International Journal of
Science Inventions Today
www.ijsit.com
ISSN 2319-5436
Research Article

# ANTIBIOFILM AND QUORUM SENSING INHIBITORY POTENTIAL OF EXCOECARIA AGALLOCHA AGAINST PSEUDOMONAS AERUGINOSA

Vijayakumar Karuppiah and Ramanathan Thiruganasambandam*

*Centre of Advanced Study in Marine Biology, Faculty of Marine Sciences, Annamalai University, Parangipettai-608 502, India*

## ABSTRACT

Quorum sensing inhibitory (QSI) potential of *Excoecaria agallocha* leaves crude ectract (EALCE) was assessed by using biomarker strain *Chromobacterium violaceum*. The EALCE at 2 mg/ml inhibited violacein production in *C.violaceum*. Further, the present study demonstration revealed that EALCE excellently affects the acyl homoserine lactone (AHL) regulated virulence production and biofilm formation including motility behavior, Las A Staphylolytic protease, Las B elastase, alginate, pyocyanin and exopolysaccharides (EPS) production in *P.aeruginosa*. The EALCE also reduced the biofilm architecture and loosening of microcolonies in in vitro biofilm formation in *P.aeruginosa*.

**Keywords:** *Chromobacterium violaceum*, quorum sensing inhibition, *Excoecaria agallocha*, antibiofilm activity

## INTRODUCTION

In generally, prokaryotes and eukaryotes cells are communicating with each other among the individuals of inter and intra species. This process occurs through a well developed system termed as quorum sensing (Ryan and Dow, 2008). In quorum sensing (QS) system, bacteria cells release chemical signal molecules termed as autoinducers, which are secreted by own cells. Synthesis of overflowing autoinducers are spread around the media which helps to increasing the bacterial growth among high cell density. The higher concentrations of autoinducers binding with cognate receptor proteins of nearby cells which results leads to forms a signal receptor complexes, which is responsible for regulates the virulence gene expression and biofilm formation in the infectious process (Ng and Bassler, 2009). This mechanism provide the ability to resist the bacterial cells towards antibiotics and pathogens increased their pathogenic factors. In Gram negative bacteria, QS system occurs through N – aetyl homoserine lactone (AHL) signal dependent mechanism (Smith and Iglewski, 2003). AHL plays akey role to controlled the numerous virulence factors of many pathogenic bacteria including *P.aeruginosa*. *Pseudomonas aeruginosa* is one of the major prevalent human pathogen which representing a life-threatening infection for global population (Gellatly and Hancock, 2013). It may cause critical threat infections including urinary tract infection, nosocomial infection and cystic fibrosis. Now a days *P.aeruginosa* is tolerance and development of resistance ability towards commercial antibiotics (Lewis, 2010). In *P.aeruginosa*, quorum sensing regulates several virulence factors including biofilm, motility factor, LasA protease, LasB elastase, pyocyanin, alginate production and rhamnolipid production (Alhede et al., 2009). *P.aeruginosa* cells are enclosed by biofilms, which is made up of exopolysaccharides and it builds a aggressive architecture and these cells exposed increased resistance ability to antibiotics and host immune system (Rybtke et al., 2015). Biofilms are much stronger than sessile cells which are able to survive and resist anibiotics at concentration of 1000 – 1500 times (Hu et al., 2006). Hence, the biofilm forming pathogen enter the host which leads to cause chronic infections for host (Bjarnsholt et al., 2008). Therefore, the urgent need for quorum sensing inhibitory compounds, which interfere the QS system and lead to suppress of virulence factors production (Kociolek, 2009).

Mangroves are derived from unique bioactive compounds, which produces structurally diverse novel natural products. Mangrove have been used as a folklore medicine world wide. Mangroves are recognized as rich source of bioactive compounds which were used as diverse pharmacological properties such as antimicrobial, antimalarial, anti viral, anti cancer and anti oxidant (Bandaranayake, 2002) and recently which exhibited the QS potential (Annapoorani et al., 2013; Musthafa et al., 2013). *Excoecaria agallocha* is a holy (Thillai) plant which is widely used folklore medicine for epilepsy, ulcers, dermatitis and leprosy (Bandaranayake, 2002; Subhan et al., 2008). Deepa et al., 2014 reported that, the crude extract derived from leaves of *E.agallocha* exhibited the antifouling activity against bacteria and diatoms. Therefore, the present study we demonstrates the crude extract of *E.agallocha* inhibited the QS and their associated virulence

factors production in multidrug resistant *P.aeruginosa*.

## MATERIALS AND METHODS

### Plant collection and extraction:

Fresh leaves of *E.agallocha L* were collected from pichavaram mangroves (latitude $11^0$ 29' N; longitude $79^0$ 46') of Tamilnadu, India. The collected leaves were washed thoroughly with sterile distilled water, shade dried and pulverized. 10 g of powdered samples were extracted with 100 ml methanol for 24 h in shaker. The solvent layer was dried and weighed. The solvent free dried extract was stored at $4^0$C. Further, the *E.agallocha L* leaves crude extract (EALCE) was assessed of QS and their mediated biofilm formation and virulence production against *P.aeruginosa*.

### Bacterial strains and growth condition:

*Chromobacterium violaceum* (MTCC 2656) was used as a marker strain to measure the QS mediated violacein production. *C.violaceum* was cultured in Luria – Bertani (LB) medium and incubated at $30^0$ C for 18 h. *P.aeruginosa* Pa01 is maintained in Brain Heart Infusion broth at $37^0$C. For biofilm assay, BHI broth was supplemented with 0.5% glucose. The cultures were maintained in BHI broth containing 20 % glycerol and stored at - $80^0$ C.

### Violacein inhibition assay:

EALCE was subjected to evaluate the anti – QS potential against *C.violaceum*. *C.violaceum* was used for screening the QS inhibitory effect of EALCE. *C.violaceum* synthesised the violet colour pigment violacein when which was involved in QS (Choo, Rukayadi & Hwang, 2006). Overnight culture (10 µl) of *C.violaceum* was placed into sterile micro titer plate containing 1 ml of LB broth along with the presence and absence of increased concentration of EALCE (2 mg/ml). The plates were incubated at $30^0$ C for 24 h, after incubation, cultures were centrifuged at 13,400 x g for 10 min. 1 ml dimethyl sulfoxide (DMSO) was then mixed to the pellets and vortexed to solubilize the violacein pigment. The violacein solubilized DMSO was centrifuged at 13,400 x g for 10 min and the supernatant was measured at OD 585 nm.

### Determination of biofilm inhibitory concentration (BIC):

The efficacy of EALCE on *P.aeruginosa* biofilm formation was assessed by the following method of Annapoorani et al. (2013). Briefly, 0.1 % of test pathogen was added in to microtitre plates containing 1 ml of NB supplemented with 1 % glucose and incubated in the presence and absence of EALCE on increasing concentrations (25 - 175 µg/ml) at $37^0$C for 24 h. After incubation plates was washed thrice with distilled water and allowed air dry. Biofilm was stained using safranin for 10 min; the plates was washed to remove

unbound stains and allowed to air dry. Stain bound bacterial biofilms was extracted with 20 % glacial acetic acid and colour intensity was absorbance at 570 nm.

## Analysis of biofilm architecture through light microscope:

To assess EALCE effect on biofilm architecture in *P.aeruginosa* was done by light microscope. In light microscope analysis, biofilms were allowed to grown on glass slides (1 x 1 cm) with presence and absence of EALCE and placed in 24 well titre plates. Biofilms formed on the glass pieces were washed thrice with distilled water and stained with Safranin for 5 min and washed to remove excess stain with distilled water. The washed slides were visualized through light microscope at 400 X magnification.

## Anti-biofilm potential of EALCE assessing through CLSM:

To confirm antibiofilm potential of EALCE was done by Confocal Laser Scanning Microscope analysis. Biofilms on glass slides as described above, the slides washed with distilled water and stained with acridine orange (0.1 %) for 60 sec. Biofilm slides visualized through CLSM, as an excitation of 515 – 560 nm and the magnification is X 200.

## XTT assay:

The effect of EALCE in cell viability of bacteria was assessed using XTT assay. An equal number of cells were inoculating 1 ml of broth with the presence and absence of EALCE and incubation for $37^0$c for 24h. After incubation, planktonic cells were taken separately. Then the cells were washed with sterile PBS (pH 7.4) and resuspended in 200µl of the same. 25 µl of XTT – menadione substrate was added to the cells and incubated in the dark at room temperature for 6 h. After the incubation the cells were read at spectrophotometrically at 490 nm.

## Growth curve assay:

The effect of EALCE affect the bacterial growth was assessed by growth curve assay. Briefly, 18 h bacterial culture was used to inoculate the appropriate medium supplemented with 1% glucose along with various concentrations (150 – 450 µg/ml) of EALCE and incubated at $37^0$C for 24 h. The growth rate was measured using spectrophotometer OD 600 nm at every 3 h interval up to 24 h.

## Dispersion of mature biofilm:

Matured biofilms are stronger than planktonic cells, its increased ability to resist against antibiotics. To assess the efficacy of EALCE in matured biofilm was observed through light microscopic by the following method of Nithya et al. (2010). Briefly, test pathogen was grown on glass slides and incubation at $37^0$C for 24

h. At the end of incubation, glass slides were treated with EALCE for 30 min at $37^0$C. After incubation, slides were washed, stained and visualized through a light microscope at 400 X magnification.

## Inhibition of EPS production:

Most of the bacterial biofilms are made up with extracellular polysaccharide, *P.aeruginosa* biofilm also depended in EPS. The biofilm architecture (strong / weak) is determined by EPS production. The efficacy of EPS production was quantified by the following method of Musthafa et al. (2012). Briefly, a test pathogen was grown on glass slides with presence and absence of EALCE and incubated at $37^0$ C for 18 h. After the incubation, the EPS was quantified by total carbohydrate assay. The glass slides were washed in 0.9 % NaCl (0.5 ml) and incubated with an equal volume of 5 % phenol (0.5 ml) was mixed with 5 volumes concentrated $H_2SO_4$. The solution was incubated at dark in 1 h and absorbance at OD 490 nm.

## *P.aeruginosa* QS mediated virulence quantification assays:

## LasA Staphylolytic assay:

LasA protease activity was assessed by measuring the ability of culture supernatants to lyse boiled *Staphylococcous aureus* cells, by the following method of Adonizio et al. (2008). Briefly, 30 ml overnight culture of *S.aureus* were centrifuged at 7000 rpm for 3 min, and the pellet was suspended in 0.002 M Tris – HCl (pH 8.5) boiled for 10 min and diluted with the same buffer to an OD of 0.8 at 595 nm. 900 µl of diluted *S.aureus* suspension was added to each 100 µl of cell free culture supernatant of *P.aeruginosa* with the presence and absence of EALCE. Finally, the aliquot solution was measured every 15 min interval for 60 min at OD 595 nm in Spectrophotometer.

## LasB elastase assay:

The elastolytic activity of *P.aeruginosa* was evaluated by using Elastin Congo Red (ECR) (Sigma, USA.) as the substrate. 100 µl of EALCE presence and absence of *P.aeruginosa* supernatant were mixed with 900 µl of ECR buffer containing 100 mM Tris, 1 mM $CaCl_2$, and pH 7.5 with 20 mg of ECR and incubated at $37^0$ C for 3 h. After incubation, the reaction was stop by using 1 ml of 0.7 mM Sodium Phosphate buffer (pH 6.0) and the tubes were placed in an ice bath for 30 min. The insoluble ECR was removed by centrifugation at 10,000 rpm for 10 min and the absorbance was measured at OD 495 nm.

## Alginate inhibition assay:

Alginate is essential for mature biofilm formation in *P.aeruginosa* was evaluated by the following method of Owlia et al. (2007). Briefly, 70 µl of sample (presence and absence of EALCE) were slowly too added to 600 µl of boric acid / $H_2SO_4$ (4: 1) solutions were placed in an ice bath. The mixture was vortexed for

10 sec and placed back in the ice bath. 20 µl of 0.2 % carbazole solution in ethanol were addedto the mixture, which was vortexed for 10 sec and the mixture were placed in a water bath at $55^0$ C for 30 min. After incubation, the solution was absorbance spectrophotometrically at OD 530 nm. The inhibition of alginate production was calculated as percentage by the following formula.

**(Control OD – Test OD / Control OD) X 100**

## Pyocyanin production:

Pyocyanin quantification was evaluated by the following method of Essar et al. (1990). *P.aeruginosa* was cultivated in presence and absence of EALCE and extracted with 3 ml of chloroform and then reextracted into 1 ml of 0.2 N HCl to get a pink colour to the deep red colour solution. The solution was observed spectrophotometrically at OD 520 nm.

## Swimming assay:

Swimming and swarming motility assay were performed by the following method of Packiavathy et al. (2014). Briefly, 3 µl of overnight culture of test pathogen was inoculated at the centre of the swimming agar medium containing 1 % tryptone, 0.5 % NaCl and 0.3 % agar with a BIC concentration of EALCE.

## Swarming assay:

In swarming assay 5 µl of overnight culture of test pathogen was inoculated at the centre of the swarming agar medium consisting of 1 % peptone, 0.5 % agar, and 0.5 % filter – sterilized D – glucose with BIC concentrations of EALCE. The plates were incubated at $30^0$ C in upright position for 16 h. After incubation, migration of swimming and swarming was recorded by measuring the swim and swarm zones of the bacterial cells.

# RESULTS

## Effect of EALCE on violacein inhibition:

The quorum sensing inhibit efficacy of EALCE was found by violacein pigment production in *C.violaceum*. EALCE at 2 mg/ml exhibited a significant inhibition of violacein production in *C.violaceum* (Figure 1)

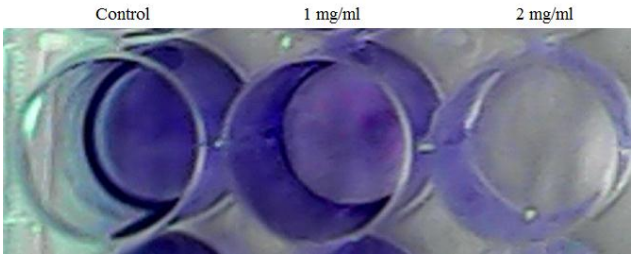


**Figure 1:** Quorum sensing inhibitory potential of EALCE against *C.violaceum*. effect of EALCE on violacein production in *C.violaceum* control and treated cultures showing progressive reduction at 2 mg/ml concentration.

## Antibiofilm efficacy of EALCE:

Biofilm quantification assay, a concentration dependent decrease in biofilm formation was observed in *P.aeruginosa*. when treated with EALCE, which exhibited 87 % reduction of biofilm formation at 150 µg/ml concentration (Figure 2)

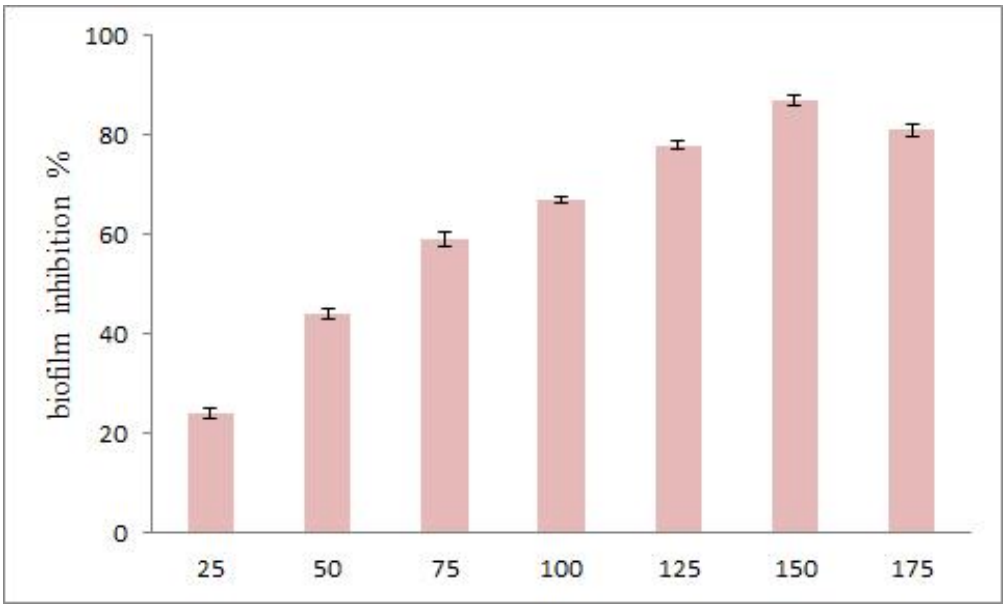


**Figure 2:** Effect of EALCE on biofilm formation of *P.aeruginosa*. Quantitative analysiss of biofilm inhibition as quantified by safranin staining and measuring absorbance at 570 nm. Data are presented as the percentage of inhibition. Mean values of triplicate independent experiments ans SD are shown.

## Antibacterial activity of EALCE:

Antibacterial efficacy of EALCE was determined, by using growth pattern of *P.aeruginosa* with the

increasing concentration of EALCE. *P.aeruginosa* growth curve was tested at the end of 24 h, EALCE was found to exhibit no antibacterial activity against *P.aeruginosa* (Figure 4). Further, *P.aeruginosa* cell viability was measured with the presence and absence of EALCE by using XTT. The obtained results showed that 150 µg/ml of EALCE does not affect the cells viability of *P.aeruginosa* (Figure 3).

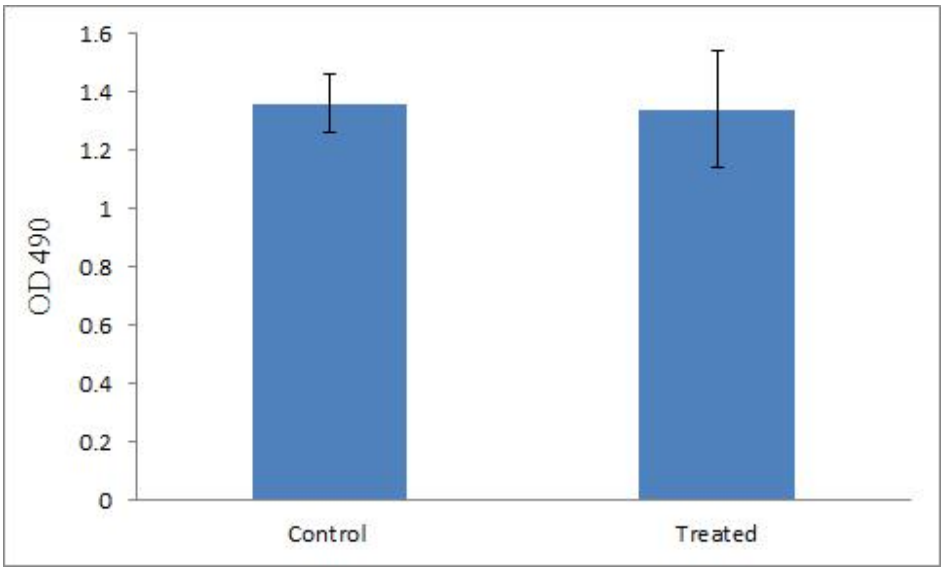


**Figure 3:** Effect of EALCE on cell viability. Quantitative measurement of cell viability was quantified by XTT assay and measuring absorbance at 490 nm. No statistically significant difference in cell viability among control and treated samples.

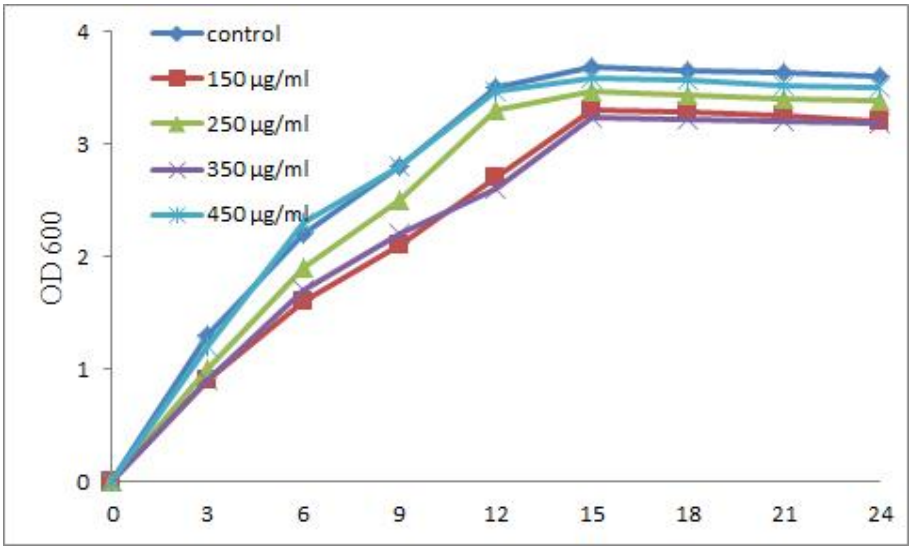


**Figure 4:** Growth curve assessment. Effect of EALCE at increasing concentration on the growth of *aeruginosa*.

## In situ analysis of biofilm formation:

Antibiofilm efficacy of EALCE was validated through microscopic techniques. In light microscopic results revealed that the significant inhibition of biofilm formation when treated with EALCE. Whereas, untreated control showed a well developed biofilm formation (Figure 5a). Further, the CLSM results showed a significant loose of biofilm architecture when treated with EALCE. Whereas, control slides showed a fine biofilm architecture (Figure 5b)

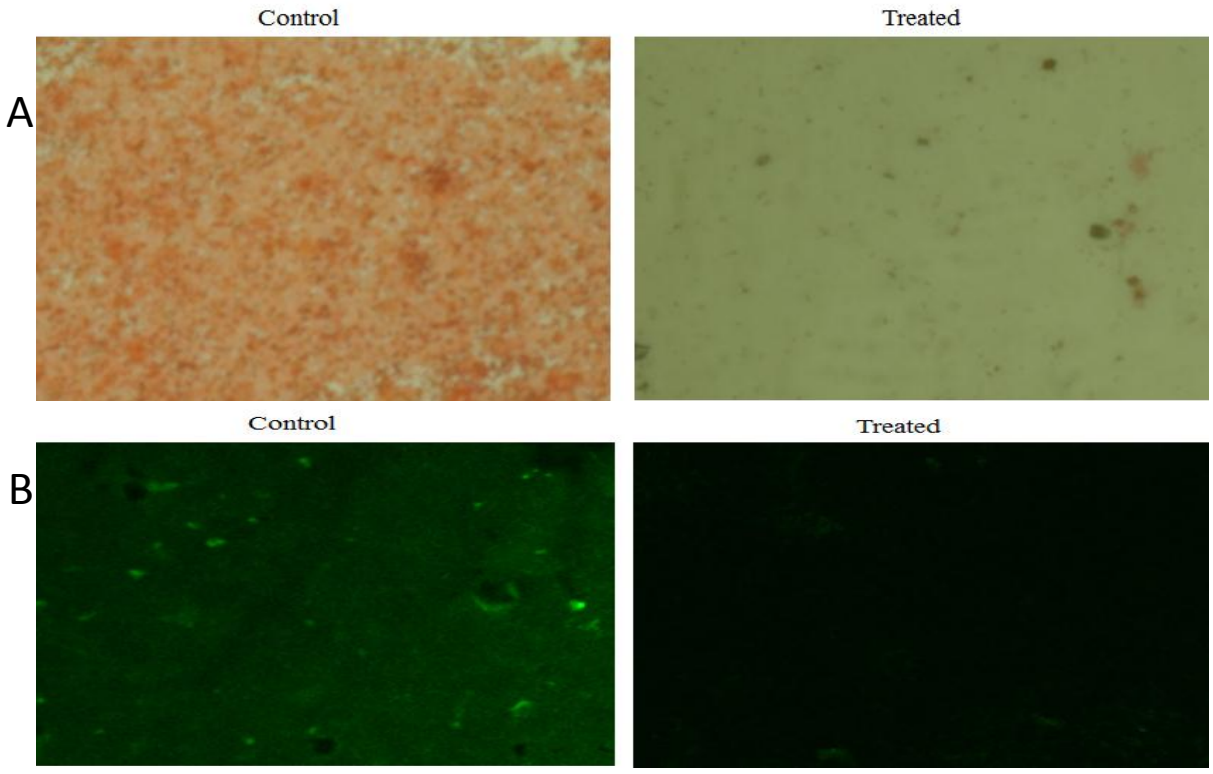


**Figure 5:** Light and confocal laser scanning microscopy images of *P.aeruginosa* biofilms grown in the presence and absence of EALCE (150 µg/ml). (A). Light microscopy images (B). CLSM images showing control and treated biofilms formation.

## EPS inhibition assy:

EPS was extracted from presence and absence of EALCE. When treated with EALCE at 150 µg/ml exhibited 89 % reduction of EPS production in *P.aeruginosa* (Figure 7).

## Motility inhibition assay:

The addition of EALCE showed a reduction of *P.aeruginosa* motility factors at dose dependent concentration. The maximum reduction of swimming and swarming motility was observed at 50 µg/ml

concentration (Figure 6).

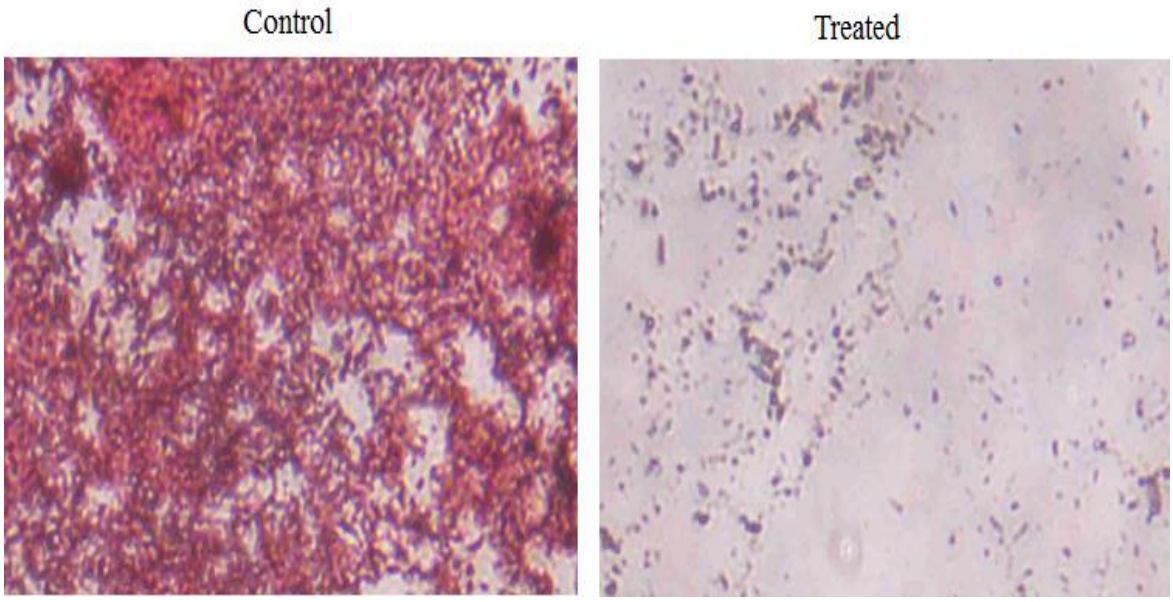


**Figure 6:** Effect of EALCE on mature biofilm architecture on *P.aeruginosa*. light microscopy images showing control and treated samples.

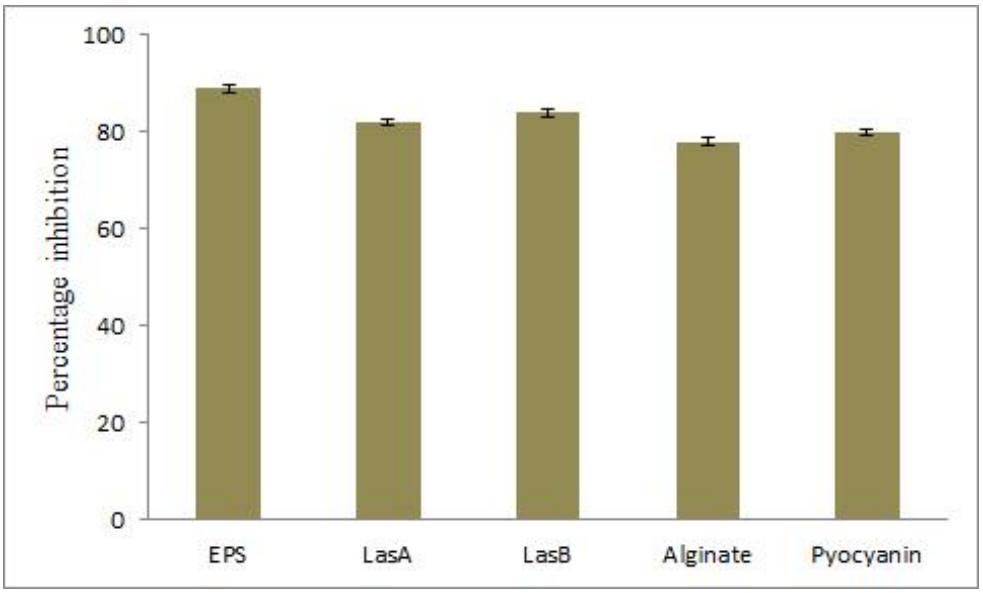


**Figure 7:** Effect of EALCE on EPS production and QS mediated virulence production on P.aeruginosa. Quantitative analysis of EPS inhibition by measuring the absorbance at 490 nm. Quantitative measurement of QS mediated virulence inhibition were quantified by Spectrophotometer absorbance. Data are presented as the percentage of inhibition. Mean values of triplicate independent experiments ans SD are shown.

## QSI potential of EALCE against P.aeruginosa:

EALCE exhibited, *P.aeruginosa* QS mediated biofilm inhibition at 150 µg/ml. At the same concentration, EALCE reduced the QS mediated virulence factors such as LasA staphylolytic protease (82 %), LasB elastase (79 %), alginate (80 %) and pyocyanin production (82 %) respectively (Fig. 7).

## DISCUSSION

The present study, this is the first report to revealed the QSI potential of *E.agallocha* leaves crude extract against QS dependent pathogens in *C.violaceum* and *P.aeruginosa*. EALCE revealed the excellent QSI potential by inhibiting the violacein pigment production in *C.violaceum*. The present data verified well with the previous findings (Annapoorani et al., 2013), who have reported the inhibition of violacein pigment production in *C.violaceum* by *Rhizophora* spp extract.

In previous studies (Viszwapriya et al., 2017) reported that the compounds exposed well known antibacterial activity could also potentially possess antipathogenic properties, which may not be related to growth inhibition of the microorganisms which report is positive correlate with our results. The EALCE revealed the ineffective on bacterial growth however, which interferes with the QS mechanism.

Naturally, QS mechanism induces the both initiation and maturation of the biofilm formation ( De Kievit et al., 2001). The exposure with EALCE at 150 µg/ml excellently reduced the biofilm formation by 87 % (Fig. 2). The obtained results compared with previous study (Sethupathy et al., 2015) who reported that, 2 – furaldehyde diethyl acetal inhibited the *P.aeruginosa* biofilm formation by 89 %. The antibiofilm efficacy of EALCE was validated through microscopic analysis. In light microscope, results revealed the excellent reduction of biofilm formation and affect the mature biofilm architecture when treated with EALCE whereas compared with control (Fig. 5a). Further, CLSM images revealed the significant reduction of biofilm biomass when treated with EALCE, result was compared with control ( Fig. 5b). In previous study (You et al., 2007) who reported that *Streptomyces albus* extract significantly inhibited the *Vibrio* spp. biofilm formation by interferes the initial adherence of planktonic cells, which report is possibly matched with our results. Hence, the active metabolites of EALCE could possibly affect the expressions of gene regulates for initial adherence, which accelerates the reduction of biofilm formation.

EPS plays a crucial role in development of biofilm architecture and maturation (Watnick and Kolter, 1999). In previous studies (Wai et al., 1998) who reported that, over production of EPS which modification in biofilm architecture which leads to increased the resistance of cells to osmotic and oxidative stress and antibiotics). Meanwhile, EPS production was controlled by QS mechanism, which interferes the QS system would result in the reduction of EPS production (Vu et al., 2009). Hence, the present study we observed EALCE revealed, the significantly reduced the EPS production by 89 % which leads it affected the biofilm

architecture and collapsed the mature biofilm architecture which was authenticated from light and CLSM images. The obtained results was compared to previous study (Packiavathy et al., 2012) who reported that *Cuminum cyminum* extract inhibited the EPS production by 70 % in *P.aeruginosa*.

Motility is a key virulence factor in P.aeruginosa pathogenesis. Motility factors are controlled by QS system. The swimming and swarming motility is essential for biofilm formation (Harshey, 2003). Hence, the reduction of the motility factors, which leads to affect the biofilm formation. In the present study, QSI potential of EALCE strongly to reduced the motility behavior in *P.aeruginosa*. The obtained results indicates, the EACLE inhibits the AHL mediated cell differentiation, which restrict the cell differentiation from normal cells to motility cells. The observed results was compared to Packiavathy et al., 2012 reported that *C.cyminum* inhibited the motility behavior in *P.aeruginosa*.

Las A staphylolytic protease, Las B elastase, alginate and pyocyanin were important virulence factors which were controlled by QS system. The QS mediated virulence factors are able to infect the chronic diseases in host. Hence, the present study revealed the efficacy of EALCE strongly inhibited the virulence production against *P.aeruginosa*. The presence of QSI compound inhibits the QS mechanism, which might affects the virulence gene expression and reduced the virulence production in physiological assays. The obtained results compared with (Sethupathy et al., 2015) who reported that, 2 – furaldehyde diethyl acetal inhibited the *P.aeruginosa* QS mediated virulence production.

## CONCLUSION

QS mediated virulence factors plays a crucial role, which were the responsible for *P.aeruginosa* pathogenesis. To the best of our knowledge, E.agallocha leaves crude extract have not been explored for its QSI activity against *C.violaceum* and *P.aeruginosa*. Therefore, it is the first report from the present study investigates the EALCE could possibly be an ideal QSI agent whereas, significantly inhibits the biofilm formation. The interferes of QS system, this impact reflects to inhibit the virulence factors production including motility behavior, Las A staphylolytic protease, Las B elastase, alginate and pyocyanin production.

## Acknowledgements:

The authors are thankful to DBT, New Delhi for the project fellowships and the authorities of Annamalai University, India for providing facilities.

## REFERENCES

1. Ryan RP, Dow JM. Diffusible signals and interspecies communication in bacteria. Microbiology. 2008, 154(7):1845-58.

2. Ng WL, Bassler BL. Bacterial quorum-sensing network architectures. Annual review of genetics. 2009, 43:197-222.
3. Smith RS, Iglewski BH. *P. aeruginosa* quorum-sensing systems and virulence. Current opinion in microbiology. 2003, 6(1):56-60.
4. Gellatly SL, Hancock RE. Pseudomonas aeruginosa: new insights into pathogenesis and host defenses. Pathogens and disease. 2013 Apr 1;67(3):159-73.
5. Lewis K. Persister cells. Annual review of microbiology. 2010 Oct 13;64:357-72.
6. Rybtke M, Hultqvist LD, Givskov M, Tolker-Nielsen T. Pseudomonas aeruginosa biofilm infections: community structure, antimicrobial tolerance and immune response. Journal of molecular biology. 2015 Nov 20;427(23):3628-45.
7. Hu JF, Garo E, Goering MG, Pasmore M, Yoo HD, Esser T, Sestrich J, Cremin PA, Hough GW, Perrone P, Lee YS. Bacterial Biofilm Inhibitors from Diospyros d Endo. Journal of natural products. 2006 Jan 27;69(1):118-20.
8. Bjarnsholt T, Givskov M. Quorum sensing inhibitory drugs as next generation antimicrobials: worth the effort?. Current infectious disease reports. 2008 Jan 1;10(1):22-8.
9. Kociolek MG. Quorum-sensing inhibitors and biofilms. Anti-Infective Agents in Medicinal Chemistry (Formerly Current Medicinal Chemistry-Anti-Infective Agents). 2009 Oct 1;8(4):315-26.
10. Bandaranayake WM. Bioactivities, bioactive compounds and chemical constituents of mangrove plants. Wetlands Ecology and Management. 2002 Dec 1;10(6):421-52.
11. Annapoorani A, Kalpana B, Musthafa KS, Pandian SK, Ravi AV. Antipathogenic potential of Rhizophora spp. against the quorum sensing mediated virulence factors production in drug resistant Pseudomonas aeruginosa. Phytomedicine. 2013 Aug 15;20(11):956-63.
12. Musthafa KS, Sahu SK, Ravi AV, Kathiresan K. Anti-quorum sensing potential of the mangrove Rhizophora annamalayana. World Journal of Microbiology and Biotechnology. 2013 Oct 1;29(10):1851-8.
13. Subhan N, Alam MA, Ahmed F, Shahid IJ, Nahar L, Sarker SD. Bioactivity of Excoecaria agallocha. Revista Brasileira de Farmacognosia. 2008 Dec;18(4):521-6.
14. Deepa M, Padmaja CK. A study on the antibacterial effects of the various extracts of Excoecaria agallocha L. Int J Pharmacol Biol Sci. 2014;4:169-78.
15. Choo JH, Rukayadi Y, Hwang JK. Inhibition of bacterial quorum sensing by vanilla extract. Letters in applied microbiology. 2006 Jun 1;42(6):637-41.
16. Nithya C, Begum MF, Pandian SK. Marine bacterial isolates inhibit biofilm formation and disrupt mature biofilms of Pseudomonas aeruginosa PAO1. Applied microbiology and biotechnology. 2010 Sep 1;88(1):341-58.
17. Musthafa KS, Balamurugan K, Pandian SK, Ravi AV. 2, 5-Piperazinedione inhibits quorum sensing-dependent factor production in Pseudomonas aeruginosa PAO1. Journal of basic microbiology. 2012 Dec 1;52(6):679-86.

18. Adonizio A, Kong KF, Mathee K. Inhibition of quorum sensing-controlled virulence factor production in Pseudomonas aeruginosa by South Florida plant extracts. Antimicrobial agents and chemotherapy. 2008 Jan 1;52(1):198-203.
19. Owlia P, Rasooli I, Saderi H, Aliahmadi M. Retardation of biofilm formation with reduced productivity of alginate as a result of Pseudomonas aeruginosa exposure to Matricaria chamomilla essential oil. Pharmacognosy Magazine. 2007 Apr 1;3(10):83.
20. Essar DW, Eberly L, Hadero A, Crawford IP. Identification and characterization of genes for a second anthranilate synthase in Pseudomonas aeruginosa: interchangeability of the two anthranilate synthases and evolutionary implications. Journal of bacteriology. 1990 Feb 1;172(2):884-900.
21. Packiavathy IA, Priya S, Pandian SK, Ravi AV. Inhibition of biofilm development of uropathogens by curcumin–an anti-quorum sensing agent from Curcuma longa. Food chemistry. 2014 Apr 1;148:453-60.
22. Viszwapriya D, Subramenium GA, Radhika S, Pandian SK. Betulin inhibits cariogenic properties of Streptococcus mutans by targeting vicRK and gtf genes. Antonie van Leeuwenhoek. 2017 Jan 1;110(1):153-65.
23. De Kievit TR, Gillis R, Marx S, Brown C, Iglewski BH. Quorum-sensing genes in Pseudomonas aeruginosa biofilms: their role and expression patterns. Applied and environmental microbiology. 2001 Apr 1;67(4):1865-73.
24. Sethupathy S, Nithya C, Pandian SK. 2-Furaldehyde diethyl acetal from tender coconut water (Cocos nucifera) attenuates biofilm formation and quorum sensing-mediated virulence of Chromobacterium violaceum and Pseudomonas aeruginosa. Biofouling. 2015 Nov 26;31(9-10):721-33.
25. You CC, Miranda OR, Gider B, Ghosh PS, Kim IB, Erdogan B, Krovi SA, Bunz UH, Rotello VM. Detection and identification of proteins using nanoparticle–fluorescent polymer 'chemical nose'sensors. Nature Nanotechnology. 2007 May 1;2(5):318-23.
26. Watnick PI, Kolter R. Steps in the development of a Vibrio cholerae El Tor biofilm. Molecular microbiology. 1999 Nov 1;34(3):586-95
27. Wai SN, Mizunoe Y, Takade A, Kawabata SI, Yoshida SI. Vibrio cholerae O1 strain TSI-4 produces the exopolysaccharide materials that determine colony morphology, stress resistance, and biofilm formation. Applied and environmental microbiology. 1998 Oct 1;64(10):3648-55.
28. Vu B, Chen M, Crawford RJ, Ivanova EP. Bacterial extracellular polysaccharides involved in biofilm formation. Molecules. 2009 Jul 13;14(7):2535-54.
29. Packiavathy IA, Agilandeswari P, Musthafa KS, Pandian SK, Ravi AV. Antibiofilm and quorum sensing inhibitory potential of Cuminum cyminum and its secondary metabolite methyl eugenol against Gram negative bacterial pathogens. Food Research International. 2012 Jan 31;45(1):85-92.
30. Harshey RM. Bacterial motility on a surface: many ways to a common goal. Annual Reviews in Microbiology. 2003 Oct;57(1):249-73