Applied Biochemistry and Biotechnology (2022) 194:434–444
https://doi.org/10.1007/s12010-021-03683-w

ORIGINAL ARTICLE

Quorum Sensing Inhibitory Potential and Molecular Docking Studies of *Phyllanthus emblica* Phytochemicals Against *Pseudomonas aeruginosa*

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Received: 27 June 2021 / Accepted: 8 September 2021 /
Published online: 6 October 2021

Abstract
Phyllanthus emblica is a traditional medicinal plant that is endowed with curative properties including anti-bacterial, anti-fungal, anti-viral, and analgesic properties. Bacteria make use of cell–cell signaling system known as quorum sensing (QS) and respond to their own population. In most gram-negative bacteria, the transcriptional regulators belonging to the Lux R protein play a crucial role in the QS mechanism by detecting the presence of signaling molecules known as N-acyl homoserine lactones (AHLs). In this present work, the anti-quorum sensing activity of *Phyllanthus emblica* was evaluated against *Pseudomonas aeruginosa*. Anti-quorum sensing efficacy of *Phyllanthus emblica* was estimated with reference to QS bio-monitoring strain *Chromobacterium violaceum*. The binding efficacy of the phytochemicals of *Phyllanthus emblica* against CviR protein from *Chromobacterium violaceum* and LasR protein from *Phyllanthus emblica* were studied.

Keywords Quorum sensing · *Phyllanthus emblica* · *Pseudomonas aeruginosa* · Molecular docking

Introduction

Globally, in the last few decades, the emergence and widespread of antimicrobial-resistant, antimicrobial drug-resistant strains of *Pseudomonas* spp. and *Staphylococcus* spp. become the alarming situation of greater public health concern [1–3]. *P. aeruginosa* is mainly

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responsible for postoperative wound infections and is more prevalent in most of the hospital-acquired infections [4–6].

In general, antibiotics are used to control these microbial infections by inhibiting their growth. However, the continuous usage and misuse of antibiotic therapy led to the emergence of multi-drug resistant strains to the tolerance against a broad spectrum of available antibiotics [7]. The development of these multiple drug-resistant bacteria has forced the scientists to search for new antibacterial agents that have become the main concern [8]. Though the search for new antimicrobial substances has resulted in novel antimicrobial chemotherapeutic agents as synthetic drugs from various sources, the higher cost production and its adverse effects have limited its usages when compared to plant-derived drugs [9–11]. Thus the search for novel anti-pathogenic agents has increased the focus on the potential compounds from plant sources that are widespread across the globe. The increase in the search for therapeutic compounds from plants is based on a fact that plants continue to survive with high bacterial density in an environment and might possess protective means against infections. Thus in recent years, the extracts from plants and the knowledge of medicinal plants have gained the attentions of many pharmaceutical industries [12–14].

Cell–cell signaling systems known as QS are used by bacteria to communicate with each other and respond to their own population. In gram-negative bacteria, the LuxR, a transcriptional regulator protein, plays a central role in the QS mechanism to detect AHLs as signalling molecules [15, 16].

In this work, the anti-quorum sensing activity of *P. emblica* was evaluated against *P. aeruginosa*. Anti-quorum sensing efficacy of *P. emblica* was estimated with reference to QS bio-monitoring strain *C. violaceum*. The binding efficacy of the phytochemicals against CviR Protein from *C. violaceum* and LasR protein from *P. aeruginosa* were studied.

Methodology

The 3D models of *P. aeruginosa* LasR (PDB ID: 2UV0) and CviR from *C. violaceum* (PDB ID: 3QP5) were retrieved from the PDB database and the conserved residues were determined with other LuxR family protein by using ClustalW at the EBI (European Bioinformatics Institute) server [17].

Ligands

The principle compounds of *P. emblica* were retrieved from Duke Ethnobotanical database, and their respective structures were obtained from PubChem database. The structures were retrieved in SDF (Structure Data File) format.

Docking Studies

The retrieved compounds in SDF file format from PubChem database were docked with the amino acids in the binding site of CviR and LasR using the default parameters. The interactions of principle compounds with LasR in the docked complex were analyzed by the pose-view of LeadIT [18]. Pose-view tools [19] were used to study the interactions of compounds with CviR and LasR in the docked complex.

Results

The 3D models of *P. aeruginosa* LasR (PDB ID: 2UV0) and CviR from *C. violaceum* (PDB ID: 3QP5) retrieved from PDB database were shown in Fig. 1. The homologies between the proteins belonging to the LuxR family (quorum sensing enhanced transcriptional regulators) were analyzed by multiple sequence alignment (Fig. 2). The binding site was determined by using the co-crystallized structures.

Docking

The Docking program FlexX, from LeadIT, was used to dock *P. emblica* compounds with the binding pocket of the LasR, CviR, and the developed model, LasR. The docking was carried out with a radius of 6.5 A^0 at the site of docking.

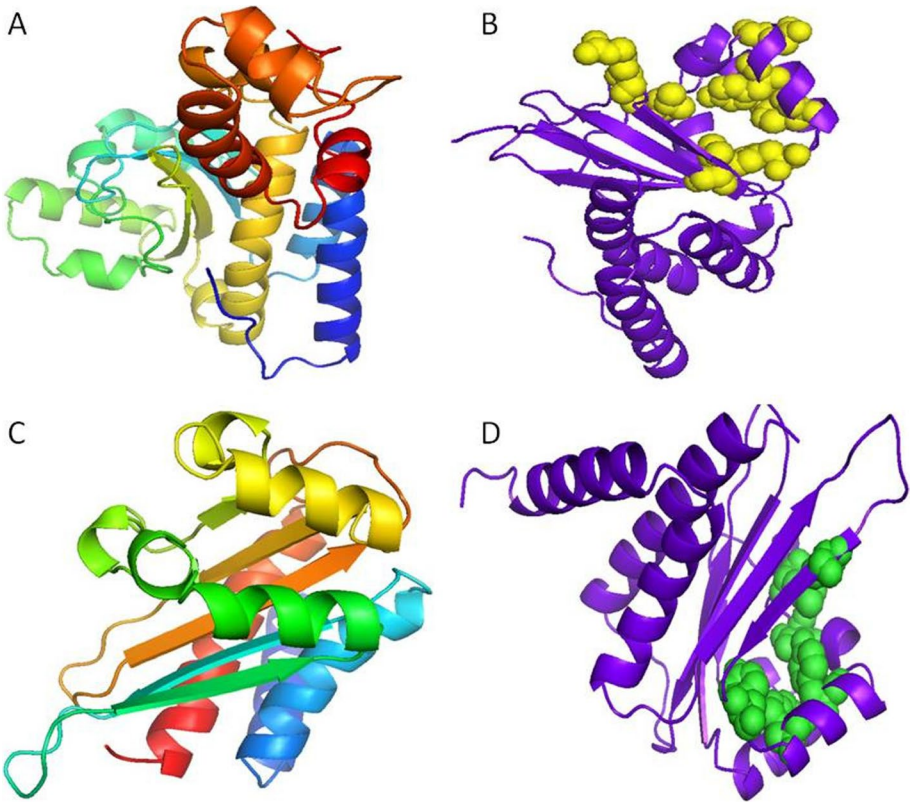


Fig. 1 3D structure of quorum sensing transcriptional activators shown in cartoons representation and the active site is highlighted as spheres. **A** CviR protein from *C. violaceum*, **B** Active site of CviR protein from *C. violaceum*. **C** LasR protein from *P. aeruginosa*. **D** Active site of LasR protein from *P. aeruginosa*

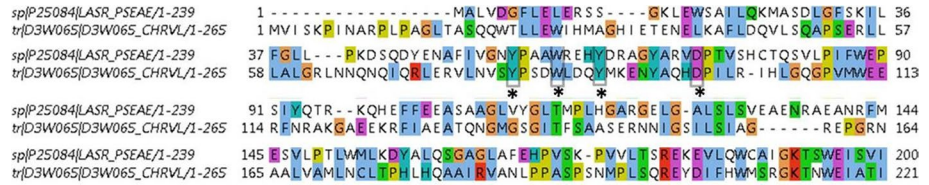


Fig. 2 The pair-wise sequence alignment between *P. aeruginosa* and *C. violaceum*. The conserved regions were shown in clustal X color format, and the conserved active site is highlighted with rectangle box and also marked with asterisk (*)

Docking Analysis

The interactions between the binding site residues of CviR and the modeled protein LasR with the compounds as ligand molecules in the docked complexes were given in Table 1. A keen observation of these interacting residues of the LuxR family proteins, the modeled LasR, and the ligand molecules revealed the most important functional groups of the ligand molecules and the amino acids LuxR family proteins favoring the interactions (Table 2). The best-docked ligand molecules and their interactions with the amino acids in the active site of CviR and the modeled protein LasR are given in Figs. 3 and 4.

Discussion

The LuxI homologs in most of the gram-negative bacteria generate the signal molecules, AHL. Usually, these signals were detected by the LuxR homologs present in them, whereas in *P. aeruginosa*, the LuxI homolog is not been found, which makes the organism not to generate the signals of their own. Hence these bacteria cannot sense the signals from the same species. Instead, it responds to the signals produced by the other pathogenic bacteria. However, it encodes a LuxR homolog, LasR which can sense the signal molecules produced by the mixed community genera [20, 21]. Thus LasR a transcriptional regulator was considered as a potential drug target.

The 3D structure of the target protein LasR from *P. aeruginosa* was not available in any of the structural database; it was developed by using the homology modeling method. The most homologous sequence in the Protein Data Bank was searched by using the BLASTP program. The BLASTP results showed that the *P. aeruginosa* transcriptional regulator LasR is homologous with the structure CviR, LuxR- type transcriptional factor from *C. violaceum* (PDB ID: 3QP5) over 40%. As all these sequences belong to the same family, the structure of 3QP5 was considered as a template structure for comparative modeling. The model was generated by using Swiss model webserver.

The multiple sequence alignment (Fig. 2) of LuxR family proteins LasR from *P. aeruginosa*, CviR from *C. violaceum*, and LasR from *Escherichia coli*, *P. aeruginosa*, and *Enterobacter aerogenes* showed that amino acids are conserved in LuxR family proteins. These alignments enlighten that LasR from *P. aeruginosa* is almost conserved. Hence, the structure of LasR from *P. aeruginosa* was considered for further docking studies.

Table 1 Docking interactions of plant compounds with the active site amino acids of CviR from *C. violaceum* and their binding scores

S. No	Compound ID	Type of interaction with active site residues		Docking score (kJ/mol)
		Bonded	Non-bonded	
1	57124935	Asp86, Trp84	Trp84, Asp86, Leu85	− 2.8686
2	6437979	-	Pro98, Asp97, Trp84, Tyr88	− 0.4569
3	5481240	Trp84, Asp97	Trp84, Ile99, Asp97, Leu100, Tyr88	− 8.2647
4	5366074	Trp84, Leu85, Asp86	Asp86, Leu85	− 8.3853
5	5281126	Trp84, Leu85, Asp86	Leu85, Tyr88, Trp84, Leu100	− 0.0456
6	5280934	Leu85, Trp84, Asp86	Trp84, Asp86, Tyr88, Leu85	− 1.4330
7	5280442	Gln87, Asp97	Gln87, Tyr88, Trp84, Leu100, Asp97	− 9.7346
8	641785	Asp97, Tyr88	Asp97, Gln87, Tyr88, Leu100	− 12.1467
9	444539	Trp84, Leu85, Asp86	Trp84, Leu85	− 11.5130
10	348962	-	Asp97, Trp84, Tyr88, Pro98	− 4.5239
11	301798	Asp97, Gln87	Gln87, Tyr88, Pro98, Trp84, Asp97	− 7.7922
12	10465	Asp86, Trp84, Leu85	Leu85, Tyr88, Asp86	− 4.4680
13	10416	Trp84, Asp86, Leu85	Leu85, Asp86	− 3.4816
14	10212	Trp84	Trp84, Asp97, Tyr88, Ile99	− 6.9059
15	1135	Trp84, Leu85, Asp86	Leu85	− 8.0549
16	985	Asp86, Leu85, Trp84	Leu85, Asp86, Tyr88	− 3.1656
17	323	Asp86, Trp84, Leu85	Trp84, Leu85	− 8.6772
Reference ligand 3-oxo-C6-HSL		Asp 97, Trp 84	Tyr 88, Trp 84, Asp 97	− 8.3776

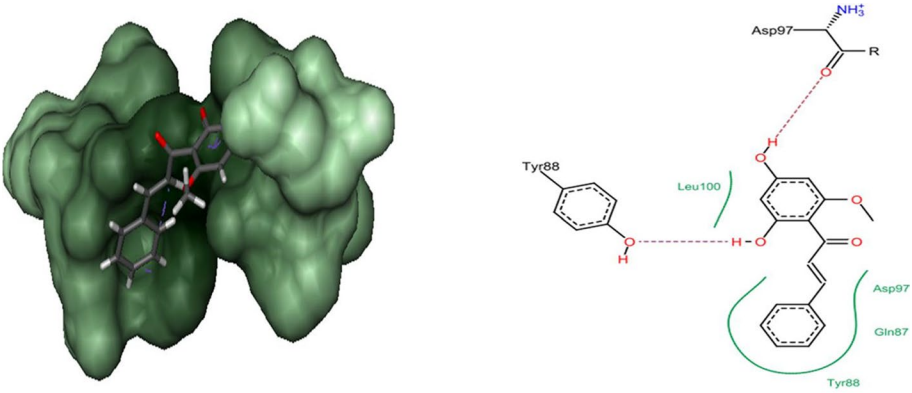
Table 2 Docking interactions of plant compounds with the active site amino acids of LasR from *P. aeruginosa* and their binding scores

S. No	Compound ID	Type of interaction with active site residues		Docking score (kJ/mol)
		Bonded	Non-bonded	
1	57124935	Trp67	Tyr71, Val82, Asp80, Tyr63, Trp67	−2.6933
2	6437979	Asp80	Tyr63, Asp80, Trp67, Tyr71	−1.2080
3	5481240	Asp80, Trp67	Trp67, Tyr63, Tyr71, Val82, Asp80	−13.5553
4	5366074	Trp67	Trp67, Val82, Tyr71, Tyr63	−7.9594
5	5281126	Trp67	Trp67, Tyr63, Tyr70	−4.1555
6	5280934	Trp67	Tyr70, Asp80, Tyr71, Trp67	−5.1023
7	5280442	Trp67	Trp67, Val82, Tyr63, Tyr71	−12.9562
8	641785	Trp67, Asp80	Asp80, Val82, Trp67, Tyr71, Tyr63	−14.8740
9	444539	Asp80	Tyr71, Tyr70, Trp67	−9.3555
10	348962	Trp67	Tyr63, Tyr71, Val82, Trp67, Asp80	−9.6899
11	301798	Asp80	Tyr63, Tyr71, Trp67	−9.9831
12	10465	Trp67	Tyr63, Tyr70, Trp67	−7.7286
13	10416	Trp67	Tyr71, Asp80, Tyr70, Trp67	−7.3933
14	10212	Trp67	Tyr71, Val82, Tyr63, Trp67	−13.2575
15	1135	Asp80, Tyr63	Trp67	−8.3390
16	985	-	Tyr63, Tyr71, Val82, Trp67	−8.1672
17	323	Trp67	Trp67, Tyr71, Val82, Tyr63	−9.7149
Reference ligand 3-oxo-octanoic acid		Trp 67, Asp 80	Tyr 71, Tyr 63, Trp 67, Asp 80	−8.3989

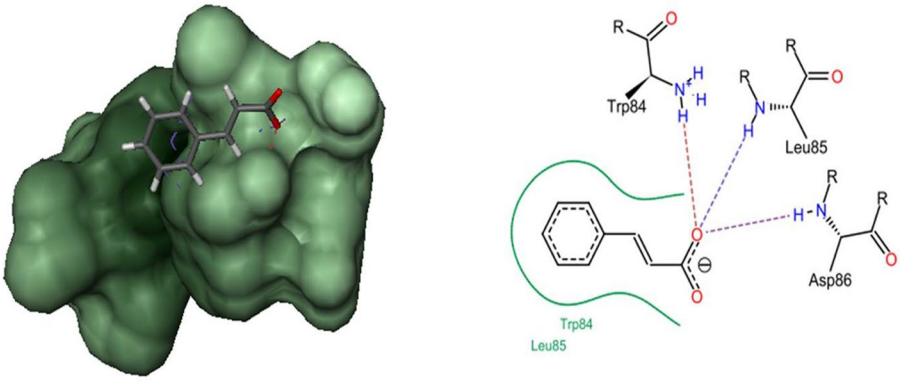
Docking Studies

A total of 19 compounds were found as the principle compounds of the *P. emblica*. The 3D structures of these compounds were retrieved as SD files from the PubChem database and were docked with the amino acids in the binding site of CviR from *C. violaceum* and LasR from *P. aeruginosa* by using FlexX. Out of these 19 compounds, 17 compounds formed docking complex with all both CviR and LasR, and its binding energies were analyzed by LeadIT (Table 1 and 2). Considering the binding energy score, the 3 best-docked compounds for each protein CviR and LasR were selected (Figs. 3 and 4), and their docking interaction with the active site residues was analyzed by using the pose view of LeadIT.

The binding interactions in the docking studies of *C. violaceum* CviR and *P. aeruginosa* LasR with the 3 best-docked compounds of the *P. emblica* exposed the similar binding of AHL residues that are responsible for quorum sensing activity. This result indicates that in *C. violaceum* CviR, it is found that tryptophan (Trp84) and aspartic acid (Asp86 & Asp97) plays a crucial role in exhibiting stronger interactions with ligands and these interactions were further supported by means of hydrophobic interactions by the contribution of tyrosine (Try88). Similarly in *P. aeruginosa* LasR, it is observed that tryptophan (Trp67) and



Docking complex and interaction of CID_641785



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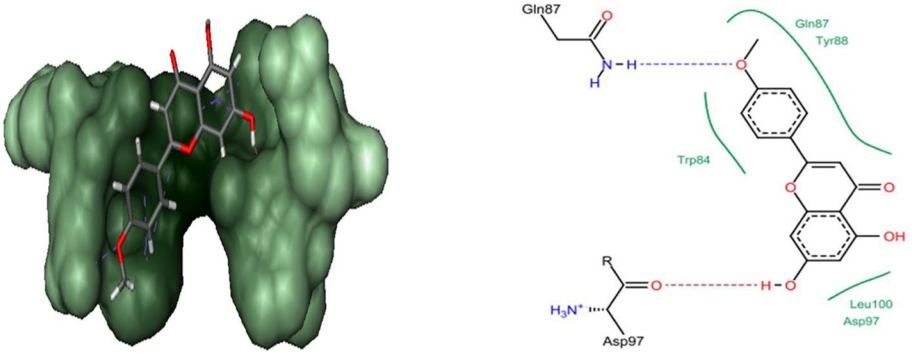
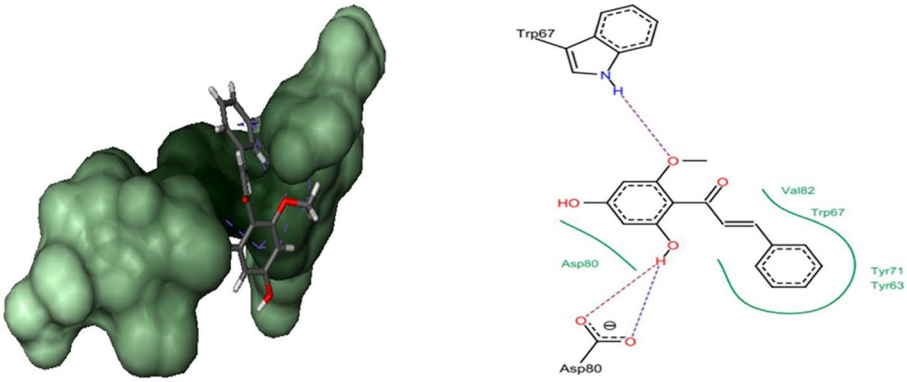
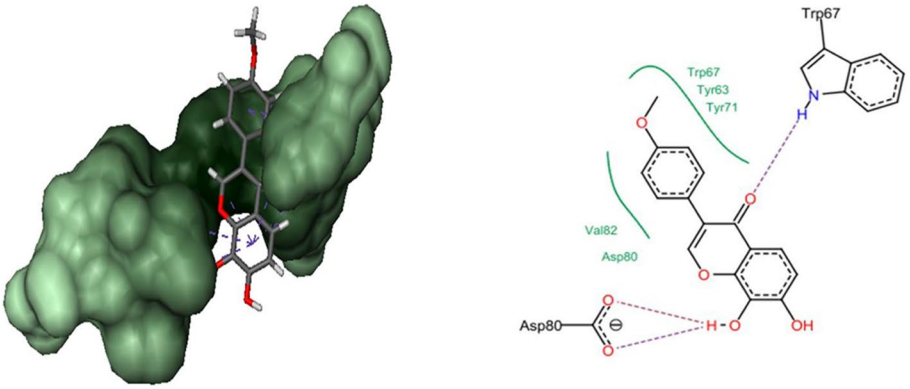


Docking complex and interaction of CID_5280442

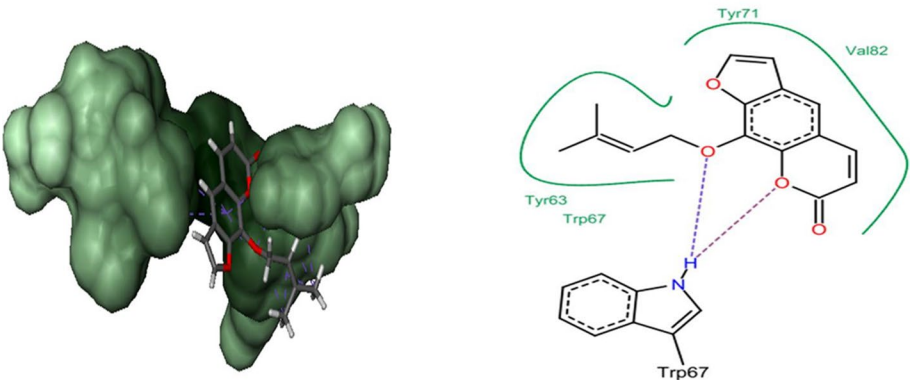
Fig. 3 Docking complex and the interactions of the best three compounds with the active site amino acids of CviR from *C. Violaceum*



Docking complex and interaction of CID_641785



Docking complex and interaction of CID_5481240



Docking complex and interaction of CID_10212

Fig. 4 Docking complex and the interactions of the best three compounds with the active site amino acids of LasR from *P. aeruginosa*

aspartic acid (Asp80) are responsible for the bonded interactions with the ligands, and the non-bonded interaction, hydrophobic is facilitated by tyrosine (Tyr 71 and Tyr 63).

The compounds CID_641785 (cardamonin), CID_444539 (cinnamic acid), and CID_5280442 (acacetin) exhibited the best docking scoring of − 12.1467 kJ/mol, − 11.5130 kJ/mol, and − 9.7346 kJ/mol, respectively, within the active site of CviR transcriptional regulator from *C. violaceum.* It is observed that natural ligand 3-oxo-C6-HSL exhibited the docking score of − 8.3776 kJ/mol. Thus among the docked compounds, it is revealed that the compound of all the three compounds CID_641785, CID_444539, and CID_5280442 is having the highest docking score when compared to that of the natural ligand. Thus these compounds can be used to inhibit the quorum sensing mechanism in *C. violaceum.*

The compounds CID_641785 (cardamonin), CID_5481240 (retusin), and CID_10212 (imperatorin) exhibited the best docking scoring of − 14.8740 kJ/mol, − 13.5553 kJ/mol, and − 13.2575 kJ/mol, respectively, within the active site of LasR transcriptional regulator from *P. aeruginosa.* It is observed that natural ligand 3-oxo-octanoic acid exhibited a docking score of − 8.3989 kJ/mol. Thus among the docked compounds, it is revealed that the compound CID_641785 is having the highest docking score when compared to that of the natural ligand. Thus this compound can be used to inhibit the quorum sensing mechanism in *P. aeruginosa.*

In a similar study, Mellini et al. [22] performed virtual screening and drug repurposing methodologies for identifying novel QS inhibitors that target *P. aeruginosa* pqs QS system. Shaker et al. [23] used a rational drug design technique to suppress *P. aeruginosa* quorum signaling system by creating effective inhibitory lead compounds against the pqsA gene's anthranilate-CoA ligase enzyme. In other study, Zhong et al. [24] identified and tested quorum sensing inhibitors (QSIs) derived from plant-based natural compounds against *P. aeruginosa*. In vitro investigations demonstrated that catechin-7-xyloside (C7X), sappanol, and butein can interact with LasR, a *P. aeruginosa* LuxR-type quorum sensing regulator.

The overall docking results of principle compounds with CviR and LasR proteins disclose the importance of the interacting amino acids tryptophan, aspartic acid, and tyrosine (Y71). The docking studies revealed the necessary crucial hydrogen bond interactions with the critical amino acids and that of the compound cardamonin (CID_641785) from *P. emblica*, with the highest binding score and might have a better inhibition activity against the quorum sensing regulation of *P. aeruginosa*.

Conclusion

P. aeruginosa, an opportunistic pathogenic bacterium causing nosocomial infections, has quickly become resistant to standard antibiotics. The ability of antibiotics resistance is due to the effective communication among the bacterial cell. This communication is enhanced by transcriptional regulators belonging to LuxR protein that plays a crucial role in the QS mechanism by detecting the presence of signaling molecules known as AHLs and regulates the pathogenicity. *P. aeruginosa* harbors a transcriptional regulator LasR (suppressor of cell division inhibition) that can recognize the AHLs to enhance the pathogenicity. Hence, LasR from *P. aeruginosa* is considered as a valid drug target. Thus in the present study, the anti-quorum sensing activity of *P. emblica* was evaluated against *P. aeruginosa*. Anti-quorum sensing efficacy of *P. emblica* was estimated with reference to QS bio-monitoring strain *Chromobacterium violaceum*. The binding

efficacy of the phytochemicals of *P. emblica* was docked with the LasR from *P. aeruginosa* and also with CviR protein from *C. violaceum*. This work discloses that amino acids tryptophan, aspartic acid, and tyrosine (Y71) were important for the interactions. The docking studies also revealed the necessary crucial hydrogen bond interactions with the critical amino acids and that of the compound cardamonin (CID_641785) with the highest binding score might be an effective inhibitor of *P. aeruginosa* pathogenesis.

Acknowledgements The authors are thankful to Tamilnadu State Council for Science & Technology (TNSCST), DOTE Campus, Chennai for the financial support and DST–FIST Centralized laboratory Muthayammal College of Arts & Science, Rasipuram, Namakkal DT, Tamil Nadu, India for providing the facilities to execute this research work.

Author Contribution Sharmila Baburam: executed the research work.
Gnanendra Shanmugam and Srinivasan Ramasamy: performed the methodology.
Maghimaa Mathanmohun: supervised the research work, and corrected the draft.

Funding This study was financially supported by the Tamilnadu State Council for Science & Technology (TNSCST), DOTE Campus, Chennai (Science & Technology Project: TNSCST/STP-PRG/AR/2018–2019).

Data availability Not applicable.

Code Availability Not applicable.

Declarations

Ethics Approval Not applicable.

Consent to Participate Not applicable.

Consent for Publication Not applicable.

Conflict of Interest The authors declare no competing interests.

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