Methanolic Extract of *Plectranthus tenuiflorus* Attenuates Quorum Sensing Mediated Virulence and Biofilm Formation in *Pseudomonas aeruginosa* PAO1

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**Abstract**

*Pseudomonas aeruginosa* is an important opportunistic pathogen which causes bacterial keratitis, cystic fibrosis and other hospital acquired infections. Its ability to form biofilm provides resistance to conventional antibiotics and further corroborates the global health concerns. The antibiotic resistance by *P. aeruginosa* is due to highly complex cellular signaling system called quorum sensing (QS). As QS controls bacterial pathogenicity and plays a crucial role in biofilm formation, it is a promising alternative target to combat the bacterial virulence. The present study aims to determine the inhibitory activity of *Plectranthus tenuiflorus* extract on QS and biofilm development in *P. aeruginosa* PAO1. The crude plant extract inhibited the production of pyocyanin, elastase, protease and chitinase by 71.96 ± 1.82, 38.74 ± 1.29, 30.84 ± 1.20 and 44.75 ± 1.40 % respectively at sub-MIC concentration of 500 µg/ml. The production of biofilm aggravating phenotypes such as exopolysaccharides, alginate and rhamnolipid were also significantly reduced. The biofilm inhibition capability of *P. tenuiflorus* was further supported by light microscopic and confocal laser scanning microscopic analysis. The phytochemicals such as phytol and mosloflavone were identified from the crude extract using gas chromatography–mass spectrometry (GC-MS). The role of these phytochemicals in down regulation of QS in *P. aeruginosa* was further confirmed by *in silico* studies targeting transcriptional receptors, LasR and RhlR of the QS regulatory network. The *in vitro* and docking studies suggest the anti QS potential of *P. tenuiflorus* in combating the bacterial pathogenesis.

**Keywords**: *Plectranthus tenuiflorus*, Quorum sensing, Biofilm, GC-MS, *Pseudomonas aeruginosa*, Molecular docking.

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INRODUCTION

Quorum sensing (QS) is a cell density dependent signaling system in bacteria that supports the survival and virulence by coordinating the production of virulence factors. Pseudomonas aeruginosa is a highly pathogenic bacterium causing pulmonary infections in the patients suffering from cystic fibrosis. The chronic bacterial infections and associated health consequences by P. aeruginosa are due to highly evolved QS regulatory network. The QS phenomenon in P. aeruginosa comprises of highly recognized and intricately co-related cascade of four signaling systems such as LasI/R, RhlI/R, quinolone based PQS and integrated quorum sensing (IQS) systems. Among the four interconnected QS hierarchy, the Las and Rhl system constitute two autoinducers synthase and their cognate receptors. The signaling molecules (autoinducers) generated from their respective AHL synthases bind with cognate receptors and control the expression of pyocyanin, exotoxins, elastolytic and proteolytic enzymes. The QS regulatory network also involves in the formation, development and maturation of highly persistent biofilm matrix. The biofilm matrix is made of exopolysaccharides, rhamnolipids and alginates, which provide resistance to conventional antimicrobial therapy. As QS mediates expression of virulence factors and controls development of biofilm, it provides novel platform for the development of anti-infective therapy.

In this context, from last few decades extensive research is being carried out in exploiting novel therapeutic agents targeting the bacterial QS and biofilm formation. Among the different strategies used to disrupt bacterial QS network, plant derived phytochemicals with proven biological activities represent an extensive class of quorum sensing inhibitors (QSIs). India represents one of the most important hotspots of medicinal plants with biodiversity. North-East India forms an important stretch of hotspot of medicinal plants with widespread distribution and rich variety of medicinal plants which have not been explored yet, thereby providing ample avenues for biomedical and pharmaceutical applications. P. tenuiflorus is an important medicinal and ornamental shrub belongs to Lamiaceae family with ethnomedicinal importance. The folkloric use of P. tenuiflorus is well established as antiseptic, antioxidant, antimicrobial agent and for the treatment of nausea, abdominal disorders and respiratory infections. In the present study, the anti QS and anti-biofilm activity of P. tenuiflorus was evaluated against P. aeruginosa PAO1 followed by docking studies.

MATERIALS AND METHODS

Chemicals and reagents

For determination of enzymatic activity, azocasein, elastin congo red (ECR) and chitin azure were purchased from Sigma Aldrich, USA. For other biological assays, chemicals like methanol, orcinol, crystal violet and acridine orange, reagents such as hydrochloric acid (HCl), sulphuric acid (H₂SO₄), calcium chloride (CaCl₂), Tris-HCl and dimethyl sulfoxide (DMSO) and the media Luria Bertani broth (LB broth), and LB agar were procured from HiMedia laboratories, India.

Collection of Plants and Extract Preparation

The leaf samples of P. tenuiflorus were collected from Manipur, India. The collected samples were cleaned and shade dried. The dried plant materials were grounded into fine powder. The powdered sample (10 g) was extracted with methanol (100 ml) by continuous stirring for 48 h. After incubation, the solution was filtered, rotaevaporated and the obtained crude extract was stored for biological activities.

Maintenance of culture

The anti QS activity of P. tenuiflorus was evaluated against biomarker strain, Chromobacterium violaceum (MTCC 2656) and test microorganism, P. aeruginosa PAO1 (MTCC 2453). The test cultures were obtained from IMTECH, Chandigarh, India.

Screening of crude extracts for anti QS activity

The preliminary anti QS activity of P. tenuiflorus crude extract was tested against C. violaceum (biomarker strain) and P. aeruginosa PAO1 using standard agar well diffusion method. Briefly, overnight bacterial culture was spread on top of the agar plates using sterile cotton swabs. Wells of 8mm diameter were created and plant extract (500 µg/ml) was loaded. The zone of inhibition was measured after 24 h of incubation.
**Determination of Minimal inhibitory concentration (MIC)**

According to Clinical and Laboratory Standards Institute (CLSI, 2014), broth macrodilution method was used to determine the MIC of *P. tenuiflorus* extract against *P. aeruginosa* PAO1. All the biological activities were carried out at sub-MIC concentration\(^\text{10}\).

**Violacein inhibition assay**

Briefly, *C. violaceum* was incubated in presence of *P. tenuiflorus* extract at 30°C for 24 h. After incubation, insoluble violacein was precipitated by centrifugation. DMSO was added to the pellet to solubilize the violacein pigment. The cell debris was removed by recentrifugation and the absorbance of violacein containing supernatant was determined at 585 nm\(^\text{11}\).

**Inhibition of QS controlled virulence in *P. aeruginosa* PAO1**

The production of pyocyanin, elastase, protease and chitinase are the important virulence factors which are regulated by QS. The inhibitory effect of *P. tenuiflorus* methanolic extract on the production of these virulence factors was evaluated. For pyocyanin inhibition, *P. aeruginosa* PAO1 was incubated in presence of *P. tenuiflorus* extract at 30°C for 18 h. Pyocyanin pigment from cell-free supernatant was extracted using chloroform (5:3 ratio). The pyocyanin containing chloroform phase (blue organic phase) was re-extracted with 0.2 M HCl. The absorbance of the upper aqueous layer was determined at 520 nm\(^\text{12}\). The ability of *P. tenuiflorus* extract on Staphylolytic activity of *P. aeruginosa* PAO1 was examined as per the standard protocol\(^\text{13}\).

For elastolytic activity, bacterial cell-free supernatant was mixed with ECR buffer (100 mM Tris, 1 mM CaCl\(_2\), pH 7.5) and incubated at 37°C for 3 h. After incubation, insoluble ECR was separated and the absorbance of the supernatant was measured at 495 nm\(^\text{14}\). For LasA protease activity, the cell free supernatant of *P. tenuiflorus* treated PAO1 was mixed with 0.3% azocasein (50 mM Tris-HCl, pH 7.8) and incubated for 30 min. After incubation, undigested substrate was pelleted and the absorbance was determined at 400 nm\(^\text{15}\). For chitinase activity, *P. aeruginosa* PAO1 was added with sodium citrate buffer (0.1 M, pH 4.8) containing chitin azure (0.5 mg/ml). The reaction mixture was incubated for 7 days with continuous stirring condition (100 rpm). After incubation, the undigested chitin azure was removed and absorbance was measured at 570 nm\(^\text{16}\).

**Inhibition of bacterial motility**

In *P. aeruginosa* PAO1, motility plays a crucial role during biofilm formation. The inhibitory potential of *P. tenuiflorus* extract on swimming and swarming motility was evaluated. Overnight grown *P. aeruginosa* PAO1 treated with plant extract was point inoculated into swimming medium (agar 0.3%, tryptone 1%, NaCl 0.5%) and swarming medium (0.5% agar, 0.5% filter sterilized glucose, 1% peptone, 0.5% NaCl) and incubated at 37°C\(^\text{17}\).

**Inhibition of biofilm formation in *P. aeruginosa* PAO1**

The process of biofilm formation and development is regulated by QS network. The biofilm is composed of carbohydrate residues like exopolysaccharides, rhamnolipids and alginate which are responsible for antibiotic resistance. The effect of methanolic extract on biofilm formation was evaluated. For biofilm inhibition, crystal violet assay was performed with slight modification. Overnight grown *P. aeruginosa* PAO1 was incubated in the freshly prepared media on treatment with plant extract for 16-18 h. After incubation, planktonic cells containing media were removed and the biofilm containing microtiter plate was washed with PBS to remove excess of planktonic cells. The biofilm cells were then stained with crystal violet (0.4%) for 10 min. After incubation, the crystal violet bound biofilms were dissolved in 95% ethanol and the optical density was measured at 540 nm\(^\text{16}\). For EPS inhibition assay, *P. tenuiflorus* extract treated *P. aeruginosa* PAO1 was centrifuged and the resulting pellet was resuspended in high salt buffer. The solution was recentrifuged and to the supernatant, chilled ethanol was added and incubated overnight (4°C). The extracted EPS was then quantified by standard phenol-sulphuric acid method\(^\text{17}\). The effect of *P. tenuiflorus* extract on alginate production was evaluated as per the standard carbazole method. In brief, *P. aeruginosa* PAO1 culture treated with *P. tenuiflorus* extract was mixed with boric acid-sulphuric acid solution. The reagent mixture was vortexed followed by addition...
of carbazole solution (0.2%) and incubated at 55°C for 30 min. After the incubation, optical density was measured at 530 nm. For rhamnolipids inhibition, modified orcinol method was followed according to the method described by Banerjee et al. (2017).

Cell surface hydrophobicity is crucial for bacterial adhesion during biofilm formation. The effect of \( P. \ tenuiflorus \) extract on bacterial CSH was evaluated by microbial adhesion to hydrocarbon (MATH) assay. Briefly, overnight culture of \( P. \ aeruginosa \) PAO1 cultivated in the presence of crude plant extract was centrifuged at 12,000 rpm for 5 min. The pellet was washed three times with phosphate buffer and finally resuspended in ice-cold phosphate buffer. The absorbance of the resuspension was measured at 600 nm \( (A_0) \). To the resuspended mixture, toluene (an aromatic hydrocarbon) was added and vigorously vortexed. After vortexing, the reaction mixture was allowed for phase separation and the absorbance of the aqueous phase was determined \( (A_1) \) and hydrophobicity \( (\%) \) was calculated:

\[
\text{Hydrophobicity (\%) = } [1 - (A_1/A_0)] \times 100
\]

The effect of \( P. \ tenuiflorus \) extract on biofilm formation in \( P. \ aeruginosa \) PAO1 was evaluated by light and confocal laser scanning microscopic (CLSM) analysis. For microscopic studies, overnight \( P. \ aeruginosa \) PAO1 culture was inoculated into fresh growth medium containing cover glass of 1 × 1 cm in 24-well Microtiter plate along with crude plant extract and incubated for 16 h. After incubation, the cover glasses were rinsed with distilled water to remove the planktonic cells. For light microscopy, the adhered biofilms on the cover glasses were stained with 0.4% crystal violet and then visualised under a light microscope at a magnification of 40×. Meanwhile, for CLSM analysis, acridine orange was used to stain the biofilm and visualised under CLSM (Model LSM710, Carl Zeiss, Jena, Germany).

Gas Chromatography-Mass Spectrometry (GC-MS) analysis

The phytochemical profile of methanolic extract of \( P. \ tenuiflorus \) extract was identified by GC-MS analysis. The phytochemicals were separated in the column using Helium as carrier gas at a constant flow of 1 ml/min. The phytochemicals present in the extract were identified based on the obtained spectrum and retention time using GC-MS NIST (2008) library.

Molecular docking studies

The docking studies were carried out in Schrodinger Maestro software v.11.5 (Schrodinger, LLC, New York, NY, 2018) and the binding affinity of identified phytochemicals from GC-MS analysis and natural autoinducers to transcriptional receptors, LasR and RhlR were analyzed. The ligand binding domain of LasR protein’s 3D-structure file (PDB ID: 2UV0) was retrieved from Protein Data Bank. As there is no experimental protein 3D-structure available for RhlR, protein sequence was retrieved from Uniprot database (ID: P54292.1) and the 3D-structures were predicted using ROBETTA web server. The obtained protein structures were validated using RAMPAGE web server (http://mordred.bioc.cam.ac.uk/rampage.php) and the best model was selected for docking studies.

The LasR and RhlR proteins were subjected to preparation in protein preparation wizard of Maestro software v.11.5. Grid generation was performed in Glide, version 7.8 in Maestro software v.11.5, for LasR protein grid were defined around the active site residues (Tyr-56, Trp-60, Asp-73, Thr-75 and Ser-129) where autoinducer C12-homoserine lactone (C12-HSL) interacts with LasR protein whereas for RhlR, the grid was generated around the active site residue Trp-68. The above prepared grids were used for docking. The ligand compounds were obtained from PubChem database and submitted for preparation in LigPrep module in Maestro software v.11.5 and the prepared protein and ligand were subjected for docking. The 2D and 3D structures were generated using LigPlot+v.1.4.5 and Chimera v.1.6.2 respectively.

Statistical Analysis

All the experiments were performed in triplicates and the data was presented as mean ± standard deviation (SD). For each assay, a control experiment (without the treatment of crude extract) was performed. All the obtained results were calculated as compared to the control.
RESULTS

Screening of the plant extracts for anti QS activity

From the preliminary agar well diffusion assay, *P. tenuiflorus* extract exhibited zone of inhibition of 18 and 16 mm against biomarker strain, *C. violaceum* and test organism, *P. aeruginosa* PAO1 respectively at a concentration of 500 µg/ml.

**Determination of MIC**

The MIC of *P. tenuiflorus* extract against *P. aeruginosa* PAO1 was found to be 750 µg/ml and 500 µg/ml was selected as the sub-MIC.

**Violacein inhibition assay**

On treatment with *P. tenuiflorus* extract, the production of violacein in *C. violaceum* was significantly reduced by 80.23 ± 2.73% as compared to control.

**Inhibition of QS regulated virulence factors in *P. aeruginosa* PAO1**

The pyocyanin production was significantly inhibited by 71.96 ± 1.82% when treated with *P. tenuiflorus* extract (Figure 1). It also showed promising ability in inhibiting Staphyloolytic activity of *P. aeruginosa* PAO1 by 18.21 ± 2.01% (Figure 1). On treatment with plant extract, LasB elastase activity was inhibited by 38.74 ± 1.29%. A significant decrease in the LasA protease and chitinase activity was observed in *P. aeruginosa* PAO1 on treatment with *P. tenuiflorus* extract with an inhibition of 30.84 ± 1.20 and 44.75 ± 1.40% respectively (Figure 1).

**Inhibition of bacterial motility**

On treatment with *P. tenuiflorus* extract, a significant decrease in swimming and swarming motility of *P. aeruginosa* was observed with a reduction of 37.93 and 54.54% respectively (Figure 2).

**Inhibition of biofilm formation in *P. aeruginosa* PAO1**

From the crystal violet based biofilm inhibition activity, it was observed that the biofilm formation was reduced by 44.03±2.00% on treatment with *P. tenuiflorus* extract. A significant reduction (41.08 ± 2.67%) in the production of EPS was observed in *P. aeruginosa* PAO1 when treated with *P. tenuiflorus* extract (Table 1). Meanwhile, on treatment with sub-MIC dose of *P. tenuiflorus* extract, alginate production in *P. aeruginosa* PAO1 was significantly inhibited by 37.13 ± 3.93%. The rhamnolipid production was also significantly altered on treatment with *P. tenuiflorus* extract with an inhibition of 34.95 ± 2.49% as compared to untreated control. The treatment with the crude extract resulted in decrease of cell to cell surface attachment of *P. aeruginosa* PAO1 with a

![Fig. 1. Effect of sub-MIC dose (500 µg/ml) of *P. tenuiflorus* extract on production of QS regulated virulence factors such as pyocyanin, Staphyloolytic activity, LasB elastase, LasA protease and chitinase activity](image1)

![Fig. 2. Effect of sub-MIC dose (500 µg/ml) of *P. tenuiflorus* extract on the inhibition of bacterial motility (swimming and swarming) as compared to untreated control. (A) Swimming motility of untreated *P. aeruginosa* PAO1, (B) Swimming motility of *P. tenuiflorus* treated *P. aeruginosa* PAO1, (C) Swarming motility of untreated *P. aeruginosa* PAO1, (D) Swarming motility of *P. tenuiflorus* treated *P. aeruginosa* PAO1](image2)
Table 1. Effect of sub-MIC dose (500 µg/ml) of *P. tenuiflorus* extract on production of QS regulated biofilm phenotypes in *P. aeruginosa* PAO1

<table>
<thead>
<tr>
<th>S. No.</th>
<th>QS regulated biofilm phenotypes</th>
<th>Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Cell Surface Hydrophobicity</td>
<td>29.19 ± 3.29</td>
</tr>
<tr>
<td>2.</td>
<td>Rhamnolipid inhibition</td>
<td>34.95 ± 2.49</td>
</tr>
<tr>
<td>3.</td>
<td>Biofilm inhibition</td>
<td>44.03 ± 2.00</td>
</tr>
<tr>
<td>4.</td>
<td>EPS inhibition</td>
<td>41.08 ± 2.67</td>
</tr>
<tr>
<td>5.</td>
<td>Alginate inhibition</td>
<td>37.13 ± 3.93</td>
</tr>
</tbody>
</table>

Table 2. List of phytochemicals identified from methanolic extract of *P. tenuiflorus* using GC-MS analysis

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Phytochemicals</th>
<th>Retention time (min)</th>
<th>Molecular formula</th>
<th>Molecular weight</th>
<th>Peak area (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Beta-D-glucopyranose, 1,6-anhydro</td>
<td>13.463</td>
<td>C₆H₁₀O₅</td>
<td>162</td>
<td>3.212</td>
</tr>
<tr>
<td>2.</td>
<td>N-Hexadecanoic acid</td>
<td>18.400</td>
<td>C₁₆H₃₂O₂</td>
<td>256</td>
<td>23.731</td>
</tr>
<tr>
<td>3.</td>
<td>Phytol</td>
<td>19.765</td>
<td>C₂₀H₄₀O₂</td>
<td>296</td>
<td>4.324</td>
</tr>
<tr>
<td>4.</td>
<td>Octadecanoic acid</td>
<td>20.246</td>
<td>C₁₈H₃₆O₂</td>
<td>284</td>
<td>2.535</td>
</tr>
<tr>
<td>5.</td>
<td>Mosloflavone</td>
<td>25.433</td>
<td>C₁₇H₁₄O₅</td>
<td>298</td>
<td>12.526</td>
</tr>
<tr>
<td>6.</td>
<td>Gamma sitosterol</td>
<td>30.080</td>
<td>C₂₉H₄₀O₅</td>
<td>414</td>
<td>4.502</td>
</tr>
</tbody>
</table>
### Table 3. Interaction of bioactive constituents of *P. tenuiflorus* extract with QS regulators, LasR and RhlR of *P. aeruginosa* PAO1 expressed in terms of molecular docking energy (kcal/mol), hydrogen bonds and interacting hydrophobic residues

<table>
<thead>
<tr>
<th>Compounds/Ligands</th>
<th>Docking score Kcal/mol</th>
<th>Hydrogen bond</th>
<th>Hydrophobic interactions</th>
<th>Docking score Kcal/mol</th>
<th>Hydrogen bond</th>
<th>Hydrophobic interactions</th>
</tr>
</thead>
<tbody>
<tr>
<td>C12-HSL (Natural ligand for LasR)</td>
<td>-8.489</td>
<td></td>
<td>Leu 36, Leu 40, Try 47, Arg 61, Val 70, Val 76, Trp 88, Tyr 93, Ala 105, Phe 101, Leu 110,</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C4-HSL (Natural ligand for RhlR)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Beta.-D-glucopyranose, 1,6-anhydro</td>
<td>-7.035</td>
<td></td>
<td>Tyr 64, Asp 73, Phe 101, Leu 110 and Thr 115</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mosloflavone</td>
<td>-7.555</td>
<td>Arg 61</td>
<td>Leu 36, Gly 38, Leu 39, Tyr 64, Asp 73, Thr 75, Phe 101, and Ser 129</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N-hexadecanoic acid</td>
<td>-3.206</td>
<td>Tyr 93</td>
<td>Leu 36, Leu 39, Leu 40, Tyr 56, Trp 60, Ala 70, Trp 88</td>
<td>-</td>
<td>His 61</td>
<td></td>
</tr>
<tr>
<td>Octadecanoic acid</td>
<td>-3.563</td>
<td>Leu 110</td>
<td>Leu 36, Leu 40, Ala 50, Ile 52, Tyr 56, Trp 60, Tyr 64, Pro 74, Asp 73</td>
<td>-</td>
<td>His 61</td>
<td></td>
</tr>
</tbody>
</table>
hydrophobicity percentage of 29.19 ± 3.29% (Table 1). From light microscopic and CLSM analysis, a significant reduction in biofilm formation was observed in presence of *P. tenuiflorus* extract with dispersed bacterial cells, compared to thick and compact biofilm in untreated control (Figure 3).

**GC-MS analysis**

The phytochemical components identified from the methanol extract of *P. tenuiflorus* using GC-MS analysis were phytol, mosloflavone, N-hexadecanoic acid, Beta-D-glucopyranose, 1,6-anhydro and gamma sitosterol along with other minor phytoconstituents (Figure 4, Table 2).

**Molecular docking studies**

Molecular docking of *P. tenuiflorus* revealed that, mosloflavone exhibited a docking score of -7.55 and -6.521 kcal/mol for LasR and RhlR respectively. The binding affinity of mosloflavone for RhlR was observed to be comparatively higher than its natural ligand suggesting the molecular target of mosloflavone (Table 3, Figure 5). Apart from mosloflavone, Beta-D-glucopyranose, 1,6-anhydro also showed greater affinity towards RhlR with a docking score of -7.499 kcal/mol as compared to -5.756 kcal/mol in case of natural ligand, C4-HSL (Figure 6).

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**Fig. 5.** Molecular docking studies of mosloflavone from crude extract of *P. tenuiflorus* to study the binding affinity with the active site of the receptor protein, LasR compared to the natural ligand (C12-HSL). (A) 2D docked conformation of C12-HSL (natural ligand) into the active site of LasR; (B) 3D docked conformation of C12-HSL into the active site of LasR, (C) 2D docked conformation of mosloflavone into the active site of LasR, (D) 3D docked conformation of mosloflavone into the active site of LasR.
DISCUSSION

Traditional medicinal plants are known for their ethnomedicinal value from several decades as the most affordable and easily accessible folkloric medicines for the treatment of various kinds of diseases including microbial infections. India represents a rich heritage of medicinal plants with enormous ethnomedicinal properties of which majority of medicinal plants remain unnoticed and unexplored. The methanolic extract of *P. tenuiflorus* was evaluated for its anti-QS and anti-biofilm activity against *P. aeruginosa* PAO1. The plant extract significantly attenuated virulence factors production and biofilm formation regulated by QS regulatory network in *P. aeruginosa* with different potency. The production of violacein pigment, an important virulence factor of biomarker strain, *C. violaceum* is regulated by QS hierarchy. The methanolic extract of *P. tenuiflorus* was evaluated for its anti-QS and anti-biofilm activity against *P. aeruginosa* PAO1. The plant extract significantly attenuated virulence factors production and biofilm formation regulated by QS regulatory network in *P. aeruginosa* with different potency. The production of violacein pigment, an important virulence factor of biomarker strain, *C. violaceum* is regulated by QS hierarchy. The methanolic extract of *P. tenuiflorus* significantly inhibited the production of violacein suggesting the efficacy in combating bacterial virulence. Pyocyanin is an important member of the phenazine compounds produced by *P. aeruginosa* and represents a major virulence determinant by generating reactive oxygen species (ROS). In the present study, *P. tenuiflorus* inhibited the pyocyanin production by 71.96%. The synergistic activity of phytochemicals like phytol and mosloflavone present in the crude extract could be responsible for pyocyanin inhibition. The production of lytic enzymes such as LasA protease and LasB elastase by *P. aeruginosa* gives an aided advantage to the bacteria during host infection by enhancing the ability to degrade the host tissues. In the present study, LasA protease and LasB elastase activity were significantly altered on treatment with *P. tenuiflorus* extract. These results suggested the ability of crude extract in impaire the ability of *P. aeruginosa* PAO1 to invade and degrade host tissues during infection. Chitinase is also an extracellular enzyme produced by *P. aeruginosa* and plays a crucial role during host invasion and survival within the host. *P. tenuiflorus* extract significantly inhibited the chitinase activity of *P. aeruginosa* PAO1.

In addition to virulence factors production, the QS regulatory network also controls the formation, development and maturation of biofilm matrix, which is mainly responsible for increased...
antibiotic resistance. The effect of crude extract on biofilm formation was quantified by crystal violet staining depicting the reduction in biofilm formation. This result was in accordance with the earlier report. EPS and alginites not only provide assistance during bacterial adhesion but also shield the bacterial cells from oxidative stress, host immunity and antimicrobial treatment. The production of EPS and alginate was inhibited when treated with P. tenuiflorus extract suggesting its role in disrupting the biofilm matrix. The production of biosurfactant, rhamnolipid plays a critical role in different stages of biofilm formation. In this context, combating rhamnolipid production could be a promising target for biofilm inhibition. The rhamnolipid production was significantly altered on treatment with P. tenuiflorus extract suggesting its ability to inhibit biofilm formation. The bacterial motility plays an important role in the bacterial pathogenicity and biofilm formation. The swimming and swarming motility of P. aeruginosa PAO1 were significantly inhibited when treated with the plant extract suggesting its efficacy in interrupting the process of biofilm formation. These results were in accordance to the earlier report. The in vitro anti-biofilm efficacy of crude plant extract was further corroborated by microscopic analysis depicting the reduction in the biofilm architecture as compared to compact biofilm architecture in the untreated control.

The presence of phytochemical in the methanolic extract of P. tenuiflorus, as identified from GC-MS analysis suggested the efficacy of crude extract in combating QS regulated virulence and biofilm formation in P. aeruginosa PAO1. The in vitro results were further validated using molecular docking studies of the identified phytochemicals for their affinity towards LasR and RhlR as compared to their respective autoinducers. Among the identified phytochemicals, mosloflavone and Beta-D-glucopyranose, 1,6-anhydro exhibited promising affinity towards LasR and RhlR respectively suggesting their role in inhibiting bacterial QS system in P. aeruginosa PAO1. The promising anti QS and anti biofilm activity exhibited by P. tenuiflorus extract was the result of synergistic activity of different phytochemicals such as phytol, mosloflavone and Beta-D-glucopyranose, 1,6-anhydro present in the extract. The in vitro results were further validated by molecular docking studies depicting the binding ability of phytochemicals to LasR and RhlR of QS regulatory network. The present study will provide a lead in the antimicrobial drug discovery for the development of anti-infectives by targeting the QS regulated bacterial virulence and biofilm formation.

CONFLICT OF INTEREST
The authors declare no conflict of interest.

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