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ORIGINAL ARTICLE

Senna alexandriana Mill as a Potential Inhibitor for Quorum Sensing-Controlled Virulence Factors and Biofilm Formation in *Pseudomonas aeruginosa* PAO1

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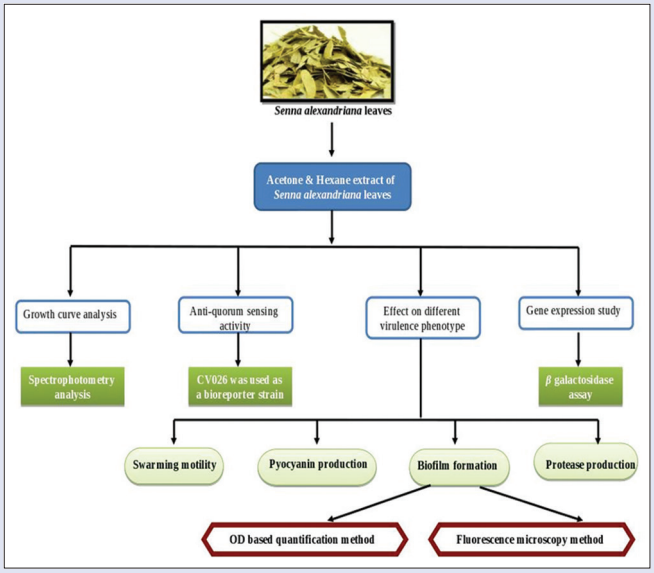
ABSTRACT

Background: *Senna alexandriana* Mill is a medicinally important plant used in laxatives and also possesses potent antimicrobial compounds, but its effect on the *Pseudomonas aeruginosa* was never analyzed. **Aim:** This study was carried out with the objective to attenuate quorum sensing (QS)-controlled virulence factors in *P. aeruginosa* and its biofilm. **Methods:** In the present study, acetone and hexane extracts of *S. alexandriana* mill were tested at sub-minimum inhibitory concentration concentrations for their anti-QS activity using bioreporter strain the *Chromobacterium violaceum CV026*. The effect of *S. alexandriana* Mill on different virulence phenotypes such as pyocyanin and protease production was investigated. Finally, the effect of the extract on *P. aeruginosa,* biofilm formation was also determined using the spectrophotometry-based method and microscopic analysis. **Results:** The extracts were tested for their activities against QS-controlled virulence factors of *P. aeruginosa* at 600 μg/ml and 800 μg/ml acetone and hexane extract concentration, respectively. Results showed that both the extracts have an inhibitory effect on different virulence factors of *P. aeruginosa,* including pyocyanin, protease production, and biofilm formation, without affecting the growth. The *P. aeruginosa* biofilm was found to be decreased by 75% and 62% in acetone and hexane extract presence. **Conclusion:** The results revealed that both acetone and hexane extracts showed anti-biofilm and anti-QS activity. It depicted the potential of *S. alexandriana* Mill against *P. aeruginosa* infection, its biofilm, and QS.

Key words: *Pseudomonas aeruginosa,* quorum sensing, *Senna alexandriana* mill, swarming motility, virulence factors

SUMMARY

- This study explored the anti-biofilm and anti-quorum sensing effect of *Senna alexandriana* Mill extract against the *P. aeruginosa*, and the obtained results



Abbreviations used: QS: quorum sensing; MIC: minimum inhibitory concentration; 3-oxo-C12HSL: N-(3-oxo-dodecanoyl)-L-homoserine lactone; PQS: Pseudomonas quinolone signal; EPS: extracellular polysaccharide layer; LB: Luria-Bertani; CV026: Chromobacterium violaceum; OD: optical density; TSB: Tryptic Soy Broth; DMSO: dimethyl sulfoxide; ANOVA: Analysis of variance; PBS: phosphate-buffered saline; DAPI: 4′,6-Diamidino-2-Phenylindole.

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INTRODUCTION

Despite increasing efforts to prevent micro-organisms from gaining antimicrobial resistance, bacteria are still developing the resistance over time, and the rate of discovery of antibiotics is decreasing continuously.[1] Hence, there is an urgent need for developing new herbal drugs, which can prevent the increasing resistance.[2,3] Quorum sensing (QS), a bacterial process of cell to cell communication, is utilized to switch on different gene expression programs concerning population density.[4] QS is thus a promising target for the development of next-generation

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antibiotics, particularly for infections caused by multidrug-resistant micro-organisms.

P. aeruginosa mainly has two quorum-sensing systems (*rhl* and *las*), which regulate the detection and synthesis of small diffusible signaling molecules called N-acyl homoserine lactones.[5] The *rhl* and *las* systems are hierarchically related to each other in such a way that the *las* system regulates the *rhl* system at both transcriptional and posttranscriptional levels.[6] QS and biofilm formation plays a pivotal role in establishing chronic and acute infections, mostly by *P. aeruginosa*.[7] Thus, antimicrobials that target the QS system and biofilm formation can prove to be good partners to the conventional antibiotics as both plants and bacteria coexisted during the evolution, so there is a possibility that plants might have evolved mechanisms to protect them from various infections.[8] This hypothesis has generated interest among the scientific community for herbal inhibitors as they impose less selection pressure on bacteria and render them more susceptible to antibiotics. *Senna alexandrina* Mill has been used as medicine by physicians from the nineteenth century.[9] Medicinal properties of *S. alexandrina* Mill are contributed mainly by the anthraquinones glycosides along with that anthraquinone and secondary metabolites, such as phenolic acids, coumarins, and flavonoids of this herb are responsible for its antimicrobial activity.[10] Phytochemical analysis of the *S. alexandrina* Mill was performed in different studies and the phytochemical constituents identified are alkaloids, glycosides, carbohydrates, phytosterols, Saponins, phenols, tannins, flavonoids, and sesquiterpenes.[10] Keeping this miracle plant's potential in mind, this work was carried out to analyze the *S. alexandrina* Mill extracts ability to inhibit QS in *P. aeruginosa* for the first time.

METHODS

Preparation of plant extracts

Dried leaves of *S. alexandrina* Mill were obtained from the Attar Ayurveda suppliers of medicinal plants. Dried leaves were grounded into a fine powder, and 5 g of dried powder was extracted separately in 500 ml of Acetone, Hexane, and Aqueous for 3 days. The solution obtained from the Soxhlet was further filtered using filter paper (Whatman No. 1). The whole extraction process was repeated thrice, and then the filtrate obtained was concentrated at 40°C under vacuum with a rotary evaporator. Crude extracts obtained were kept at 4°C for further use.

Bacterial strains

This study used the *P. aeruginosa* PAO1 bacterial strain. GFP-PAO1 and reporter strain *Escherichia coli* pJN105 LpSC11 were kindly provided by Dr. Greenberg (University of Washington). N-(3-oxo-dodecanoyl)-L-homoserine lactone (3-oxo-C_{12} HSL), gentamycin, ampicillin were procured from Sigma-Aldrich. L-arabinose was purchased from Himedia.

Screening for quorum sensing inhibition

To determine the anti-QS activity of the extract, *Chromobacterium violaceum* (CV026), a mini-Tn*5* transposon mutant, was used as a reporter strain. In brief, the overnight grown CV026 culture was spread on prepared Luria-Bertani (LB) agar plates containing hexanoyl homoserine lactone and kanamycin to prepare uniform lawn. Wells were punched in the agar, and 50 µl of the extracts were put into the punched wells and were incubated in the upright position for 24 h.[3]

Growth curve

To observe the effect of different extracts on *Pseudomonas aeruginosa* PAO1 growth. *P. aeruginosa* was incubated with acetone (600 µg/ml) and hexane extract (800 µg/ml) of *S. alexandrina* Mill for 24 h, growth was measured at 600 nm at an interval of 2 h till the micro-organism reaches a stationary phase. LB medium supplemented with the extract alone was taken as a control to normalize the variation in the absorbance.

β-galactosidase assay

For gene expression studies, *P. aeruginosa* PAO1 was grown at 37°C in the presence of acetone and hexane extracts of *S. alexandrina* Mill dissolved in dimethyl sulfoxide (DMSO), alongside cinnamon oil, was used as a positive control. After overnight incubation, the treated culture was centrifuged for 15 min at 10,000 rpm. The obtained supernatant was filtered through a 2 µm syringe filter and checked for the presence of 3-oxo-C_{12} HSL. Briefly overnight grown *E coli* pJN105LpSC11 was inoculated into LB broth supplemented with gentamycin (20 µg/ml) and ampicillin (100 µg/ml) and grown to optical density (OD_{600}) 0.2, then 0.25% L-arabinose was added for the induction of LasR expression and further incubated till the OD_{600} reaches 0.5. After attaining a 0.5 OD sterile test, bacterial supernatant was mixed with 1:100 dilution of the *E coli* pJN105LpSC11 and incubated for 2 h at 37°C, β-galactosidase activity was measured using Miller assay.[11,12]

Effect of *Senna alexandrina* mill on different virulence phenotypes

Pyocyanin assay

A culture of *P. aeruginosa* PAO1 was standardized to OD_{600} 1.0 and then inoculated into 25 ml of LB supplemented with acetone and hexane extracts of *S. alexandrina* Mill along with the cinnamon oil and DMSO as a positive and negative control and then grown overnight at 37°C. Supernatants were collected after centrifugation at 10,000 rpm and filter sterilized. Pyocyanin was extracted using chloroform, followed by the 0.2 M HCl, and the pink color obtained was measured at 520 nm.[13]

Protease assay

Briefly overnight grown culture in the presence of acetone extract and hexane extract of *S. alexandrina* Mill along with the cinnamon oil and DMSO as a positive and negative control, were centrifuged at 15,000 g and 4°C for 15 min. The supernatant was collected and filter sterilized. 300 µl of the filter-sterilized supernatant was mixed with 200 µl of 2% azocasein solution. By adding 10% trichloroacetic acid, the reaction was stopped, precipitating undigested substrate, and centrifuged at 15,000 g for 10 min. The supernatant obtained was mixed with 1M NaOH, and activity was measured at 430 nm against the blank, which is run parallel and normalized by the culture OD at 600 nm. To avoid the confusion on false results because of the extracts, extracts diluted in LB without growth were run in parallel with the same procedure. The Skim milk agar plate assay was also performed to confirm the inhibition of protease activity by extracts.[14]

Motility assay

Briefly swarming medium contents are glucose (1%), bacto peptone (0.5%), yeast extract (0.2%), and bacto agar (0.5%). Swarming agar was supplemented with 200 µl extract, and 5 µl of overnight grown culture was inoculated onto the middle of the agar plate and incubated overnight at 37°C. Swarming motility was assessed by measuring the dendrites length.[15]

Quantification of biofilm

P. aerugiosa PAO1 was grown overnight at 37°C with shaking. The overnight grown *P. aeruginosa* PAO1 culture was diluted to OD_{600} 1 and was then inoculated to a fresh Tryptic Soy Broth (TSB). 200 µl of inoculated broth was dispensed in different wells of a microtiter plate containing acetone (600 µg/ml) and hexane extracts (800 µg/ml) along with the 0.2 µl cinnamon oil and 1% DMSO which serve as a positive and negative control. The plate was incubated for 24 h at 37°C, growth observed after 24 h was discarded and replaced with fresh medium and incubated again at 37°C for 24 h. Growth observed was discarded, and wells were washed thrice with PBS (7.4) and stained with 200 µl of 0.4% crystal violet (CV) for 30 min. The CV stain was discarded, and the microtiter plate wells were rinsed with distilled water twice to remove the unbound dye, and 100 µl of DMSO was added to each microtiter plate well for solubilizing the stain, and absorbance was read at 590 nm using Teccan Microplate reader.[16]

Microscopic analysis

Biofilm formation was carried out in the presence of acetone and hexane extracts of *S. alexandrina* Mill. cinnamon oil and DMSO were taken as positive and negative control. Briefly, the overnight grown culture of g-PAO1 was diluted in fresh TSB to OD_{600} 1 and inoculated to the petri plates containing sterilized coverslips and incubated for 24 h. Afterward, the growth observed was discarded and replaced with fresh medium and incubated further for 24 h, the supernatant was removed, and the incubated coverslips were washed gently with 1X PBS thrice to remove the weakly adhered cells, and the biofilm obtained was stained with 0.4% CV for 30 min and rinsed thrice in autoclaved distilled water to eliminate the unbound stain and observed under ×100. For fluorescent staining, g-PAO1 biofilm was stained with 4',6-diamidino-2-phenylindole (DAPI) and visualized under a fluorescence microscope.[17]

Statistical analysis

All the microbiological experiments were performed in triplicates, and the results are expressed as mean values ± standard mean error. The significant values were investigated using one-way analysis of variance, and only values at $P < 0.05$ were considered statistically significant.

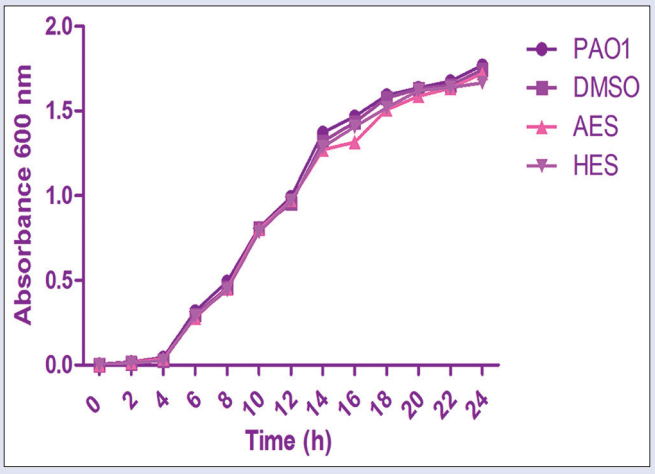

Figure 1: Growth curve analysis in the presence of acetone and hexane extract of *Senna alexandrina* Mill

RESULTS

Senna extracts inhibits quorum sensing

Different extracts of *S. alexandrina* Mill (acetone, hexane, aqueous) were tested for their anti- QS activity using CV026, and clear halo was observed around the wells containing the acetone and hexane extracts. DMSO was used as solvent control, and no zone was observed around the well-containing DMSO. Acetone and hexane extracts were selected for further studies on QS inhibition in *P. aeruginosa* PAO1.

Growth curve study

The growth curve study revealed that there is no effect on the growth of the *P. aeruginosa* PAO1 when grown in the presence of acetone extract of *S. alexandrina* Mill at 600 µg/ml concentration and hexane extract of *S. alexandrina* Mill at 800 µg/ml [Figure 1]. The growth pattern of PAO1 was treated with acetone and hexane extract of *S. alexandrina* Mill resembled that of the untreated control, which further suggests that this concentration can be used for assessing the effect of senna extract on QS of *P. aeruginosa* PAO1. Concentrations were selected on the basis of minimum inhibitory concentration (MIC) and only sub-MIC concentrations can be used for the QS inhibition to prevent the selection pressure exhibited at the concentration above MIC. In addition, the positive control used in the study is purified oil and these are crude extracts therefore we selected these concentrations.

β galactosidase assay

E coli pJN105LpSC11 was used as a bioreporter strain to investigate the effect of *S. alexandrina* Mill on QS in *P. aeruginosa* PAO1 as β galactosidase activity (expressed in terms of Miller Units) can be measured as a function of 3-oxo-C_{12} HSL. It was observed that 3-oxo-C_{12} HSL level in the supernatant of acetone and hexane extracts treated culture was reduced to 61% and 43% than the untreated culture, as shown in Figure 2, which shows that β galactosidase activity in the

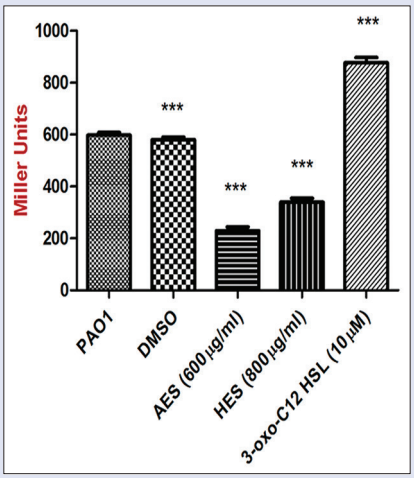

Figure 2: Effect of acetone and hexane extract of *Senna alexandrina* Mill on *Pseudomonas aeruginosa* QS. The effect is measured using biosensor strain *E coli pJN105 LpSC11* which specifically detects N-(3-oxo-dodecanoyl)-L-homoserine lactone in the supernatant of treated cultures. All the experiments were performed in triplicates, and the error bar represents the standard error mean of three measurements. ***indicates the samples which were significant (one-way analysis of variance $P < 0.0001$)

treated culture was nearly equivalent to the positive control cinnamon oil and lower than the untreated control.

Effect of *Sennaalexandrina* Mill on different virulence phenotypes

Pyocyanin assay

Pyocyanin is a blue-colored phenazine derivative produced by *P. aeruginosa* and helps in the successful establishment of infections by generating reactive oxygen species that kill defense cells. While studying the effect of *S. alexandriana* Mill extract on the pyocyanin production, it was observed that the production of pyocyanin was reduced to about 58% and 45%, respectively, when compared with the untreated control, as shown in Figure 3.

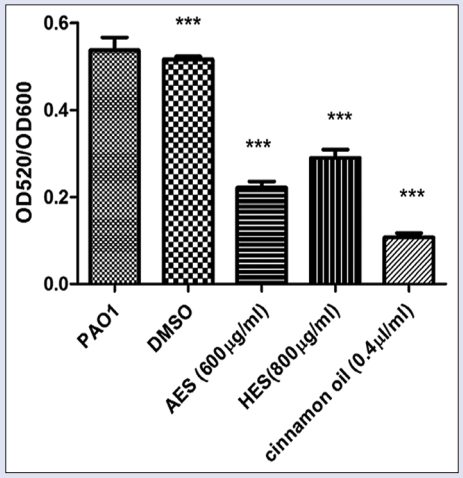

Figure 3: Effect of acetone and hexane extract of *Senna alexandrina* Mill on pyocyanin. All the experiments were performed in triplicates, and the error bar represents the standard error mean of three measurements. ***indicates the samples which were significant (one-way analysis of variance $P < 0.0001$)

Protease assay

Protease plays a vital role in *P. aeruginosa* infection by helping in penetration of the tissue by degrading laminin. As shown in Figure 4, a sharp decrease in protease production was observed when *P. aeruginosa* PAO1 was grown in the presence of acetone and hexane of *S. alexandriana* Mill in comparison with the untreated control. Obtained results were further confirmed by using skim milk agar assay, which supports the results obtained.

Motility assay

Swarming motility is a type of movement of bacteria by which it travels through a semisolid medium with the help of flagella in search of nutrients. Swarming helps bacteria to translocate it to a site of infection. *P. aeruginosa* swarming motility was significantly decreased in the presence of acetone and hexane extract of *S. alexandriana* Mill as compared to the control, as shown in Figure 5, in which the length of the dendrites in the treated sample was less than the untreated control. As swarming in *P. aeruginosa* is dependent on rhamnolipid production, which acts as a surfactant, a decrease in swarming might be due to the reduced rhamnolipid production.

Quantification of biofilm

Micro-organisms grow in biofilm mode by secreting the extracellular polysaccharide layer (EPS) layer to provide protection against various antimicrobial treatments as biofilm formation in *P. aeruginosa* was controlled partially by QS. Hence, the effect of acetone and hexane extract of *S. alexandriana* Mill on biofilm formation was examined by using CV assay, and it was observed that biofilm formation was reduced to about 75% and 62% in the presence of acetone and hexane extract of *S. alexandriana* Mill respectively when compared with the control [Figure 6].

Microscopic analysis

Microscopic analysis reveals the changes in the architecture of biofilm. The effect of hexane and acetone extract of *S. alexandriana* Mill was further confirmed by light microscopy and fluorescence microscopy. It was observed that cells were in dispersed form in the treated samples compared to the confluent growth in control samples without treatment,

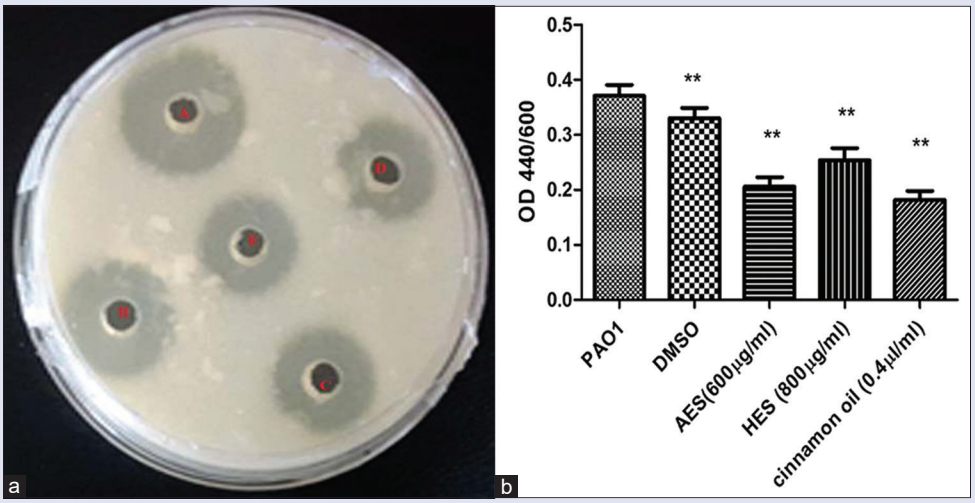

Figure 4: Effect of acetone and hexane extract of *Senna alexandrina* Mill on protease production (a) Skim milk agar assay (a) *Pseudomonas aeruginosa* PAO1 (b) dimethyl sulfoxide Acetone extract of *Senna alexandrina* Mill Hexane extract of *Senna alexandrina* Mill *Cinnamon oil* (b) Azocasein assay. All the experiments were performed in triplicates, and the error bar represents the standard error mean of three measurements. ***indicates the samples which were significant (one-way analysis of variance $P < 0.001$)

which might be due to a reduction in EPS production by extracts. For fluorescence studies, g-PAO1 was used for the formation of biofilm and stained with DAPI for the examination of the effect of extracts; it was observed that biofilm formation was reduced significantly in the presence of acetone extract as compared to the hexane extract and untreated control and extracellular DNA content of the treated biofilm was higher as compared with the untreated control which might be due to the lysis of cells by extract as shown in Figure 7.

DISCUSSION

With the increasing demand for new therapies to cope up with the multidrug-resistant micro-organisms, QS inhibition emerged as a new strategy for the development of compounds that inhibit different virulence traits responsible for the pathogenesis of micro-organisms.[18] Herbal plants have been used for the centuries for the treatment of various microbial infections and have been proven to be beneficial because of low cytotoxicity and less selection pressure on the micro-organisms. Therefore, search for medicinally important plants and plant-derived compounds are an attractive alternative for the treatment of various pathogens.

Among the different extracts of *S. alexandrina* Mill acetone and hexane were found positive for QS inhibition. Both the extracts at a tested concentration (600 µg/ml and 800 µg/ml) showed no effect on the growth of *P. aeruginosa*. However, previous reports have shown that extracts of *S. alexandrina* Mill possess antimicrobial activity against both bacteria and fungi, including *P. aeruginosa* as *Cassia alata* has been shown to possess anti-biofilm activity against *P. aeruginosa* by inducing changes in the EPS.[19,20] Most of the virulence phenotypes such as elastase, pyocyanin, rhamnolipid, protease along with other factors in *P. aeruginosa* are regulated by QS circuits *las*IR, *rhl*IR, and PQS system.[21] Our results revealed that acetone and hexane extract of *S. alexandrina* Mill are interfering with both the *rhl* pathway as well as *las* pathway. Decreased production of protease and pyocyanin in the presence of acetone and hexane extract of *S. alexandrina* Mill describes their anti-QS potential. Swarming motility of *P. aeruginosa* is another important virulence factor controlled partially by QS, which helps bacteria to migrate on semisolid surfaces and assist in colonizing the surfaces. Reduced swarming in the presence of extracts might be due to the reduced production of rhamnolipid, which acts as a surfactant and helps in motility. Biofilm formation plays a crucial role in the pathogenesis of micro-organisms.[22] The present study revealed a decrease in the biofilm production in the presence of acetone and hexane extracts of *S. alexandrina* Mill without affecting growth rate, the findings are consistent with the previous findings in which biofilm formation was found deceased in the presence of berberine.[23] Quantitative results obtained were further confirmed by light and fluorescent microscopy. Fluorescent microscopy was done to reveal the alterations in the structure of biofilm in the presence of extracts by staining g-PAO1 with DAPI. In the present study, it was observed that the treatment of g-POA1 biofilm with acetone and hexane extract of *S. alexandrina* Mill increased the extracellular DNA content in comparison to the control; treatment with extracts may have caused the lysis of cells and release of nuclear DNA into the cytoplasm.

CONCLUSION

P. aeruginosa is an opportunistic Gram-negative pathogen responsible for causing various diseases in the immune-compromised patients and difficult to eradicate because of various mechanisms operating in it to provide multidrug resistance. QS controls various virulence factors contributing to the pathogenesis of *P. aeruginosa*. Although antibiotics can be used against these pathogenic bacteria but due to the development of resistance against the antibiotics makes their eradication more complicated. In this context, herbal compounds that interfere with the QS and biofilm process will be a promising alternative for controlling *P. aeruginosa* associated infection. Thus, the present study reveals that *S. alexandrina,* Mill which has been used for various laxative preparations, must be containing compounds that can target QS-controlled virulence phenotypes of *P. aeruginosa* PAO1 and its biofilm formation. In the future, the results may be considered for performing *in vivo* and clinical investigations.

Acknowledgements

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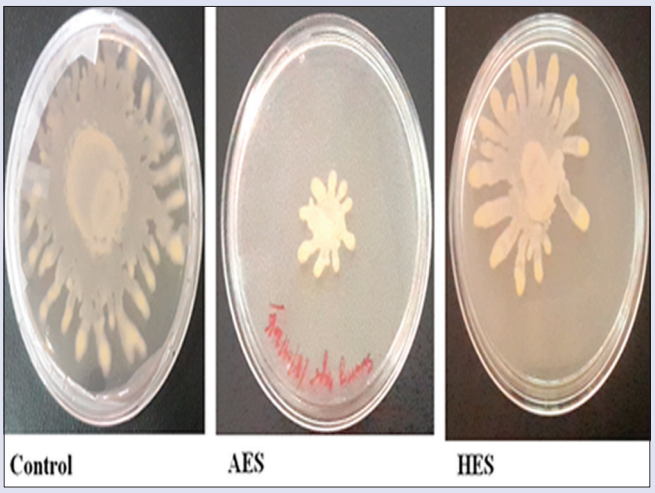

Figure 5: Effect of acetone and hexane extract of *S. alexandriana* Mill on swarming motility of *Pseudomonas aeruginosa*

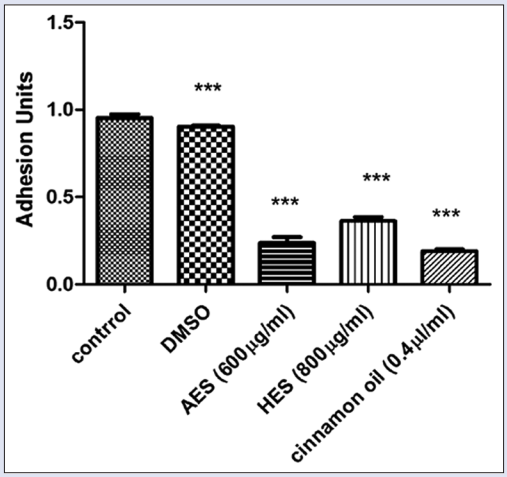

Figure 6: Effect of acetone and hexane extract on biofilm formation is represented in adhesion units (OD_{590}/OD_{600}). All the experiments were performed in triplicates, and the error bar represents the standard error mean of three measurements. ***indicates the samples which were significant (one-way analysis of variance $P < 0.001$)

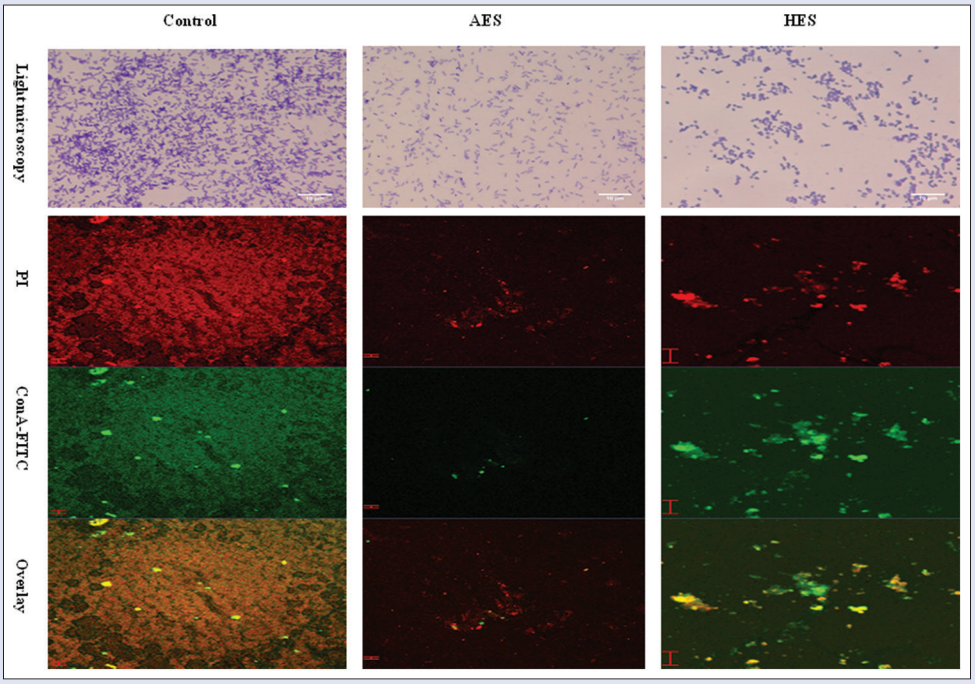

Figure 7: Microscopic analysis of biofilm in the presence of acetone extract of *Senna alexandrina* Mill and hexane extract of *Senna alexandrina* Mill extracts of *Senna alexandrina* Mill

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Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Hancock RE, Nijnik A, Philpott DJ. Modulating immunity as a therapy for bacterial infections. Nat Rev Microbiol 2012;10:243-54.
2. Koriem KMM, Arbid MS, Saleh HN. Antidiarrheal and protein conservative activities of Psidium guajava in diarrheal rats. J Integr Med. 2019;17:57-65. [doi: 10.1016/j.joim.2018.12.001].
3. Rasmussen TB, Givskov M. Quorum-sensing inhibitors as anti-pathogenic drugs. Int J Med Microbiol 2006;296:149-61.
4. Ng WL, Bassler BL. Bacterial quorum-sensing network architectures. Annu Rev Genet 2009;43:197-222.
5. Williams P, Cámara M. Quorum sensing and environmental adaptation in *Pseudomonas aeruginosa*: A tale of regulatory networks and multifunctional signal molecules. Curr Opin Microbiol 2009;12:182-91.
6. Medina G, Juárez K, Díaz R, Soberón-Chávez G. Transcriptional regulation of *Pseudomonas aeruginosa* rhlR, encoding a quorum-sensing regulatory protein. Microbiology (Reading) 2003;149:3073-81.
7. Byrd MS, Pang B, Hong W, Waligora EA, Juneau RA, Armbruster CE, *et al.* Direct evaluation of *Pseudomonas aeruginosa* biofilm mediators in a chronic infection model. Infect Immun 2011;79:3087-95.
8. Cos P, Vlietinck AJ, Berghe D Vanden, Maes L. Anti-infective potential of natural products: How to develop a stronger *in vitro* "proof-of-concept." J Ethnopharmacol 2006;106:290-302.
9. Franz G. The senna drug and its chemistry. Pharmacology 1993;47 Suppl 1:2-6.
10. Umadevi M, Kumar KPS, Bhowmik D, Duraivel S. Senna – A medical miracle plant. J Med Plants Stud 2013;1:56-74.
11. Shaw PD, Ping G, Daly SL, Cha C, Cronan JE Jr., Rinehart KL, *et al.* Detecting and characterizing N-acyl-homoserine lactone signal molecules by thin-layer chromatography. Proc Natl Acad Sci U S A 1997;94:6036-41.
12. Low KB. Experiments in Molecular Genetics. Jeffrey H. Miller." The Quarterly Review of Biology.1974;49:151. [doi:10.1086/408025].
13. O'Malley YQ, Reszka KJ, Spitz DR, Denning GM, Britigan BE. *Pseudomonas aeruginosa* pyocyanin directly oxidizes glutathione and decreases its levels in airway epithelial cells. Am J Physiol Lung Cell Mol Physiol 2004;287:L94-103.
14. Hentzer M, Riedel K, Rasmussen TB, Heydorn A, Andersen JB, Parsek MR, *et al.* Inhibition of quorum sensing in *Pseudomonas aeruginosa* biofilm bacteria by a halogenated furanone compound. Microbiology (Reading) 2002;148:87-102.
15. Kohler T, Curty LK, Barja F, Delden CV, Peche`Re JC. Swarming of *Pseudomonas aeruginosa* is dependent on cell-to-cell signaling and requires flagella and pili. J Bacteriol 2000;182:5990-6.
16. O'Toole GA. Microtiter dish biofilm formation assay. J Vis Exp. 2011;30:2437. doi: 10.3791/2437.
17. Zhao T, Liu Y. N-acetylcysteine inhibit biofilms produced by *Pseudomonas aeruginosa*. BMC Microbiol 2010;10:140.
18. Bjarnsholt T, Jensen PØ, Jakobsen TH, Phipps R, Nielsen AK, Rybtke MT, *et al.* Quorum sensing and virulence of *Pseudomonas aeruginosa* during lung infection of cystic fibrosis patients. PLoS One 2010;5:e10115.
19. El-Morsy TH. Antibiotic Properties of Leaf Extracts of Sennaalexandrina (L). J Am Sci 2013;9:288-92.
20. Saito ST, Trentin Dda S, Macedo AJ, Pungartnik C, Gosmann G, Silveira Jde D, *et al.* Bioguided fractionation shows Cassia alata extract to inhibit Staphylococcus epidermidis and *Pseudomonas aeruginosa* growth and biofilm formation. Evid Based Complement Alternat Med 2012;2012:867103.
21. Glessner A, Smith RS, Iglewski BH, Robinson JB. Roles of *Pseudomonas aeruginosa* las and rhl quorum-sensing systems in control of twitching motility. J Bacteriol 1999;181:1623-9.
22. De Kievit TR, Gillis R, Marx S, Brown C, Iglewski BH. Quorum-sensing genes in *Pseudomonas aeruginosa* biofilms: Their role and expression patterns. Appl Environ Microbiol 2001;67:1865-73.
23. Huang X, Zheng M, Yi Y, Patel A, Song Z, Li Y, *et al.* Inhibition of berberine hydrochloride on candida albicans biofilm formation. Biotechnol Lett 2020;42:2263-9.