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ANTI QUORUM SENSING AND ANTI BIOFILM POTENTIAL OF *ANOGEISSUS ACUMINATA* AND *MALLOTUS ROXBURGHIANUS* MUELL. AGAINST *PSEUDOMONAS AERUGINOSA* PAO1

Sairengpuii Hnamte[1], Pattnaik Subhaswaraj[1], Sampath Kumar Ranganathan[2], Dinakara Rao Ampasala[2], Gangatharan Muralitharan[3], Busi Siddhardha[1]*

Address(es):
[1]Department of Microbiology, School of Life Sciences, Pondicherry University, Puducherry-605014, India.
[2]Centre for Bioinformatics, School of Life Sciences, Pondicherry University, Puducherry-605014, India.
[3]Deparment of Microbiology, School of Life Sciences, Bharathidasan University, Tiruchirappali- 620024, India.

*Corresponding author: siddhardha.busi@gmail.com

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ABSTRACT

Pseudomonas aeruginosa causes severe persistent infections in immunocompromised patients by showing tolerance conventional antibiotic therapy by virtue of specialized hierarchy of quorum sensing (QS) network. The purpose of the study was to evaluate the efficacy of two Mizoram medicinal plants i.e., *Anogeissus acuminata* and *Mallotus roxburghianus* Muell. in attenuating QS regulated *P. aeruginosa* virulence. The crude leaf extracts were tested for their ability to combat QS associated virulence phenotypes such as pyocyanin production, LasB elastase and Staphylolytic activity and production of biofilm determinants such as alginate and exopolysaccharide in *P. aeruginosa* PAO1. The gas chromatography mass spectrometry (GC-MS) analysis revealed the presence of phytocompounds, the synergistic activity of which is responsible for the anti QS activity. The *in silico* studies provide the binding efficacy of obtained phytochemical with QS receptor protein, LasR. The *in vitro* studies followed by *in silico* analysis demonstrated the efficacy of phytochemical of *A. acuminata* and *M. roxburghianus* Muell. in competitively inhibiting the binding of natural ligand with LasR and thereby altering production of virulence phenotypes. The efficacy in down regulating bacterial virulence shown by *A. acuminata* and *M. roxburghianus* Muell. provides promising alternatives to develop next generation anti-pathogenic agents.

Keywords: Antibiotic resistance, Biofilm, *Pseudomonas aeruginosa*, Quorum sensing, Virulence factors

INTRODUCTION

Microbial pathogens possess significant public health concern by showing multi drug resistance (MDR) to conventional antibiotics. In this context, the current antimicrobial approach has been shifted towards development of anti-pathogenic agents targeting quorum sensing (QS) regulated bacterial virulence rather than targeting bacterial killing (**Maisuria *et al.*, 2016**). The phenomenon of QS is a cellular signaling process between bacterial cells coordinating the social behaviour through a network of discrete signaling mechanism controlling expression of different pathophysiological functions in the form of virulence factors and formation & development of biofilm (**Vasavi *et al.*, 2016**). *Pseudomonas aeruginosa* causes severe respiratory infections, urinary tract infections (UTIs) and other hospital acquired infections in the immunocompromised individuals under the influence of highly specialized QS regulatory network (**Datta *et al.*, 2016**). The QS system in *P. aeruginosa* comprises of two major LuxI/R QS machinery such as Las and Rhl system. The Las system comprises of LasR transcriptional activator which operates through a signal molecule, N-3-oxo-dodecanoyl-homoserine lactone (C_{12}-HSL) whereas RhlR, as transcriptional regulator activated by a signal molecule called N-butanoyl-homoserine lactone (C_4-HSL) constitute Rhl system. Both LasI/R and RhlI/R systems are intriguingly correlated with each other and coordinate the expression of virulence phenotypes such as proteases, elastases, cytotoxic pyocyanin, exopolysachharides (EPS) and rhamnolipid (**Aybey and Demirkan, 2016**). The ability of *P. aeruginosa* to form biofilm aids as an arsenal to the bacteria to provide tolerance against conventional antimicrobial therapy. In this context, targeting QS associated virulence could provide alternative strategies to combat QS regulated pathogenicity (**Luo *et al.*, 2016**).

From last few decades, a number of synthetic, semi-synthetic and natural compounds were exploited to attenuate bacterial virulence in *P. aeruginosa* (**Ouyang *et al.*, 2016**). Among the natural products, plant derived phytochemicals are exploited the most owing to their diverse chemical features, widespread bioactivities and are of potential importance in biomedical and pharmaceutical industries (**Silva *et al.*, 2016**). In the present study, two medicinal plants, *Anogeissus acuminata* and *Mallotus roxburghianus* Muell. from Mizoram, India were evaluated for their anti QS and anti-biofilm activity. *A. acuminata* locally known as Zairum is being actively used for stomach ache, diarrhea, as antiseptic and skin diseases like eczema, skin ulcers and dermatitis (**Lalfakzuala *et al.*, 2007; Hemamalini *et al.*, 2011**). Meanwhile, *M. roxburghianus* Muell. (locally known as Zawngtenawhlung) has been used for antioxidant, anti-inflammatory and gastroprotective properties (**Roy *et al.*, 2016; Sagun *et al.*, 2017**). The aim of the present study is to evaluate the efficacy of selected medicinal plants from Mizoram, India in combating *P. aeruginosa* PAO1 pathogenicity.

MATERIALS AND METHODS

Collection of Plants and Extract Preparation

A total of twenty-five medicinal plants from different parts of Mizoram, India were collected, cleaned thoroughly, dried and grounded in the form of powder. The powdered plant materials were extracted with different solvents such as methanol, ethanol, chloroform and petroleum ether.

Maintenance of culture

The bacterial strains *Chromobacterium violaceum* (MTCC 2656) and *P. aeruginosa* PAO1 used for QS inhibition study were obtained from Microbial Type Culture Collection (MTCC), IMTECH, Chandigarh, India. *C. violaceum* was used as a biomarker strain for preliminary anti QS assay.

Preliminary screening of crude plant extracts for anti-QS activity

All the crude plant extracts were evaluated for their anti QS potential by agar well diffusion method against biomarker strain, *C. violaceum* and test organism, *P. aeruginosa* PAO1. Briefly, overnight bacterial culture was spread on top of the agar plates using sterile cotton swabs. Wells of 8mm diameter were prepared and

various concentrations of plant extracts were loaded into the wells and incubated at 37°C for 24 h. DMSO was used as control (**Vasavi *et al.*, 2016**).

Determination of Minimal Inhibitory Concentration (MIC)

The MIC of the selected plant extracts (*A. acuminata* and *M. roxburghianus* Muell.) against *P. aeruginosa* PAO1 was fixed as per the Clinical and Laboratory Standards Institute (CLSI) standards. All the Anti-QS assays were performed with sub-MIC dose.

Anti QS activity against biomarker strain, *C. violaceum*

Violacein inhibition activity

For violacein inhibition assay, overnight *C. violaceum* was grown on treatment with sub-MIC of crude plant extracts at 30 °C for 24 h. After incubation, the insoluble violacein was precipitated by centrifuging the culture at 10,000 rpm for 10 min. The pellet was resuspended with DMSO and vortexed for 30 sec to solubilize the violacein. The solution was then recentrifuged (10,000 rpm, 10 min) and the violacein present in the supernatant were determined at 595 nm (**D'Almeida *et al*., 2017**).

Anti QS activity of plant extracts against *P. aeruginosa* PAO1

Pyocyanin inhibition activity

For pyocyanin inhibition assay, cell free supernatant was collected from *P. aeruginosa* PAO1 treated with sub-MIC of crude plant extracts. The supernatant was mixed with chloroform in the ratio of 3:2 and was left for phase separation. The pyocyanin from the organic phase was reextracted with 0.2 N HCl. The absorbance of the aqueous phase was measured at 520 nm (**Luo *et al*., 2016**).

Elastolytic activity

The effect of crude plant extracts on elastolytic activity was determined according to **Luo *et al.* (2016)**. Briefly, cell free supernatant of overnight bacterial culture was mixed with Elastin Congo Red buffer (pH 7.5) in the ratio of 1:9 and incubated at 37 °C for 3 h with continuous shaking condition. After incubation, equal volume of sodium phosphate buffer (0.7 M, pH 6.0) was added and placed for 30 min in an ice water bath followed by recentrifugation (10,000 rpm, 10 min) to remove insoluble ECR. The optical density was determined at 495 nm.

Staphylolytic activity

The ability of *P. aeruginosa* PAO1 to lyse Staphylococcus aureus cells, Staphylolytic activity was performed. *S. aureus*, grown overnight was boiled (10 min) followed by centrifugation at13,000 rpm, 10 min. The obtained pellet was suspended in high salt buffer (pH 4.5) and to the suspension *P. aeruginosa* PAO1 cell free supernatant mixed in 1:9 ratio. The absorbance of mixed suspension was determined at 495 nm **(Alasil *et al*., 2015)**.

Anti biofilm activity of plant extracts against *P. aeruginosa* PAO1

Exopolysaccharides (EPS) inhibition activity

For EPS inhibition activity, to the cell free supernatant ethanol was added and incubated at 4 °C for 24 h to precipitate the EPS. After incubation, the obtained EPS was resuspended in distilled water and quantified by phenol-sulfuric acid method as described by **(Rasamiravaka *et al.*, 2015)**.

Alginate inhibition activity

Briefly, overnight *P. aeruginosa* PAO1 grown in presence of sub-MIC of plant extracts was mixed with NaCl (0.85%) and the mixture was centrifuged. To the supernatant, cetyl pyridinium chloride (2%) was added and precipitated (10,000 rpm, 20 min). The obtained pellet was dissolved in 1M NaCl, precipitated again with cold isopropanol, followed by recentrifugation. The pellet was resuspended in saline and stored at 4°C. To the suspension, 25mM sodium tetraborate solution was added followed by heating (100°C for 10 min), cooled at room temperature for 15 min. To the solution, carbazole solution (0.125%) was added and heated followed by cooling and the optical density was measured at 550 nm (**Rasamiravaka *et al*., 2015**).

Biofilm formation activity

Anti-biofilm activity of plant extracts was determined according to **Luciardi *et al*. (2016).** Briefly, *P. aeruginosa* in presence of crude plant extracts was grown for 16 h. The planktonic cells were carefully removed and rinsed with sterile phosphate buffer saline (PBS). The biofilms attached on the surface were stained with crystal violet (0.4%) for 10 min. After incubation, to the crystal violet bound biofilm, absolute ethanol was added and optical density was measured at 590 nm.

Microscopic observation of biofilm by confocal laser scanning microscopy (CLSM)

The effect of crude plant extracts on biofilm formation was analyzed by CLSM analysis as per the description given by **Sethupathy *et al*. (2015)**. For CLSM analysis, 24 h biofilms attached to microtiter plate stained with acridine orange for 10 min. After incubation, sterile PBS was used to remove excess stain and **image of the biofilm was analyzed under microscope at 20X magnification**.

Gas Chromatography-Mass Spectrometry (GC-MS) analysis

The phytochemical investigation of crude extract of two plants was determined using GC-MS analysis. The phytochemicals present in the spectrum was identified by NIST library database.

Molecular docking studies

The *in silico* analysis was carried out in Schrodinger maestro 9.2 to analyze the binding affinity of plant metabolites observed from GC-MS analysis towards LasR protein as compared to its autoinducer. The ligand binding domain of LasR protein (PDB ID: 2UV0) was obtained from Protein Data Bank. For LasR protein, grids were defined around the active aminoacid residues such as Tyr56, Trp60, Asp73, Thr75 and Ser129 where C_{12}-HSL interacts with LasR protein **(Kim *et al*., 2015)**. The ligand compounds were obtained from PubChem database and submitted for preparation in Ligprep module 2.5 in Schrodinger suite and the prepared protein and ligand were subjected for docking. The best posed ligand was selected based on hydrophobic and hydrogen bond interaction around the specified active site residues within the predefined grid box.

Statistical analysis

All the experimental data presented as mean ± standard deviation (SD) of three independent experiments. One-way analysis of variance (ANOVA) was performed to determine the significance between both the plant extracts in combating virulence. For statistical analysis, $p<0.05$ was considered as significant.

RESULTS

Screening of the plant extracts for anti-QS activity

Among the 25 plants screened with four different solvent system, methanolic extract of *A. acuminata* and ethanolic extract of *M. roxburghianus* Muell. were selected for further bioassays on the basis of zone of inhibition (S1).

Determination of MIC

The MIC for *A. acuminata* (methanol) and *M. roxburghianus* (ethanol) was found to be 1000 µg/mL and 750 µg/mL selected as the sub-MIC. All the bioassays were performed with sub-MIC dose.

Violacein inhibiton Assay

On treatment with sub-MIC dose of *A. acuminata* and *M. roxburghianus* Muell., the relative production of violacein was significantly reduced by 70.46±3.67 and 58.89±4.32% respectively as compared to control (**Figure 1**).

Elastolytic activity

On treatment with sub-MIC concentration of *A. acuminata* and *M. roxburghianus* Muell., a significant decrease in the elastolytic activity of test organism, *P. aeruginosa* PAO1 was observed with a reduction of 59.85±1.82 and 51.65±3.88% respectively (**Figure 2**).

Staphylolytic activity

On treatment with sub-MIC dose *A. acuminata* and *M. roxburghianus* Muell. a significant reduction in Staphylolytic activity was observed with a reduction of 39.67±3.06 and 32.39±2.23% respectively as compared to control (**Figure 2**).

Anti-biofilm activity of plant extracts

EPS inhibition activity

A significant decrease in EPS production in *P. aeruginosa* PAO1 was observed on treatment with sub-MIC level of *A. acuminata* and *M. roxburghianus* Muell. with a reduction of 65.07±3.63 and 52.88±3.03% respectively as compared to control (**Figure 3**).

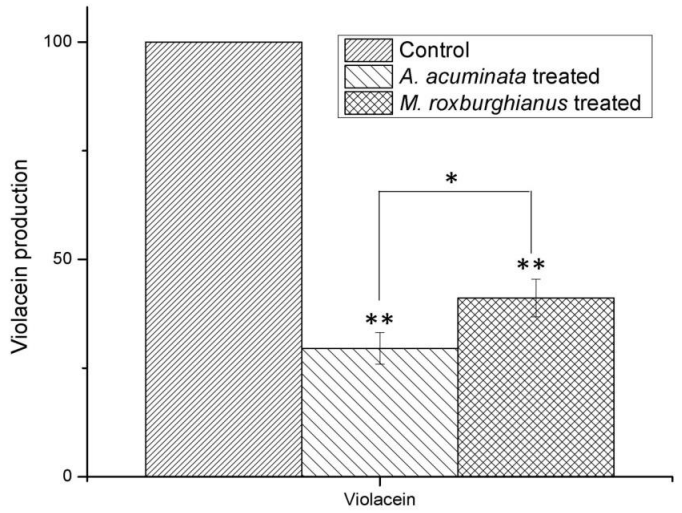

Figure 1 Effect of crude plant extract of *A. acuminata* and *M. roxburghianus* Muell. on violacein production in the biomarker strain *C. violaceum* (MTCC 2656) as compared to untreated control (normalized into 100% violacein production). Tukey's Q-test demonstrates significance between the different test groups of different sub-MIC values. *values are significantly different at $p<0.05$, **values are significantly different at $p<0.01$, ***values are significantly different at $p<0.001$, ^{NS}values are not significantly different.

Anti QS activity of plant extracts

Pyocyanin inhibition activity

A. acuminata exhibited 65.24±1.72% and *M. roxburghianus* Muell. exhibited 53.97±2.15% of reduction in pyocyanin production as compared to untreated control (**Figure 2**).

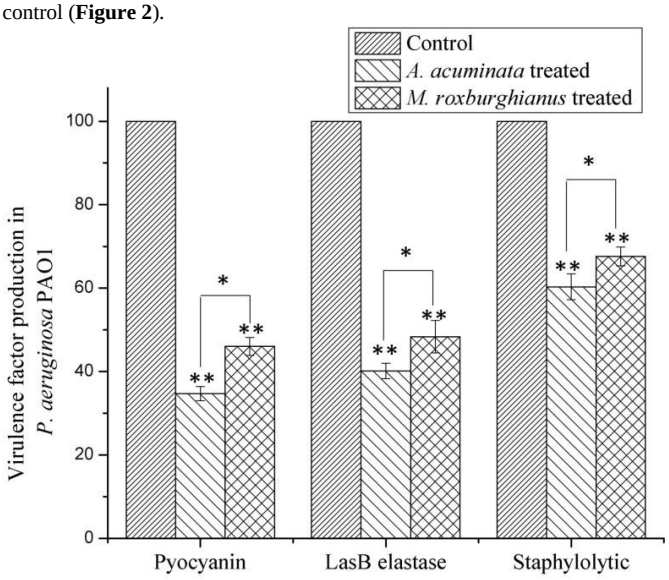

Figure 2 Effect of *A. acuminata* and *M. roxburghianus* Muell. Extract on pyocyanin production, LasB elastase and Staphylolytic activity of *P. aeruginosa* PAO1 as compared to untreated control (normalized into 100%). *values are significantly different at $p<0.05$, **values are significantly different at $p<0.01$, ***values are significantly different at $p<0.001$, ^{NS}values are not significantly different.

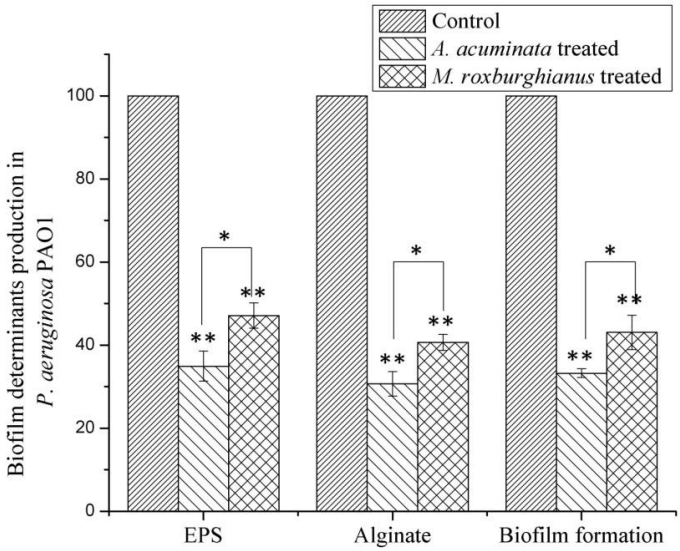

Figure 3 Effect of *A. acuminata* and *M. roxburghianus* Muell. on production of biofilm determinants (EPS and alginate) and biofilm formation in *P. aeruginosa* PAO1 as compared to untreated control (normalized into 100%). *values are significantly different at $p<0.05$, **values are significantly different at $p<0.01$, ***values are significantly different at $p<0.001$, ^{NS}values are not significantly different.

Alginate inhibition activity

At sub-MIC of 750 µg/mL, *A. acuminata* reduced the production of alginate by 69.31±2.93% as compared to 59.33±1.91% on treatment with crude extract of *M. roxburghianus* Muell. (**Figure 3**).

Biofilm formation activity

On treatment with sub-MIC level of *A. acuminata* and *M. roxburghianus* Muell., a significant decrease in the biofilm formation was observed with a reduction of 66.78±1.05 and 56.96±4.15% respectively (**Figure 3**).

CLSM analysis of biofilm

The CLSM analysis confirmed the alteration of biofilm architecture on treatment with sub-MIC dose of *A. acuminata* and *M. roxburghianus* Muell. as compared to the control with larger aggregation of cells and thicker biofilm (**Figure 4**)

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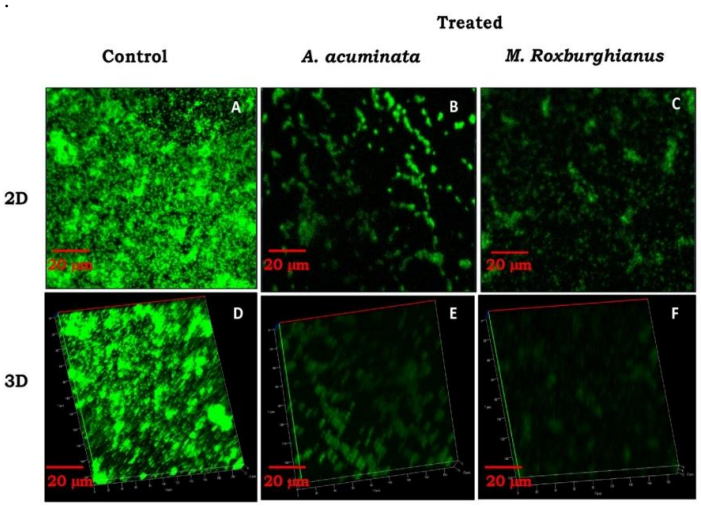

Figure 4 Effect of crude plant extract of *A. acuminata* and *M. roxburghianus* Muell. on biofilm formation as observed by CLSM analysis. A. 2D image of untreated *P. aeruginosa* PAO1 biofilm, B. 2D image of *A. acuminata* treated *P. aeruginosa* PAO1 biofilm, C. 2D image of *M. roxburghianus* Muell. treated *P. aeruginosa* PAO1 biofilm, D. 3D image of untreated *P. aeruginosa* PAO1 biofilm, E. 3D image of *A. acuminata* treated *P. aeruginosa* PAO1 biofilm, F. 3D image of *M. roxburghianus* Muell. treated *P. aeruginosa* PAO1 biofilm

GC-MS analysis

From the GC-MS analysis, *A. acuminata* crude extract comprised of several phytochemicals of diverse chemical class such as phytol, N hexadecanoic acid, oleyl alcohol, squalene, α- tocopherol and betulin. Meanwhile, the presence of phytol, ethyl palmitate, squalene, α-tocopherol, dihydrostachysterol, betulin and

sulfurous acid and 2-propyl tridecyl ester in the ethanolic extract of *M. roxburghianus* Muell. (**Table1**).

Table 1 List of phytochemicals present in the crude extracts of *A. acuminata* and *M. roxburghianus* Muell. by GC-MS analysis

Sl no.	Crude plant extract	Compound name	Chemical class	Retention Time	Area %	Reported biological activity	References
1.	*Anogeissus acuminata*	Phytol	Diterpene alcohol	16.68	9.32	Anti-inflammatory, anti-QS activity	**(Pejin *et al*., 2015)**
2.		N-hexadecanoic acid	Saturated fatty acid	18.30	18.68	Antioxidant and anti-inflammatory activity	**(Abubakar and Majinda, 2016)**
3.		Oleyl alcohol	Unsaturated fatty alcohol	19.81	7.28	Antibacterial activity	**(Jafari *et al*., 2014)**
4.		1-Hexyl-1-Nitrocyclohexane	Ketone	20.015	13.47	Antimicrobial, anti-inflammatory activity	**(Selvamangai and Bhaskar, 2012)**
5.		Squalene	Polyunsaturated hydrocarbon	24.76	7.14	Antioxidant, anticancer activity	**(Kim and Karadeniz, 2012)**
6.		α- tocopherol	Vitamin E	27.11	20.00	Antioxidant, hepatoprotective activity	**(Palipoch *et al*., 2014)**
7.		Betulin	Pentacyclic triterpene	29.12	11.19	Immunomodulatory activity	**(Pfarr *et al*., 2015)**
8.	*Mallotus roxburghianus* Muell.	Phytol	Diterpene alcohol	16.57	25.4	Anti-inflammatory, anti-QS activity	**(Pejin *et al*., 2015)**
9.		Ethyl palmitate	Fatty acid esters	18.21	17.59	Antioxidant, nematicidal activity	**(Kumar *et al*., 2010)**
10.		Squalene	Polyunsaturated hydrocarbon	24.75	11.48	Antioxidant, anticancer activity	**(Kim and Karadeniz, 2012)**
11.		Sulfurous acid, 2-propyl tridecyl ester	Propyl ester	25.32	1.91	Pharmaceuticals	**(Vivekraj *et al*., 2015)**
12.		α- tocopherol	Vitamin E	27.11	14.83	Antioxidant, hepatoprotective activity	**(Palipoch *et al*.,2014)**
13.		Dihydrotachysterol	Vitamin D4	29.14	5.48	For treatment of hypocalcemia and hypothyroidism	
14.		Betulin	Pentacyclic triterpene	29.89	7.47	Immunomodulatory activity	**(Pfarr *et al*.,2015)**

Molecular docking studies

In silico analysis of the phytochemicals present in *A. acuminata* and *M. roxburghianus* Muell. revealed that phytol (a common phytochemical in both crude extracts) exhibited a docking score of -7.042 kcal/mol suggesting its affinity for LasR. In addition to phytol, sulfurous acid, 2-propyl tridecyl ester also showed promising affinity with a score of -6.669 kcal/mol (**Figure 5, Table 2**).

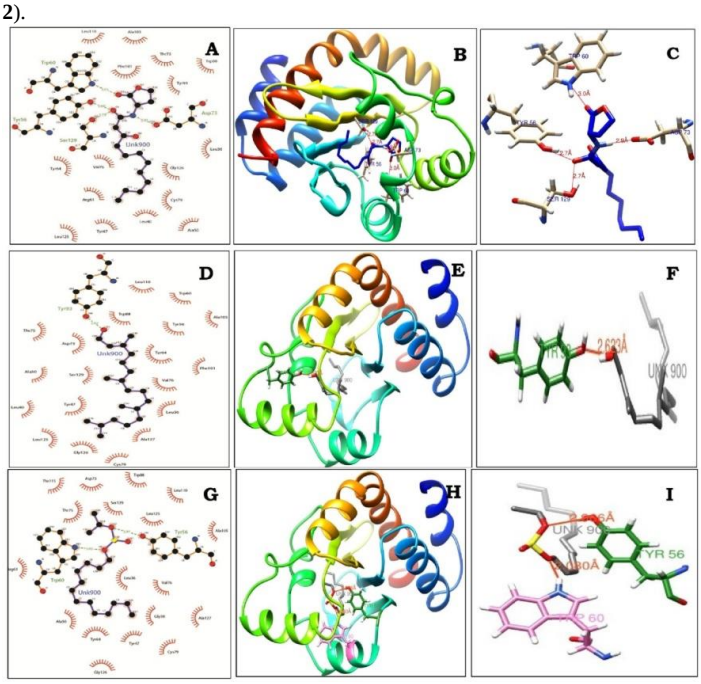

Figure 5 Docking studies: A & B. 2D and 3D docked conformation of C12-HSL into the active site of LasR; C. 3D docked conformation of C12-HSL into the active site of LasR depicting the H-bond interaction, D&E. 2D and 3D docked conformation of phytol into the active site of LasR, F. 3D docked conformation of phytol into the active site of LasR depicting the H-bond interaction, G&H. 2D and 3D docked conformation of sulfurous acid, 2-propyl tridecyl ester into the active site of LasR, I. 3D docked conformation of sulfurous acid, 2 - propyl tridecyl ester into the active site of LasR.

Table 2 Details of Docking score, hydrogen bonds and interacting residues revealed through molecular docking of selected compounds against transcriptional regulator, LasR.

Sl no.	Plant	Compounds	Docking score Kcal/mol	Hydrogen bond	Hydrophobic interactions
1.		3-oxo-C12-HSL (Natural ligand for LasR)	-8.489	Tyr56, Trp60, Asp73, Ser129	Leu36,Try47, Tyr64, Val70, Val76, Trp88, Tyr93,Ala105, Phe101,Leu110,
3.		Phytol	-7.042	Tyr 93	Tyr 47,Ile 52,Ala 70,Leu 36
4.		N-hexadeccanoic acid	-1.979	Tyr56	Ala 70, Ile 52,Ala 50
5.	*A. acuminata*	R-(-)-4-Methylhexanoic acid	-4.498	Tyr 56,Thr 75,Ser 129	Ile 84, Ala 44, Tyr 72, Tyr 64
6.		Bicyclo[4.1.0Hepatan E, 7Pentyl	-5.421	-	Leu36,Tyr64,Asp73,Thr75,Val76,Trp88,Ser129
7.		1-Hexyl-2 Nitrocyclohexane	-3.958	Tyr 56	Leu36,Trp60,Tyr64,Asp73,Thr75, Trp88,Ser129
8.		Phytol	-7.042	Tyr93	Tyr 47,Ile 52, Ala 70, Leu 36
9.	*M. roxburghianus* Muell.	1-Hexyl-2 Nitrocyclohexane	-3.958	Tyr 56	Leu36,Trp60,Tyr64,Asp73,Thr75, Trp88,Ser129
10.		Hexadecanoic acid ethyl ester	-6.005	Tyr 56	Leu36,Tyr47,Ala50,Ile52,Tyr64,Asp73,Trp88
11.		Sulfurous acid,2-propyl tridecyl ester	-6.669	Trp-60, Tyr-56	Leu36,Gly38,Ala50,Tyr64,Thr75,Leu125,Ser129

DISCUSSION

From ancient times, medicinal plants and their phytoderivatives have been investigated for their inherent biomedical applications including QS inhibition by targeting bacterial virulence and biofilm formation. In the present study, two medicinal plants from Mizoram, India i.e. *A. acuminata* and *M. roxburghianus* Muell. were investigated for their ability to attenuate *P. aeruginosa* PAO1 virulence and biofilm formation amply supported by *in silico* analysis. In the present study, *C. violaceum* was used as a reporter strain to identify the efficacy of *A. acuminata* and *M. roxburghianus* Muell. in attenuating the production of violacein pigment. The methanolic extract of *A. acuminata* and ethanolic extract of *M. roxburghianus* Muell. significantly reduced the production of violacein as compared to control. The present results are in accordance with the earlier report suggesting the efficacy of medicinal plants in inhibiting violacein production **(Tiwari *et al*., 2017)**. Pyocyanin is a class of phenazine compound produced by *P. aeruginosa*, acts as one of the major virulence determinants and is responsible for ROS generation and acute cytotoxicity. A significant decrease in the production of pyocyanin was observed on treatment with sub-MIC of *A. acuminata* and *M. roxburghianus* which was comparatively better than earlier study (**Chong *et al*., 2011**). In addition to pyocyanin production, sub-MIC dose of *A. acuminata* and *M. roxburhianus* Muell. also significantly modulated the elastolytic and Staphylolytic activity thereby suggesting the efficacy of test plant in modulating host immune system during host infection **(Haripriyan *et al*.,2018)**.

During the biofilm formation, EPS plays crucial role in constituting the biofilm matrix and alginate, an important member of EPS family is responsible for maintaining the biofilm integrity **(Powell *et al*., 2018).** A significant reduction in EPS and alginate polysaccharides production was observed on treatment with plant extracts suggesting their role in disrupting biofilm dynamics and thereby enhancing the susceptibility of biofilm cells to the conventional antibiotics treatment **(Harimawan and Ting, 2016).** The biofilm disruption ability of crude plant extracts was further supported by CLSM analysis where the architecture of treated biofilm cells was significantly thinner as compared to thicker and highly compact biofilms in the untreated control.

The GC-MS analysis of the concentrated ethanol and methanol extract of the two plants resulted in the identification of an array of phytocompounds such as phytol, hexadecanoic acid, squalene, α- tocopherol, betulin etc. which have already been reported for a diverse range of pharmaceutical and biomedical applications. The promising anti QS and anti-biofilm activities of both the plant extracts is the result of synergistic activities of the phytochemicals present in the crude extracts thereby enhancing the activities. The presence of large quantity of phytol in both the crude extracts suggested their efficacy in attenuating *P. aeruginosa* PAO1 virulence and biofilm formation **(Pejin *et al*., 2015)**. The anti QS activity of plant extracts was validated by *in silico* analysis, which provide an insight into the QS inhibition by certain phytochemicals present in the crude extracts by competitive binding to LasR, transcriptional regulator for production of virulence phenotypes **(Kim *et al*., 2015)**.

From the preliminary anti QS and anti-biofilm activity, it was observed that both *A. acuminata* and *M. roxburghianus* Muell. extracts significantly altered the QS regulated virulence in *P. aeruginosa* PAO1. The presence of phytoconstituents such as phytol, squalene, betulin, hexadecanoic acid and their synergistic activities were mainly responsible for significant modulation of QS regulated virulence phenotypes and biofilm architecture. In addition, *in silico* molecular docking studies further corroborated the binding affinity of the phytoconstituents to LasR and thereby altering bacterial virulence in *P. aeruginosa* PAO1. The present study thus provides promising alternatives to develop anti-pathogenic agents against QS associated bacterial infections.

CONCLUSION

Our findings feature the importance of these medicinal plants as a rich source of phytocompounds for inhibiting QS. Therefore, further investigation and evaluation for these metabolites and their mechanisms are still required. This study can be regarded as an initiatory step to overcome the current problems associated with drug therapy and provide encourage research for possible use of plant metabolites as anti-QS agents.

Conflict of interest: The authors hereby declare no conflict of interest.

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