In vitro Quorum Quenching Activity of Eleusine indica Crude Ethanolic Extract against Pseudomonas aeruginosa and Serratia marcescens

Allan John R. Barcena,1 Eunice Maricar M. Baldovino,1 Justin Grace Bañez,1 Czarina Ann B. Baptisma,1 Aldwin Matthew M. Baronda,1 Renren B. Barroga,2 Jumela Mica Q. Bautista,1 Gabriel Roberto G. Baybay,1 Rafael Mariano G. Baybay,1 Vibiene Norma C. Bernal,1 Katherine Adrielle R. Bersola,1 Katrina Ysabelle T. Bolaños,1 Hans Joren L. Bondoc,1 Julius Ervin S. Buitizon,1 Alec Xavier D. Bukuhan,1 John Patrick B. Bulaong,1 Jan Louise DC. Cabrera,1 Nikko H. Cabrestante,1 Gian Carlo M. Cabuco,1 Jose Paciano B.T. Reyes, MD2 and Fresthel Monica M. Climacosa, MD, PhD3

1College of Medicine, University of the Philippines Manila
2Department of Pharmacology and Toxicology, College of Medicine, University of the Philippines Manila
3Department of Medical Microbiology, College of Public Health, University of the Philippines Manila

ABSTRACT

Introduction. Nosocomial contaminants such as Pseudomonas aeruginosa and Serratia marcescens are increasingly developing resistance to many antibiotics. One of the promising alternatives that may complement, if not substitute, the use of antibiotics is quorum quenching, the process of interfering with chemical signals that mediate communication between microorganisms. Eleusine indica, a ubiquitous grass used traditionally to treat infections, has been shown to contain metabolites, such as fatty acid derivatives and p-coumaric acid, capable of quorum quenching. To date, there has been no study on the quorum quenching activity of E. indica.

Objectives. This study aimed to determine the in vitro activity of crude ethanolic extract of E. indica leaves against selected quorum-sensing regulated virulence factors of P. aeruginosa and S. marcescens.

Methodology. E. indica leaves were collected, washed, air-dried, and homogenized. Following ethanolic extraction and rotary evaporation, the extract was screened for antimicrobial activity through disk diffusion test and broth microdilution assay. The quorum quenching activity of the extract against P. aeruginosa was measured through swarming motility assay, while the activity against S. marcescens was measured through swarming motility and pigment inhibition assays. The quorum quenching assays were conducted in triplicates, and analysis of variance (ANOVA) was performed to identify differences among the treatment groups.

Results. Disk diffusion test revealed that no zones of inhibition formed against both P. aeruginosa and S. marcescens for varying concentrations of up to 200 mg/mL of the crude extract. Likewise, the MIC of the extract against both P. aeruginosa and S. marcescens was determined to be >200 mg/mL. However, it was shown that the extract, at 50 mg/mL, has statistically significant activity (p<0.05) against the swarming motility of P. aeruginosa, and it is 71.6% as effective in reducing the swarming area of the bacteria compared to cinnamaldehyde. This was not observed when the extract was tested against the swarming motility of and pigment production by S. marcescens.

Conclusion. In this study, the quorum quenching activity of the crude ethanolic extract of E. indica leaves was found to be effective against P. aeruginosa but not against S. marcescens. The compounds that will be identified by further studies may conceivably be used as an adjunct therapy in P. aeruginosa infections and as coating agents in medical devices.

Key Words: Eleusine indica, Pseudomonas aeruginosa, quorum quenching, swarming motility, Serratia marcescens, prodigiosin
INTRODUCTION

The inappropriate use of antibiotics worldwide is accelerating the development of antimicrobial resistance in various microorganisms. With a growing number of antibiotics losing their efficacy, infections are becoming harder to treat, and the increasing number of infections due to resistant microorganisms has led to increased morbidity, mortality, length of hospitalization, and overall healthcare expenditure across the globe. In fact, among the most important focal points for the spread of resistance are hospitals where resistant microbes cause severe nosocomial infections. One of the promising alternatives that may complement, if not substitute, the use of antibiotics in treating infections is quorum quenching—the process of extinguishing the signals that mediate quorum sensing.

Quorum sensing is a chemically mediated type of communication among bacterial cells that allows them to function as a unit. The compounds that mediate this process are called autoinducers, and most Gram-negative bacteria utilize a specific class of compounds called acyl-homoserine lactone (AHL) autoinducers. In an AHL system, the signal molecule is produced constitutively at low levels insufficient to trigger a single bacterium’s receptors. However, when a bacterial population reaches a threshold density, the concentration of the signal can shift the equilibrium towards the binding of the signal to the receptor. The signal-receptor complex then acts as a transcription factor that promotes further production of the signal and expression of specific virulence factors such as swarming motility, pigment production, and biofilm formation, depending on bacterial density.

By attenuating this communication system, inhibition of quorum sensing imposes lesser selective pressure for the development of resistance when compared to directly killing the pathogen. With antibiotics, especially when misused, a drug-resistant strain is given a survival advantage wherein it is allowed to proliferate without having to compete with non-resistant strains. In quorum sensing inhibition or quorum quenching, however, even if a bacterium eventually develops resistance against the inhibitor, it still has to compete with the whole bacterial population for survival. In addition, the majority of the bacterial population will be unable to produce the threshold signal to induce expression of the virulence factors by both non-resistant and resistant strains.

As AHL autoinducers mediate quorum sensing in most Gram-negative microorganisms, the process of quorum sensing in Gram-negative pathogens, such as Pseudomonas aeruginosa and Serratia marcescens, may be disrupted by various quorum quenching mechanisms that interfere with the AHL system. These mechanisms include inhibition of AHL synthase expression, direct inhibition of AHL molecules, degradation of the AHL autoinducers, and utilization of synthetic analogs that mimic AHL molecules. Interestingly, a variety of plant-derived molecules and compounds, which include halogenated furanones, gamma-aminobutyric acid (GABA), pyrogallol, curcumin, ursolic acid, rosmarinic acid, fatty acid derivatives, phenolic compounds, flavanones, flavonoids, and flavonols, have been shown to inhibit quorum sensing in human pathogens through mechanisms previously mentioned. Elesine indica, a ubiquitous grass, locally known as paragis, was reported to contain some of these quorum-quenching compounds. This grass has been used in many communities as a treatment for vaginal and urinary tract infections. A study by Penaloza et al. in 2018, which utilized mass spectrometry (MS) and nuclear magnetic resonance (NMR) spectroscopy, found that the aerial parts of E. indica contain fatty acid derivatives and p-coumaric acid. Several of these fatty acids have demonstrated activity against the swarming motility of P. aeruginosa in a study by Inoue et al. in 2008, while p-coumaric acid has demonstrated activity against Agrobacterium tumefaciens NTLA, Chromobacterium violaceum 5999, Pseudomonas chlororaphis in a study by Bodini et al. on soil bacteria in 2009.

To date, there has been no study on the efficacy of E. indica in combating bacterial infections through quorum sensing inhibition. This study aimed to determine the effects of crude ethanolic extract of E. indica leaves against the growth and production of quorum sensing-regulated virulence factors of P. aeruginosa and S. marcescens.

METHODS

Plant collection and extraction

E. indica leaves were collected from the University of the Philippines Diliman. Soil sample (1 kg) was also collected for heavy metal analysis. The leaves were thoroughly washed with distilled water and were air-dried for seven days. Following blender homogenization, the leaves were soaked in 95% ethanol at a 1:2 w/v ratio for three days. The solution was then filtered using Whatman filter paper no. 4 into a round bottom flask. The filtrate was heated at 40°C at 100 rpm using a rotary evaporator until the solvent has completely evaporated.

Antimicrobial susceptibility testing

To determine the susceptibility of P. aeruginosa and S. marcescens to the extract, disk diffusion test and broth microdilution assay were performed.

Disk diffusion test

Mueller-Hinton (MH) agar plates were inoculated evenly with the test microorganisms—P. aeruginosa and S. marcescens. Filter paper disks (6 mm in diameter) were loaded with 20 µL of the different concentrations of the crude extract. Disks loaded with sterile water were used as the negative control, while disks loaded with 10 µg of gentamicin were used as positive control following the standard protocol of the Clinical and Laboratory Standards, Institute (CLSI). The disks were then placed in the appropriate plate quadrants. Each setup was prepared in quintuplicates for
each test microorganism, and the plates were incubated at 37°C for 24 hours.

**Broth microdilution assay**

On a sterile 96-well plate, 50 µL of the 200 mg/mL crude plant extract was pipetted into 3 columns for *P. aeruginosa* and 3 columns for *S. marcescens*. The first well in each column contained 200 mg/mL of the crude extract and each succeeding well down each column contained half of the previous well's concentration. The 8th well contained only 1.56 mg/mL of crude extract. To each well, 40 µL of Mueller-Hinton broth and 10 µL of the bacterial suspension were added to obtain an organism concentration of 1x10⁶ CFU/mL per well. The positive control contained dilutions of gentamicin, while the negative control only contained 90 µL of MH broth and 10 µL of inoculum. The plate was incubated for 24 hours.

**Swarming motility assay for *P. aeruginosa* and *S. marcescens***

Swarming motility media infused with the crude extract (50 mg/mL) was prepared and dispensed into Petri plates. Sterile distilled water was used as the negative control, and 50 mg/mL cinnamaldehyde was used as the positive control. After drying for 60 minutes, 5 µL of bacteria suspended in buffered saline with an OD₆₀₀ of 3.0 (1.5x10⁵ CFU/mL) was inoculated in the middle of each plate. Motility was observed after incubation for 24 hours. Using ImageJ, the surface area covered by the bacteria was determined. The experiment was performed in triplicates.

**Pigment inhibition assay for *S. marcescens***

*S. marcescens* was inoculated into brain heart infusion (BHI) broth with 1% inoculum and either 50 mg/mL of the extract, sterile distilled water (negative control), or 50 mg/mL cinnamaldehyde (positive control). After 24 hours of incubation, the samples were subjected to centrifugation (8,000 rpm; 5 minutes) to extract prodigiosin, an antibiotic and pro-apoptotic pigment produced by *S. marcescens*. The supernatant was discarded, and the pellet was resuspended in acidified ethanol (4% 1 M HCl in ethanol). The remaining cell debris was removed by re-centrifugation, and the supernatant was transferred to a 96-well plate for measurement of absorbance at 534 nm. Three replicates were prepared per test. A blank was prepared for each set-up to eliminate the effect of the extract’s color on the absorbance values.

**Data collection and analysis**

Analysis of variance (ANOVA) was used to compare the different treatment groups. Simple ANOVA, followed by Tukey post-hoc testing, was performed for data that satisfied the Levene’s test of homogeneity (p<0.05). On the other hand, ANOVA-Welch test, followed by Games-Howell post-hoc testing, was performed for data which did not satisfy the test of homogeneity.

**RESULTS**

**Heavy metal testing**

The sample of the soil from which the plant samples were collected did not contain cadmium and nickel. On the other hand, the soil sample contained natural levels of arsenic (0.568 mg/kg) and lead (37 mg/kg). The natural level of arsenic in soils ranges from 1 to 40 mg/kg, while the natural level of lead in soils ranges from 15 to 40 mg/kg.

**Susceptibility testing**

The mean zone of inhibition of gentamicin is 26.1 ± 0.65 for *P. aeruginosa* and 21.7 ± 1.25 for *S. marcescens*. Both values are within the susceptible range based on the standard protocol by the Clinical and Laboratory Standards, Institute (CLSI) and the American Society of Microbiology (ASM). No zones of inhibition formed for the negative control, and varying concentrations of the crude extract up to 200 mg/mL. Similarly, we found that both *P. aeruginosa* and *S. marcescens* were susceptible only to the positive control upon performing the MIC test. The MIC of the positive control is less than the minimum tested concentration of gentamicin, which is 1.56 µg/mL, for both *P. aeruginosa* and *S. marcescens*; this is consistent with the breakpoint value recommended by CLSI for gentamicin. On the other hand, no inhibition was observed for varying concentrations of the crude extract up to 200 mg/mL. For the succeeding tests, one maximal dose (50 mg/mL) was used due to limitations in the amount of extract produced.

**Quorum sensing inhibition assay for *P. aeruginosa***

The swarming surface area of *P. aeruginosa* was measured in mm² using ImageJ. The mean and standard deviation of the surface area for the 50 mg/mL crude extract is 71.29 and 3.60, respectively; for 50 mg/mL cinnamaldehyde, the mean and standard deviation are 14.49 and 0.56, while for distilled water, 214.53 and 34.17. Since the data set did not satisfy the assumption for homogeneity confirmed by Levene's test for homogeneity (p<0.05), the Welch's F was used to compare the mean swarming surface area of *P. aeruginosa* exposed to 50 mg/mL crude extract, 50 mg/mL cinnamaldehyde, and distilled water; Games-Howell test was used for post-hoc analysis.

Figure 1 shows that the 50 mg/mL crude extract (71.29 ± 3.60) was able to inhibit the swarming motility of *P. aeruginosa*. There was a significant difference between all the groups, and posthoc comparisons using the Games-Howell test indicated that the swarming surface area of the cinnamaldehyde-exposed *P. aeruginosa* is significantly the smallest (14.49 ± 0.56) followed by the 50 mg/mL crude extract (71.29 ± 3.60), and distilled water had the highest surface area (214.53 ± 34.17). Hence, the 50 mg/mL crude extract has quorum quenching activity against the swarming motility of *P. aeruginosa*, although less compared to the positive control. The positive control caused a 93.25%
reduction in the swarming area versus negative control, while the crude extract at 50 mg/mL caused a 66.77% reduction. Compared to the positive control, the extract at 50 mg/mL is 71.6% as effective in reducing the swarming area of *P. aeruginosa*.

---

**Figure 1.** Mean surface area of *P. aeruginosa* swarming motility. The surface area covered by cinnamaldehyde-exposed *P. aeruginosa* is the smallest (14.49 ± 0.56), followed by the 50 mg/mL crude ethanolic extract (mean = 71.29 ± 3.60) and then distilled water (214.53 ± 34.17).

**Figure 2.** Mean surface area of *S. marcescens* swarming motility. The surface area of the cinnamaldehyde-exposed *S. marcescens* is the smallest (7.57 ± 0.38) followed by the 50 mg/mL crude extract (144.38 ± 45.18) and distilled water (179.42 ± 20.77).

**Figure 3.** Pigment inhibition assay. Cinnamaldehyde has the lowest mean spectrophotometric absorbance value at 534 nm (0.054 ± 0.002), followed by distilled water (0.19 ± 0.03), then the crude extract, which has the highest absorbance value (0.23 ± 0.02).

---

**Quorum sensing inhibition assays for *S. marcescens***

Figure 2 shows that the mean swarming surface areas covered by *S. marcescens* exposed to 50 mg/mL crude extract, 50 mg/mL cinnamaldehyde, and distilled water, are 144.38, 7.57, and 179.42, respectively. The outcome variable was found to be normally distributed and the assumption for equal variances was fulfilled based upon the result of the Levene’s test. Posthoc comparisons using Tukey HSD test revealed that there is a significant difference between positive control (7.57 ± 0.38) and the other two groups, but no significant difference between negative control (179.42 ± 20.77) and the 50 mg/mL crude extract (144.38 ± 45.18). Thus, the crude extract at 50 mg/mL had no quorum quenching activity against the swarming motility of *S. marcescens*.

The inhibition of prodigiosin production by *S. marcescens* was determined by spectrophotometry at 534 nm where a higher absorbance indicates the presence of a greater amount of prodigiosin. The mean absorbance measurements for 50 mg/mL crude extract, 50 mg/mL cinnamaldehyde, and distilled water are 0.23, 0.05, and 0.19, respectively. Levene’s test revealed that the groups do not assume equal variances (p<0.05), hence, Welch’s F was used to compare the mean absorbance values for each treatment. Figure 3 shows that there is a significant difference between positive control and the other two groups, but no significant difference between negative control and extract. Posthoc analysis using the Games-Howell test revealed that cinnamaldehyde (0.05 ± 0.002) is significantly different from the 50 mg/mL crude extract and distilled water. However, there is no significant difference between the 50 mg/mL crude extract (0.23 ± 0.02) and distilled water (0.19 ± 0.03). Therefore, the crude extract at 50 mg/mL had no quorum quenching activity against the swarming motility of *S. marcescens*.
extract at 50 mg/mL did not inhibit the quorum sensing-dependent production of prodigiosin by *S. marcescens*.

**DISCUSSION**

**Susceptibility testing**

The results of the susceptibility tests show that the crude extract has no direct antimicrobial activity against *P. aeruginosa* and *S. marcescens* in all concentrations (up to 200 mg/mL) tested. The antimicrobial effect of *E. indica* on *P. aeruginosa* has been reported in the literature, but this is the first study to explore the antimicrobial effect of *E. indica* on *S. marcescens*. While the results appear to contradict the findings of previous studies on the antimicrobial activity of *E. indica* against *P. aeruginosa*, it is important to note that these studies have used different extraction methods and solvents. For example, the study by Al-Zubairi et al. in 2011 reported that *E. indica* hexane, dichloromethane, and ethyl acetate extracts have significant activity against *P. aeruginosa*.12 Another study by Alaekwe et al. in 2015, which tested the aerial parts of *E. indica* and found that the extracts exhibited antibacterial activity on different species of bacteria, including *P. aeruginosa*, has used chloroform and methanol as extraction solvents.13 Thus, the use of ethanol as a solvent in this study may have affected the antimicrobial activity of the extract. Although it appears to be an unsuitable solvent for the extraction of antimicrobial compounds, it allows for the separation of compounds that may be tested for their quorum quenching activity.

**Quorum sensing inhibition assay against *P. aeruginosa***

It was shown that the extract (at 50 mg/mL) has statistically significant activity (p<0.05) against the swarming motility of *P. aeruginosa*. Swarming, defined as movement across a semisolid medium, is important in the spread of infection and evasion of the host response. It is highly dependent on the cooperative production of proteases and surfactants, and it is easily measured through swarming motility assays.

The swarming motility of *P. aeruginosa* is controlled by a multi-layered quorum sensing network consisting of at least four interconnected systems—namely Las, Rhl, Iqs, and Pqs. The Las system is responsible for the production of elastases and proteases that are important in the breakdown of nutritional substrates and movement of bacterial cells across protein-rich tissues; this requires LasI synthase to produce N-(3-oxododecanoyl)-L-homoserine lactone autoinducers which activate the LasR transcription factor. The Rhl system, on the other hand, is responsible for the production of rhamnolipids, the biosurfactants necessary for swarming and translocation of *P. aeruginosa* in semisolid surfaces; this system involves the RhlR synthase that produces N-butryl-L-homoserine lactone autoinducers required by RhlR transcriptional regulators. The Las and Rhl systems work in synchrony with the other systems—Iqs (integrated QS signal) and Pqs (*Pseudomonas* quinolone signal)—to produce important virulence factors, which not only include swarming motility but also biofilm formation and antibiotic production.14

Due to the dependence of quorum sensing systems on AHL autoinducers, direct inactivation of autoinducers or blockage of autoinducer receptors may ultimately lead to quorum sensing inhibition. AHL analogs, including holo-ACP, L/D-S-adenosylhomocysteine, sinefungin, and butyryl-S-adenosylmethionine, have been found to interfere with AHL production and, consequently, quorum sensing.15 Even molecules that have retained only a part of the cognate AHL molecule have been implicated in significantly lowering the LasR-controlled expression of important effector proteins. Several compounds that can act as quorum inhibitors have been isolated from fungi and plants, and the ability of the crude extract at 50 mg/mL to interfere with the swarming motility of *P. aeruginosa* suggests the presence of quorum sensing inhibitors in *E. indica* leaves as well.

A study by Penalozal et al. in 2018 using NMR spectroscopy reported that the aerial parts of *E. indica* contained four specific compounds—flavonoids schaftoside, isoschaftoside, vitexin, and p-coumaric acid.7 They have also detected 29 unknown compounds which they grouped into fatty acid derivatives, amino acids, carbohydrates, and aromatics.8 Among all these components, flavonoids, p-coumaric acid, and fatty acid derivatives have the potential to promote the inhibition of the *P. aeruginosa* quorum-sensing network. A few plants, such as *Centella asiatica* L. and *Psidium guajava* L., which have been shown to inhibit the swarming motility of *P. aeruginosa* in a dose-dependent manner, have been reported to contain rich amounts of flavonoids, including kaempferol, quercetin, apigenin, rutin, and naringin.16 While the aforementioned flavonoids have not yet been identified to be present in *E. indica*, it is postulated that other flavonoids which are known to be present in *E. indica* also have potential to inhibit quorum sensing. On the other hand, p-coumaric acid has demonstrated quorum quenching activity against *Agrobacterium tumefaciens* NTL4, *Chromobacterium violaceum* 5999, and *Pseudomonas chlororaphis* in a study by Bodini et al. on soil bacteria in 2009.11 It is postulated to inhibit biofilm formation and swarming motility in selected bacteria by decreasing the expression of promoters of genes important for biosurfactant expression. Fatty acids may also account for the quorum sensing inhibition against *P. aeruginosa*. Inoue et al. showed that branched-chain fatty acids and unsaturated fatty acids, such as anteiso-C15:0, iso-C15:0, anteiso-C17:0, iso-C17:0, oleic acid, and vaccenic acid, inhibited the swarming motility of *P. aeruginosa*.10 The inhibitory activity seemed to correspond to the length of the chain with a length greater than fourteen carbons exhibiting more suppressive activity.10

Contamination with specific heavy metals, such as cadmium and nickel, may also produce quorum quenching...
activity. However, the results of the heavy metal testing showed that the sample of soil from which the plant samples were collected did not contain cadmium and nickel. On the other hand, the soil sample contained natural levels of arsenic and lead. There are no reports in the literature that arsenic and lead can act as quorum sensing inhibitors.

**Quorum sensing inhibition assay against S. marcescens**

Swarming motility is also a quorum sensing-regulated virulence factor of *S. marcescens*. The quorum sensing network of *S. marcescens* has three key regulators—namely Swr, Sma, and Spn; both the Swr and Sma systems are the main controllers of swarming motility. The Swr system primarily regulates the production of serrawettin, proteases, and S-layer proteins, while the Sma system regulates the production of caseinases and chitinases. The most notable surfactant molecule involved in the swarming motility of *S. marcescens* is serrawettin, an extracellular lipopeptide that reduces surface tension. Studies involving surfactant-defective phenotypes showed that knockouts had reduced swarming capability, which was quickly restored by supplementing the medium with serrawettin.

Unlike in the case of *P. aeruginosa*, the crude extract at 50 mg/mL has no significant activity against the swarming motility of *S. marcescens*. The swarming motility of *S. marcescens* is less studied, and some of the compounds that have been shown to inhibit the swarming motility of *P. aeruginosa*, such as flavonoids and fatty acids, are not known to inhibit the swarming motility of *S. marcescens*. However, a study in 2014 showed that biofilm formation, a phenotype closely related to swarming motility, by *S. marcescens* can be inhibited by a phenolic compound—phenol, 2,4-bis(1,1-dimethylethyl)—produced by *Vibrio alginolyticus*. This just shows that quorum sensing is specific and that inhibitors of quorum sensing in one species may not work for other bacteria. Other known inhibitors of swarming motility in *Serratia* species include halogenated furanone, Cn-CPA, and *Anethum graveolens* methanolic extract. Halogenated furanone, produced by *Delisea pulchra* was observed to have inhibitory effects on *S. liquefaciens* swarming motility. Cn-CPA, which interferes with quorum sensing-regulated virulence factors of *S. marcescens*, was found to inhibit the swarming motility and biofilm formation of *S. marcescens*; it was also found to be more effective than the halogenated furanone. *A. graveolens* methanolic extract, on the other hand, has been shown to downregulate quorum sensing-regulated expression of transcription of factors involved in adhesion and motility.

While the motility of both *P. aeruginosa* and *S. marcescens* has an established association with their virulence, the mechanisms involved in each species’ swarming motility are different. The swarming cell of *P. aeruginosa* is elongated, some possessing two polar flagella with or without pili. *S. marcescens*, on the other hand, has an elongated, multinucleated, aseptate, and hyper-flagellated swarming cell. Thus, inhibition of flagellar movement may be a more important target in the swarming motility of *S. marcescens*. A study by Devi et al. in 2018 shows that *B. subtilis* R-18 PE extract inhibited swimming and swarming in *S. marcescens* by reducing flagella-mediated movements. The biosurfactants that *P. aeruginosa* and *S. marcescens* produce are also different, specifically rhamnolipid for *P. aeruginosa* and serrawettin for *S. marcescens*.

Prodigiosin production is also a quorum sensing-regulated virulence factor of *S. marcescens*. Of the three key regulators of the quorum sensing network of *S. marcescens*, the Spn system is the one primarily involved in regulating the production of prodigiosin in addition to nuclease production. Similar to swarming motility, the crude extract at 50 mg/mL has no significant activity against the prodigiosin production of *S. marcescens*. The prodigiosin production of *S. marcescens* is also less studied, and some of the compounds that have been shown to inhibit the swarming motility of *P. aeruginosa*, such as flavonoids, phenolic compounds, and fatty acids, are not known to inhibit prodigiosin production of *S. marcescens*. However, it has been shown that a few known compounds may inhibit the production of prodigiosin in *S. marcescens*. For example, a study by Morohoshi et al. found that N-nonanoyl-cyclopentylamide (C9-CPA) exhibited a strong inhibitory effect on prodigiosin production by *S. marcescens*. Similarly, this confirms that inhibitors of quorum sensing in *P. aeruginosa* may not work for other bacteria, such as *S. marcescens*.

**CONCLUSION**

Since the discovery of quorum sensing in many pathogenic bacteria, quorum quenching mechanisms have been explored as a promising approach to combat the global health problem of antibiotic resistance and to develop novel medicines and combination therapies. In this research, the quorum quenching activity of *E. indica* at a concentration of 50 μg/mL was found to be effective against the swarming motility *P. aeruginosa* but not against the swarming motility and prodigiosin production of *S. marcescens*. With this, usage of *E. indica* quorum quenching activity offers a promising addition in treating *P. aeruginosa*-associated infections in which quorum sensing plays a significant role, such as microbial keratitis and cystic fibrosis opportunistic infection. However, the application of *E. indica* quorum quenching activity against *S. marcescens* remains unproven. Nonetheless, *E. indica* could still be explored as an adjunct therapy in nosocomial infections, which are most commonly caused by *P. aeruginosa*, especially with the rapid development of resistance by *P. aeruginosa* to multiple classes of antibiotics that are currently used in most hospitals around the world.

Aside from having direct therapeutic applications, quorum quenching compounds that will be isolated from *E. indica* ethanolic extract may also be applied on medical
devices such as dressings and catheters to prevent nosocomial infections. We look forward to more studies on the quorum quenching activity of *E. indica* ethanolic extract using other concentrations to determine the presence of a dose-response relationship. We also look forward to studies on identifying the specific mechanisms involved in the inhibition of *P. aeruginosa* swarming motility following isolation for possible large-scale synthetic production, and on exploring the effect of *E. indica* ethanolic extract on other pathogens.

**Statement of Authorship**

All authors have approved the final version submitted.

**Author Disclosure**

All authors declared no conflicts of interest.

**Funding Source**

This study was funded by the Department of Pharmacology and Toxicology, College of Medicine, University of the Philippines Manila.

**REFERENCES**