract was able to inhibit it at 78.35% (1.14 ± 0.067 and 0.32 ± 0.035 of OD_{495} value, respectively).

Since the absorbance of the dye was shown to be decreasing as the extract concentration increases, this might suggest inhibition on biofilm formation in this assay due to increasing concentration of cell death in the

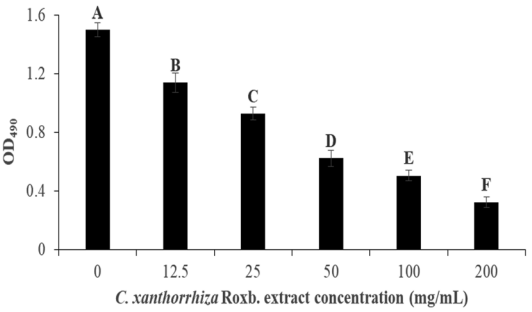

Fig. 4. The inhibition of the *P. aeruginosa* biofilm formation by *C. xanthorrhiza* Roxb. extract via absorbance at OD_{490}. The alphabetical grouping represent the significance different between extract concentration (Those that share similar letter is not significant different).

biofilm[88]. In the study, up to 50% localized killing and lysis were observed in the biofilm center where the microcolonies are formed. There are several explanations that can attribute to biofilm cell death such as due reactive oxygen species (ROS) such as hydrogen peroxide (H_2O_2) and nitric oxide (NO) concentration accumulation in the quorum leading to localized lysis and biofilm cell deaths[88,89].

Normally, NO concentration is regulated by the production of pyocyanin pigment via redox reaction[90]. Pyocyanin is also reported to facilitate biofilm formation via the release of extracellular DNA (eDNA) through H_2O_2 facilitated cell death[91]. In the early stages of biofilm development to form stable and mature biofilm, more than 50% of the biofilm matrixes are produced by eDNA[92,93]. eDNA plays a crucial factor by regulating the interconnection of *P. aeruginosa* cells[57] such as promoting strong adherence towards the surface and providing a stable structure in the exopolysaccharides (EPS) of biofilm[92,93] and involved in cell-to-cell communication[92-94]. Furthermore, eDNA also facilitates biofilm expansion via twitching motility by maintaining a coherent cell alignment[95] and act as a nutrient source during starvation[96,97].

Production of biofilm in *P. aeruginosa* has been reported to involve an inverse regulation between swarming versus biofilm and pyocyanin[98]. C-di-GMP, a compound that is found to be positively regulated in production of pyocyanin and biofilm was reported to be negatively regulated when swarming motility is high[99-102]. Such claim is also reported in the study by Kim and Park[68] where by the ginger extract were able to reduce *P. aeruginosa* biofilm formation but increases the swarming activity.

Swarming and biofilm formation relationship involves the type of cell attachment to the surface. While swarming motility requires reversible and motile attachment, biofilm formation involves sessile, non-motile attachment. Reduction in biofilm formation occurs when the concentration of non-motile sessile cell attachment to the surface is decreasing[98,103]. The conversion between the two often regulated via expression of sadB and sadC genes that control the production of c-di-GMP concentration[98,103]. Since BifA is a C-di-GMP phosphodiesterase, interference on its activation can reduce c-di-GMP concentration thus reducing the signal C-di-GMP from being transmitted to Pel protein and CheIV chemotaxis-like cluster components[104].

However, a contradiction was raised in the recent studies whereby both inhibitions on both quorum sensing regulation virulence were observed[105,106]. Both inhibition on swarming and biofilm formation can also be explained when the production of its signaling molecules are interrupted. This is supported by the study by Krishnan *et al.*[37] whereby the presence of rhl inhibitor in clove extract were able to reduce swarming and biofilm production. Furthemore, *C. xanthorrhiza* Roxb. ethanolic extract was able to act as a quorum quenching agent by affecting the GacA/GacS system, a super QS regulator reported to aid the four QS systems in *P. aeruginosa*[107]. The super QS regulator can be affected by inactivating the free Rsma and increasing RetS activity, which will negatively regulate the production of AIs and repress biofilm formation, respectively[108,109]. Therefore, this could suggest that apart from inhibiting biofilm maturation, swarming motility and pyocyanin pigment production by affecting the concentration of c-di GMP associated mechanisms, the *C. xanthorrhiza* Roxb. extract also interfered with the production of the QS autoinducers signaling molecules and systems, thus affecting the activity of swarming, pyoycanin and biofilm formation.

CONCLUSION

In conclusion, as the extract concentration chose did not exhibited or only slightly show the antimicrobial activity against *P. aeruginosa* bacterial growth, the inhibition on the quorum sensing mediated virulence and biofilm formation could be due to the intrinsic quorum quenching activities by the extract. The ethanolic extract of *C. xanthorrhiza* Roxb. effect on the *P. aeruginosa* quorum sensing is concentration dependent whereby at 200 mg/mL, the extract were able to inhibit 72.12% of *P. aeruginosa* swarming, 84.30% of pyocyanin production and 78.35% of the biofilm formation. These highlight the extracts' potential as a good anti-quorum sensing agent.

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CONFLICTS OF INTEREST

The authors declares that there is no conflict of interest.

AUTHORS' CONTRIBUTION

AFO substantially contributed in investigation and writing original draft. YR substantially contributed in conception, design of the work and interpreted microbiological data. YR and SR did the draft editing and reviewed the manuscript.

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DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript.

ETHICS STATEMENT

This article does not contain any studies with human participants or animals performed by any of the authors.

REFERENCES

1. Fukuda K. Food safety in a globalized world. *Bull. World Health Organ*, 2015; **93**: 212. https://doi.org/10.2471/BLT.15.154831.
2. Gustavsson J., Cederberg C., Sonesson U., Otterdijk R.V., Meybeck, A. Global Food Losses and Food Waste: Extent, Causes and Prevention, 2011. Rome: Food and Agriculture Organization of the United Nations, Natural Resources Management and Environment Department, 2011. www.fao.org/docrep/014/mb060e/mb060e00.htm. Accessed 7 July, 2018.
3. Adams MS., Moss MO. Food Microbiology. 2nd Ed. Cambridge, UK: The Royal Society of Chemistry, 2000.
4. Mortality and Global Health estimate. https://www.who.int/gho/en/, Accessed 7 July 2018.
5. Scallan E., Hoekstra RM., Angulo FJ., Tauxe RV., Widdowson M.A., Roy S.L., Griffin P.M. Foodborne illness acquired in the United States—major pathogens. *Emerg. Infect. Dis*., 2011; **17**(1): 7–15. https://doi.org/10.3201/eid1701.P11101.
6. Teisl MF., Roe BE. Consumer willingness-to-pay to reduce the probability of retail foodborne pathogen contamination. *Food Policy*, 2010; **35**: 521–530. https://doi.org/10.1016/j.foodpol.2010.07.003.
7. Abdul-Mutalib NA, Syafinaz AN., Sakai K., Shirai Y. An overview of foodborne illness and food safety in Malaysia. *Int. Food Res. J.*, 2015; **22**(3): 896–901.
8. Soon JM., Singh H., Baines R. Foodborne diseases in Malaysia: A review. *Food Control*, 2011; **22**: 823–830. https://doi.org/10.1016/j.foodcont.2010.12.011.
9. Levinson W. Review of Medical Microbiology and Immunology, pp. 142-159. 13th Ed. The McGraw Hill Education, New York, 2014.
10. Sigma Aldrich. Mechanism of Action – Antibiotics. https://www.sigmaalrich.com, Accessed 20 May, 2018.
11. Bhunia AK. Introduction of Foodborne Pathogens, pp. 1-16. In Foodborne Microbial Pathogens. 1st Ed. Springer, New York, 2008. https://doi.org/10.1007/978-1-4939-7349-1_1.
12. Dong Y., Wang L., Zhang LH. Quorum-quenching microbial infections: Mechanisms and implications. *Philos. Trans. R. Soc. Lond. B. Biol. Sci*., 2007; **362**(1483): 1201–1211. https://doi.org/10.1098/rstb.2007.2045.
13. Zhang LH., Dong YH. Quorum sensing and signal interference: Diverse implications. *Mol. Microbiol.*, 2004; **53**(6): 1563–1571. https://doi.org/10.1111/j.1365-2958.2004.04234.x.
14. Ahmad A., Viljoen AM., Chenia HY. The impact of plant volatiles on bacterial quorum sensing. *Lett. Appl. Microbiol.*, 2015: **60**(1): 8–19. https://doi.org/10.1111/lam.12343.
15. Skandamis PN., Nychas GJE. Quorum sensing in the context of food microbiology. *Appl. Environ. Microbiol.,* 2012; **78**(16): 5473–5482. https://doi.org/10.1128/AEM.00468-12.
16. Szabo M., Varga GZ., Hohmann J., Schelz Z., Szegedi E., Amaral L., Molnar J. Inhibition of quorum-sensing signals by essential oils. *Phytother. Res.,* 2010; **24**(5): 782–786. https://doi.org/10.1002/ptr.3010.
17. Bai AJ., Rai VR.Bacterial Quorum Sensing and Food Industry.*Compr. Rev. Food Sci*. *Food Saf.,* 2011; **10**(3):183–193. https://doi.org/10.1111/j.1541-4337.2011.00150.x.
18. Hossain Z. Bacteria, pp. 490–491. In Motarjemi Y, Moy G, and Ewen CDT (eds.), Encyclopedia of Food Safety, 1st Ed. Academic Press, Cambridge, Massachusetts, 2013. https://doi.org/10.1016/B978-0-12-378612-8.00109-8.
19. Lee J., Zhang L. The hierarchy quorum sensing network in *Pseudomonas aeruginosa. Protein and Cell*, 2014; **6**(1): 26–41. https://doi.org/10.1007/s13238-014-0100-x.
20. Lew KF., Goh GL., Son R., Rukayadi Y. Effect of Javanese turmeric (*Curcuma xanthorrhiza* Roxb.) extract on natural microflora of oyster mushroom (*Pleurotus sajur-caju*) and its sensory acceptability. *Int. Food Res. J.*, 2015; **22**(6), 2446–2451.
21. Musfiroh I., Muchtaridi M., Muhtadi A., Diantini A., Hasanah AN., Udin LZ., Ibrahim S. Cytotoxicity studies of xanthorrhizol and its mechanism using molecular docking simulation and pharmacophore modelling. *J. Appl. Pharm. Sci.*, 2013; **3**(6): 7-15.
22. Lim TK. *Curcuma zanthorrhiza*. pp. 384. In Edible Medicinal and Non-Medicinal Plants. Cham: Springer International Publishing, New York, 2016.

https://doi.org/10.1007/978-3-319-26065-5_16.

23. Park JH., Park KK., Kim MJ., Hwang JK., Park SK., Chung WY. Cancer chemoprotective effects of *Curcuma xanthorrhiza. Phytother. Res.*, 2008; **22**(5): 695–698. https://doi.org/10.1002/ptr.2418.
24. Jantan I., Saputri FC., Qaisar MN., Buang F. Correlation between chemical composition of *Curcuma domestica* and *Curcuma xanthorrhiza* and their antioxidant effect on human low-density lipoprotein oxidation. *Evid. Based Complement. Alternat. Med.*, 2012; 438356. https://doi.org/10.1155/2012/438356.
25. Sylvester WS., Son R., Lew KF., Rukayadi Y. Antibacterial activity of Java turmeric (*Curcuma xanthorrhiza* Roxb.) extract against *Klebsiella pneumoniae* isolated from several vegetables. *Int. Food Res. J.*, 2015; **22**(5): 1770–1776.
26. Casilag F., Lorenz A., Krueger J., Klawonn F., Weiss S., Haussler S. LasB elastase of *Pseudomonas aeruginosa* acts in concert with alkaline protease AprA to prevent flagellin-mediated immune recognition. *Infect. Immun.*, 2015; **84**(1): 162–171. https://doi.org/10.1128/IAI.00939-15.
27. Ab Halim MR., Mahmud MZT., Mahmud R., Ismail S. Standardization and phytochemical studies of *Curcuma xanthorrhiza* Roxb. *Int. J. Pharm. Pharm. Sci.*, 2012; **4**(3): 606–610.
28. Soniya M., Kuberan T., Anitha S., Sankareswari P. *In vitro* antibacterial activity of plant extracts against Gram positive and Gram negative pathogenic bacteria. *I. J. Microbiol. Immun. Res.*, 2013; **2**(1): 1–5.
29. Naz R., Ayub H., Nawaz S., Islam ZU., Yasmin T., Bano A., Roberts TH. Antimicrobial activity, toxicity and anti-inflammatory potential of methanolic extracts of four ethnomedicinal plant species from Punjab, Pakistan. *BMC Complement. Altern Med.,* 2017; **302**: 1-13. https://doi.org/10.1186/s12906-017-1815-z.
30. Clinical and Laboratory Standards Institute (CLSI). 2005. Performance Standards for Antimicrobial Disk Susceptibility Tests; Approved Standard, pp. M2-A8. 8th Ed. Villanova, PA.
31. Hazan R., Que YA., Maura D., Rahme LG. A method for high throughput determination of viable bacteria cell counts in 96-well plates. *BMC Microbiol.*, 2012; 12: 259. https://doi.org/10.1186/1471-2180-12-259.
32. Rashid MH., Kornberg A. Inorganic polyphosphate is needed for swimming, swarming, and twitching motilities of *Pseudomonas aeruginosa*. *Proc. Nat. Acad. Sci.,* 2000; **97**(9): 4885–4890. https://doi.org/10.1073/pnas.060030097.
33. King M., Guragain M., Sarkisova S., Patrauchan M. Pyocyanin extraction and quantitative analysis in swarming *Pseudomonas aeruginosa.* Bio-Protocol,. https://doi.org/10.21769/BioProtoc.2042.
34. Karpagam S., Sudhakar T., Lakshmipathy M. Microbicidal response of pyocyanin produced by *Pseudomonas aeruginosa* toward clinical isolates of fungi. *Int. J. Pharm. Pharm. Sci.,* 2013; **5**(3): 870–873.
35. Ugurlu A., Yagci AK., Ulusoy S., Aksu B., Bosgelmez-Tinaz G. Phenolic compounds affect production of pyocyanin, swarming motility and biofilm formation of *Pseudomonas aeruginosa. Asian Pac. J. Tropic. Biomedic,* 2016; **6**(8): 698-701. https://doi.org/10.1016/j.apjtb.2016.06.008.
36. Ozcan D., Kahraman H. Pyocyanin production in the presence of calcium ion in *Pseudomonas aeruginosa* and recombinant bacteria*. Turkish J. Sci. Technol.,* 2015; **10**(1): 13–19.
37. Krishnan T., Yin WF., Chan KG. Inhibition of quorum sensing-controlled virulence factor production in *Pseudomonas aeruginosa* PAO1 by ayurveda spice clove (*Syzygium aromaticum*) bud extract. *Sensors,* 2012; **12**(4): 4016–4030. https://doi.org/10.3390/s120404016.
38. Datta S., Debanjan Jana B., Tilak Raj Maity B., Aveek Samanta B., Rajarshi Banerjee B. Piper betle leaf extract affects the quorum sensing and hence virulence of *Pseudomonas aeruginosa* PAO1. *Biotech,* 2016; **6:**18. https://doi.org/10.1007/s13205-015-0348-8.
39. Chong YM., Yin W., Ho CY., Mustafa MR., Hadi A., Hamid A., Awang K., Narrima P., Koh CL., Appleton DR., Chan KG. *Malabaricone C* from *Myristica cinnamomea* exhibits anti-quorum sensing activity. *J. Nat. Prod.,* 2011; **74** (10): 2261-2264. https://doi.org/10.1021/np100872k.
40. Sarkisova S., Patrauchan MA., Berglund D., Nivens DE., Franklin MJ. Calcium-induced virulence factors associated with the extracellular matrix of mucoid *Pseudomonas aeruginosa* biofilms. *Society,* 2005; **187**(13): 4327–4337. https://doi.org/10.1128/JB.187.13.4327-4337.2005.
41. Essar DW., Eberly L., Hadero A., Crawford IP. Identification and characterization of genes for a 2nd Anthranilate Synthase in *Pseudomonas aeruginosa*: Interchangeability of the 2 anthranilate synthases and evolutionary implications. *J. Bacteriol.,* 1990; **172**(2): 884–900. https://doi.org/10.1128/jb.172.2.884-900.1990.
42. Varposhti M., Abdi Ali A., Mohammadi P., Saboora A. Effects of extracts and an essential oil from some medicinal plants against biofilm formation of *Pseudomonas aeruginosa. J. Med. Microbiol. Infect. Dis.*, 2013; **1**(1): 36–40.
43. Azwanida NN. A review on the extraction methods use in medicinal plants, principle, strength and limitation. *Med. Aromat. Plants*, 2015; **4**: 196.
44. Do QD., Angkawijaya AE., Tran-Nguyen PL., Huynh LH., Soetaredjo FE., Ismadji S., Ju YH. Effect of extraction solvent on total phenol content, total flavonoid content and antioxidant activity of *Limnophila aromatica. J. Food Drug Anal.*, 2014; **22**(3): 296–302. https://doi.org/10.1016/j.jfda.2013.11.001.
45. Aida W. Effect of ethanol concentration, extraction time and extraction temperature on the recovery of phenolic compounds and antioxidant capacity of *Centella asiatica* extracts. *Int. Food Res. J.*, 2011; **18**: 571–578.
46. Dai J., Mumper R.J. Plant phenolics: Extraction, analysis and their antioxidant and anticancer properties. *Molecules*, 2010; **15**: 7313-7352. https://doi.org/10.3390/molecules15107313.
47. Whitcombe T. Why is ethanol the only relatively safe alcohol for human consumption?. *MadSci Network: Biochemistry,* 2000. http://www.madsci.org/posts/archives/2000-11/973793825.Bc.r.html, Accessed 17

July 2018.

48. Saifudin A., Rahayu HV., Teruna HY. "Standarisasi bahan obat alam". Pp. 16. Graha Ilmu. Yogyakarta, 2011.
49. Tarigan J., Zuhra CF., Sihotang H. Skrining fitokimia tumbuhan yang digunakan oleh pedagang jamu gendong untuk merawat kulit wajah di Kecamatan Medan Baru. *J. Biol. Sumatera*, 2008; **1**(3): 1–6.
50. Diastuti H., Syah YM., Juliawaty LD., Singgih M. Antibacterial *Curcuma xanthorrhiza* extract and fractions*. J. Math. Fund. Sci.*, 2014; **46**(3): 224–234. https://doi.org/10.5614/j.math.fund.sci.2014.46.3.2.
51. Harborne J. Phytochemical methods. A guide to modern techniques of plants analysis. 2nd Ed. Chapman and Hall, London, 1996.
52. Mangunwardoyo W., Deasywaty, Usia T. Antimicrobial and identification of active compound *Curcuma xanthorrhiza* Roxb. *Int. J. Basic Appl. Sci. IJBAS-IJENS,* 2012; **12**(1): 69–78.
53. Selim S., Hassan S., Al Soumaa K., EL-Anzy S. Prevalence, antibiotic resistance and in vitro activity of yogurt against some Gram negative pathogenic bacteria isolated from Arar Hospital, KSA. *Life Sci. J.*, 2013; **10**: 1450–1456.
54. Hanouda T., Baker JR. Antimicrobial mechanism of action of surfactant lipid preparation in enteric Gram negative bacilli. *J. Appl. Microbiol.*, 2000; **89**: 397–403. https://doi.org/10.1046/j.1365-2672.2000.01127.x.
55. Hidayathulla S., Keshava CK., Chandrashekar KR. Phytochemical evaluation and antibacterial activity of *Pterospermum diversifolium* Blume. *Int. J. Pharm. Sci.*, 2011; **3**(2): 165–167.
56. Al-Rubiay KK., Jaber NN., Al Mhaawe BH., Alrubaay LK. Antimicrobial of henna extract. *Oman Med. J.*, 2008; **23**: 4 – 8. https://doi.org/10.4176/081215.
57. Nohynek LJ., Alakomi A., Kankonen MP., Heinonen M., Helander I.M., Oksman- Caldentey KM., Puupponen-Pimia RH. Berry phenolics: Antimicrobial properties and mechanisms of action against severe human pathogens. *Nutr. Cancer*, 2006; **54**(1): 18–32. https://doi.org/10.1207/s15327914nc5401_4.
58. Taylor PK., Yeung ATY., Hancock REW. Antibiotic resistance in *Pseudomonas aeruginosa* biofilms: Towards the development of novel anti-biofilm therapies. *J. Biotechnol.*, 2014; **191**: 121–130. https://doi.org/10.1016/j.jbiotec.2014.09.003.
59. Hertiani T., Palupi SI., Sanliferianti., Nurwindasari D.H. *In vitro* test on antimicrobial potency against *Staphyloccocus aureus, Escherichia coli, Shigella dysentriaea* and *Candida albicans* of some herbs traditionally used cure infection diseases*. Pharmacon,* 2003; **4**(2): 89–95.
60. Hancock REW., and Bell A. Antibiotic uptake into Gram-negative bacteria*. Eur. J. Clin. Microbiol. Infect. Dis.*, 1988; **7**: 713–720. https://doi.org/10.1007/BF01975036.
61. Khan MSA., Zahin M., Hasan S., Husain FM., Ahmad I. Inhibition of quorum sensing regulated bacterial functions by plant essential oils with special reference to clove oil. *Lett. Appl. Microbiol.,* 2009; **49**(3): 354–360. https://doi.org/10.1111/j.1472-765X.2009.02666.x.
62. Kɤhler T., Curty LK., Barja F., van Delden C., Pech ɒre JC. Swarming of *Pseudomonas aeruginosa* is dependent on cell-to-cell signaling and requires flagella and pili. *J. Bacteriol.,* 2000; **182**(21): 5990–6. https://doi.org/10.1128/JB.182.21.5990-5996.2000.
63. Davey ME., Caiazza NC., O'Toole GA. Rhamnolipid surfactantproduction affects biofilm architechture in *Pseudomonas aeruginosa* PA01. *J. Bacteriol.*, 2003; **185**(3): 1027-1036. https://doi.org/10.1128/JB.185.3.1027-1036.2003.
64. Nogales J., Dominiguez-Ferreras A., Amaya-Gomez CV., van Dillewijn P., Cuellar V., Sanjuan J., Olivares J., Soto M.J. Transcriptome profiling of a *Sinorhizobium meliloti* fadD mutant reveals the role of rhizobactin 1021 biosynthesis and regulation genes in the control of swarming. *BMC Genomics*, 2010; **11**: 157. https://doi.org/10.1186/1471-2164-11-157.
65. Rahim R., Ochsner UA., Olvera C., Graninger M., Messner P., Lam J.S., Soberɳn-Chavez G. Cloning and functional characterization of the *Pseudomonas aeruginosa rhl*C gene that encodes Rhamnosyltransferase 2, an enzyme responsible for di-rhamnolipid biosynthesis. *Mol. Microbiol.*, 2001; **40**(3): 708–18. https://doi.org/10.1046/j.1365-2958.2001.02420.x.
66. Ochsner UA., Reiser J. Autoinducer-mediated regulation of rhamnolipid biosurfactant synthesis in *Pseudomonas aeruginosa. Proc. Nat. Acad. Sci.*, 1995; **92**: 6424–6428. https://doi.org/10.1073/pnas.92.14.6424.
67. Caiazza NC., Shanks RMQ., O'toole GA. Rhamnolipids modulate swarming motility patterns of *Pseudomonas aeruginosa*. *J. Bacteriol.*, 2005; **187**(21): 7351–7361. https://doi.org/10.1128/JB.187.21.7351-7361.2005.
68. Kim HS., Park HD. Ginger extract inhibits biofilm formation by *Pseudomonas aeruginosa* PA14. *PLoS ONE,* 2013; **8**: 9. https://doi.org/10.1371/journal.pone.0076106.
69. Rasmussen TB., Bjarnsholt T., Skindersoe ME., Hentzer M., Kristoffersen P., Kɤte M., Givskov M. Screening for quorum-sensing inhibitors (QSI) by use of a novel genetic system, the QSI selector. *J. Bacteriol.*, 2005; **187**(5): 1799–814. https://doi.org/10.1128/JB.187.5.1799-1814.2005.
70. Caiazza NC., Merritt JH., Brothers KM., O'Toole GA. Inverse regulation of biofilm formation and swarming motility by *Pseudomonas aeruginosa* PA14. *J. Bacteriol.*, 2007; **189**(9): 3603–12. https://doi.org/10.1128/JB.01685-06.
71. Pratt LA., Kolter R. Genetic analysis of *Escherichia coli* biofilm formation: Roles of flagella, motility, chemotaxis and type I pili. *Mol. Microbiol.*, 1998; **30**(2): 285–293. https://doi.org/10.1046/j.1365-2958.1998.01061.x.
72. O'May C., Tufenkji N. The swarming motility of *Pseudomonas aeruginosa* is blocked by *Cranberry proanthocyanidins* and other tannin-containing materials. *Appl. Environ. Microbiol.*, 2011; **77**(9): 3061–3067. https://doi.org/10.1128/AEM.02677-10.
73. Vattem DA., Mihalik K., Crixell SH., McLean RJC. Dietary phytochemicals as quorum sensing inhibitors. *Fitoterapia*, 2007; **78**(4): 302–310. https://doi.org/10.1016/j.fitote.2007.03.009.
74. Huber B., Eberl L., Feucht W., Polster J. Influence of polyphenols on bacterial biofilm formation and quorum-sensing. *Z. Naturforsch,* 2003; **58**: 879–884. https://doi.org/10.1515/znc-2003-11-1224.
75. Serrano J., Puupponen-Pimiה R., Dauer A., Aura

AM., Saura-Calixto F. Tannins: Current knowledge of food sources, intake, bioavailability and biological effects. *Mol. Nut. Food Res.*, 2009; **53**(S2): S310–S329. https://doi.org/10.1002/mnfr.200900039.

76. Haslam E. Natural polyphenols (vegetable tannins) as drugs: Possible modes of action. *J. Nat. Prod.*, 1996; **59**(2): 205–15. https://doi.org/10.1021/np960040+.
77. Hagerman AE., Butler LG. The specificity of proanthocyanidin-protein interactions. *The J. Biol. Chem.*, 1981; **256**(9): 4494–7.
78. Borges A., Saavedra MJ., Simγes M. The activity of ferulic and gallic acids in biofilm prevention and control of pathogenic bacteria. *Biofouling*, 2012; **28** (7): 755–767. https://doi.org/10.1080/08927014.2012.706751.
79. Reszka KJ., O'Malley Y., McCormick ML., Denning GM., and Britigan BE. Oxidation of pyocyanin, a cytotoxic product from *Pseudomonas aeruginosa,* by microperoxidase 11 and hydrogen peroxide. *Free Radical Biology and Medicine*, 2004; **36**(11): 1448–1459. https://doi.org/10.1016/S0891-5849(04)00224-2.
80. Prijaya P., Philip R., Singh ISB. Cloning and overexpression of Phz genes encoding phenazine biosynthetic pathway for the enhanced production of pyocyanin in *Pseudomonas aeruginosa* MCCB117. Cochin University of Science and Technology, Shodhganga. India, 2013.
81. Borges A., Serra S., Cristina Abreu A., Saavedra MJ., Salgado A., Simγes M. Evaluation of the effects of selected phytochemicals on quorum sensing inhibition and in vitro cytotoxicity. *Biofouling*, 2014; **30**(2): 183–195. https://doi.org/10.1080/08927014.2013.852542.
82. Karatuna O., Yagci A. Analysis of quorum sensing-dependent virulence factor production and its relationship with antimicrobial susceptibility in *Pseudomonas aeruginosa* respiratory isolates. *Clin. Microbiol. Infect.*, 2010; **16**(12): 1770–1775. https://doi.org/10.1111/j.1469-0691.2010.03177.x.
83. Mustafa RA., Abdul Hamid A., Mohamed S., Bakar FA. Total phenolic compounds, flavonoids, and radical scavenging activity of 21 selected tropical plants. *J. Food Sci.*, 2010; **75**: C28-35. https://doi.org/10.1111/j.1750-3841.2009.01401.x.
84. Moure A., Cruz JM., Franco D., Manuel Dom ɒnguez J., Sineiro J., Domɒnguez H., Nאסez M.J., Carlos Parajף J. Natural antioxidants from residual sources. *Food Chem.*, 2001; **72**: 145–171. https://doi.org/10.1016/S0308-8146(00)00223-5.
85. Okwu D.E. Evaluation of the chemical composition of indigenous. Spices and flavouring agents. *Global J. Pure Appl.Sci.*, 2001;**7**: 455–459. https://doi.org/10.4314/gjpas.v7i3.16293.
86. Kim JE., Kim HE., Hwang JK., Lee HJ., Kwon HK., Kim BI. Antibacterial characteristics of *Curcuma xanthorrhiza* extract on *Streptococcus mutans* biofilm. *J. Microbiol.*, 2008; **46**: 228–232. https://doi.org/10.1007/s12275-007-0167-7.
87. Tunney MM., Ramage G., Field TR., Moriarty TF., Storey DG. Rapid colorimetric assay for antimicrobial susceptibility testing of *Pseudomonas aeruginosa. Antimicrob. Agents Chemother.*, 2004; **48**(5): 1879-1881. https://doi.org/10.1128/AAC.48.5.1879-1881.2004.
88. Webb JS., Thompson LS., James S., Charlton T., Tolker-Nielsen T., Koch B., Givskov M., Kjelleberg S. Cell death in *Pseudomonas aeruginosa* biofilm development. *J. Bacteriol.*, 2003; **185**(15): 4585-4592. https://doi.org/10.1128/JB.185.15.4585-4592.2003.
89. Barraud N., Hassett DJ., Hwang SH., Rice SA., Kjelleberg S., Webb JS. Involvement of nitric oxide in biofilm dispersal of *Pseudomonas aeruginosa. J. Bacteriol.*, 2006; **188**(21): 7344–7353. https://doi.org/10.1128/JB.00779-06.
90. Samanta S., Thavasi R., Jayalakshmi S. Phenazine pigments from *Pseudomonas aeruginosa* and their application as antibacterial agent and food colourants. *Res. J. Microbiol.*, 2008; **3**(3): 122–128. https://doi.org/10.3923/jm.2008.122.128.
91. Das T., Manefield M. Pyocyanin Promotes Extracellular DNA Release in *Pseudomonas aeruginosa*. *PLOS ONE*, 2012; **7**(10): e4671B. https://doi.org/10.1371/journal.pone.0046718.
92. Allesen-Holm M., Barken KB., Yang L., Klausen M., Webb JS., Kjelleberg S., Tolker-Nielsen TA. Characterization of DNA release in *Pseudomonas aeruginosa* cultures and biofilms. *Mol. Microbiol.*, 2006; **59**: 1114–1128. https://doi.org/10.1111/j.1365-2958.2005.05008.x.
93. Whitchurch CB., Tolker-Nielsen T., Ragas PC., Mattick JS. Extracellular DNA required for bacterial biofilm formation. *Science*, 2002; **295**(5559): 1487. https://doi.org/10.1126/science.295.5559.1487.
94. Flemming HC., Wingender J. The biofilm matrix. *Nat. Rev. Microbiol.,* 2010; **8**(9): 623–633. https://doi.org/10.1038/nrmicro2415.
95. Gloag ES., Turnbull L., Huang A., Vallotton P., Wang H., Nolan LM., Whitchurch CB. Self-organization of bacterial biofilms is facilitated by extracellular DNA. *Proc. Nat. Acad. Sci.*, 2013; **110**(28): 11541–11546. https://doi.org/10.1073/pnas.1218898110.
96. Mulcahy H., Charron-Mazenod L., Lewenza S. *Pseudomonas aeruginosa* produces an extracellular deoxyribonuclease that is required for utilization of DNA as a nutrient source. *Environ. Microbiol.,* 2010; **12(**6): 1621–9. https://doi.org/10.1111/j.1462-2920.2010.02208.x.
97. Finkel SE., Kolter R. DNA as a nutrient: novel role for bacterial competence gene homologs. *J. Bacteriol.*, 2001; **183**(21): 6288–93. https://doi.org/10.1128/JB.183.21.6288-6293.2001.
98. Merighi M., Lee VT., Hyodo M., Hayakawa Y., Lory S. The second messenger bis-(3'-5')-cyclic-GMP and its PilZ domain-containing receptor Alg44 are required for alginate biosynthesis in *Pseudomonas aeruginosa*. *Mol. Microbiol.*, 2007; **65**(4): 876–895. https://doi.org/10.1111/j.1365-2958.2007.05817.x.
99. Merritt JH., Brothers KM., Kuchma SL., O 'toole GA. SadC reciprocally influences biofilm formation and swarming motility via modulation of exopolysaccharide production and flagellar function. *J. Bacteriol.*, 2007; **189**(22): 8154–8164. https://doi.org/10.1128/JB.00585-07.
100. Rאmling U., Gomelsky M., Galperin MY. C-di-GMP: The dawning of a novel bacterial signalling system. *Mol. Microbiol.*, 2005; **57**(3): 629–639. https://doi.org/10.1111/j.1365-2958.2005.04697.x.
101. D'Argenio DA., Miller SI. Cyclic di-GMP as a bacterial

second messenger. *Microbiology*, 2004; **150**(8): 2497–2502. https://doi.org/10.1099/mic.0.27099-0.

102. Jenal U. Cyclic di-guanosine-monophosphate comes of age: A novel secondary messenger involved in modulating cell surface structures in bacteria. *Curr. Opin. Microbiol.*, 2004; **7**: 185–191. https://doi.org/10.1016/j.mib.2004.02.007.
103. O'toole GA. How *Pseudomonas aeruginosa* regulates surface behaviors. *Microbe,* 2008; **3**(2): 65–71. https://doi.org/10.1128/microbe.3.65.1.
104. Verstraeten N., Braeken K., Debkumari B., Fauvart M., Fransaer J., Vermant J., Michiels J. Living on a surface: Swarming and biofilm formation. *Trends Microbiol.*, 2008; **16**(10): 496–506. https://doi.org/10.1016/j.tim.2008.07.004.
105. Vasavi H., Arun A., Rekha P. Anti-quorum sensing activity of flavonoid- rich fraction from *Centella asiatica* L. against *Pseudomonas aeruginosa* PAO1. *J. Microbiol. Immun. Infect.*, 2016; **49**: 8–15. https://doi.org/10.1016/j.jmii.2014.03.012.
106. Issac A., Sybiya VP., Palani A., Khadar SM., Shunmugiah KP., Arumugam VR. 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. https://doi.org/10.1016/j.foodres.2011.10.022.
107. Parkins MD., Ceri H., Storey DG. *Pseudomonas aeruginosa* GacA, a factor in multihost virulence, is also essential for biofilm formation. *Mol. Microbiol.*, 2001; **40**(5): 1215–26. https://doi.org/10.1046/j.1365-2958.2001.02469.x.
108. Kay E., Humair B., Dᵻnervaud V., Riedel K., Spahr S., Eberl L., Haas D. Two GacA-dependent small RNAs modulate the quorum-sensing response in *Pseudomonas aeruginosa. J. Bacteriol.*, 2006; **188**(16): 6026–33. https://doi.org/10.1128/JB.00409-06.
109. Reimmann C., Beyeler M., Latifi A., Winteler H., Foglino M., Lazdunski A., Haas D. The global activator GacA of *Pseudomonas aeruginosa* PAO positively controls the production of the autoinducer N-butyryl-homoserine lactone and the formation of the virulence factors pyocyanin, cyanide, and lipase. *Mol. Microbiol.*, 1997; **24**(2): 309–19. https://doi.org/10.1046/j.1365-2958.1997.3291701.x.