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Research Article

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In silico Identification of Polyphenolic Compounds from the Grape fruit as Quorum sensing inhibitors

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ABSTRACT

Quorum sensing (QS) is the well adapted cell to cell communication system present mainly in all the pathogenic bacterial species. The mechanism of QS is population-density-dependent and the system expresses at particular threshold signal. This system regulates the production of N*-acylated l-homoserine lactones (AHLs) as autoinducers (AIs) which mediate the QS signalling pathway. QS activity is responsible for the production of virulence factors, formation of bacterial biofilm and directly associated with the development of drug resistance. Phenolic compounds from the ginger rhizome (Zingiber officinale Roscoe) viz. [6]-gingerol, [6]-shogaol and isoxazoline derivative of [6]-gingerol exhibited QS inhibitory activity against Chromobacterium violaceum and Pseudomonas aeruginosa and thus found to be the promising leads in the domain of anti pathogenic drugs. In this work we have focussed our attention on the identification of mode of binding of phenolic compounds (those showing anti QS activity) of ginger rhizome in the active site pockets of CviR and LasR receptor protein. Based on this template, molecular docking of analysis of polyphenolic compounds (stilbenes, flavonols, flavan-3-ols) which are abundantly present in Vitis vinifera (common grape vine) was carried out. Out of 9 studied bioactives majorly all of them were found to be effectively stabilizing the domain of LasR receptor protein and binding with greater affinity (-6.8 to -11.4 kcal/mol) in comparison to natural ligand. The best binding affinity was shown by quercetin and myricetin which belongs to flavonols. However, in general polyphenolic compounds have shown less binding affinity against CviR receptor protein. Further, molecular electrostatic surface potential (MESP) of the investigated compounds have shown that polyphenols carry structural complementary features which are responsible for the binding interaction with the target proteins. Present study illustrated the potential of polyphenolic compounds present in Vitis vinifera to act as prospective leads for the further development of novel QS inhibitors as antimicrobial therapeutics.*

Key words: Molecular docking studies, CviR and LasR receptor, Quorum sensing inhibitors, MESP studies

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INTRODUCTION

Quorum sensing (QS) is the mechanism responsible for the cell to cell communication in the bacterial species which is mediated by the release of small molecule called autoinducers. These autoinducers belongs to *N*-acyl homoserine lactone chemical class and responsible for the production of variety of biochemical processes like bioluminescence, virulence expression and biofilm formation.[1] The structural representation of few autoinducers was shown in Figure 1. This phenomenon is mainly responsible for the pathogenesis of bacterial species and thus inhibiting this process is highly demandable. Developing quorum sensing inhibitors proves out a milestone in the field of adjuvant therapy for antimicrobial treatment.[2, 3] Several chemical classes were identified as quorum sensing inhibitors (QSI) but none out of them have seen the face of the clinic owing to their toxic effects in experimental model.[4, 5]

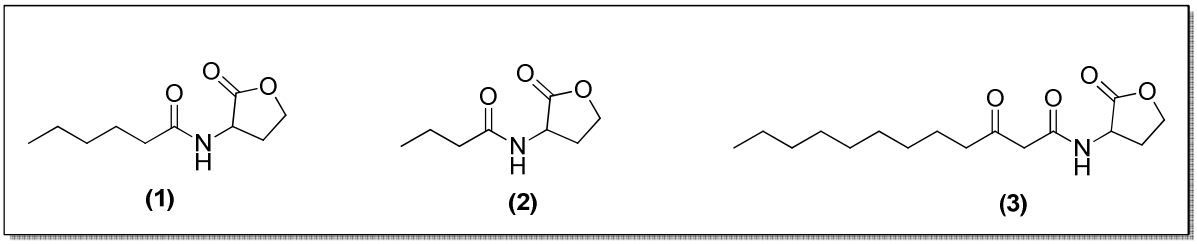


Figure 1. Representative structure of a few acyl homoserine lactone based autoinducres

Quorum sensing in *P. aeruginosa* and *C. violaceum* is well established and also exploited well for finding out hits/leads which can curb the infection caused by these two agents.[6] The clinical importance of QSIs is becoming essential owing to increase burden of antibiotic resistance.[7] The traditional antibiotics are fading away in terms of their efficacy and becoming resistant. This situation will be more worsen in the coming years due to overuse and misuse of antibiotics which has been accelerated enormously. Moreover, the repeated chances of outbreak of new infectious disease or re-appearance of old infection can impose a serious threat on the human health and global economy. In addition to this, the mechanism of QS is also noticed in bacteria which are commonly associated with spoilage of food. Thus spoilage of food by bacterial species forming biofilm is also a significant problem in food industry. Thus the exploration of molecules from natural or synthetic sources which can quench the process of QS has immense industrial value. [8]

In present scenario, the identification of novel lead molecules by the use of modern computational techniques based on the three-dimensional structure of the target protein has been increasingly utilized. Particularly, molecular docking studies employed in the computer-aided drug discovery aid help in predicting the optimal conformation and key interactions of promising small molecules to its receptor. This approach can be well used to model the protein small molecule interaction at atomic level, which renders information about the behaviour of small molecules in the binding site of the target protein. The drug design scientists are in continuous search of leads/drugs which can bind the receptor protein with high binding affinity and minimal toxicity.

Recently, long alkyl chain containing phenolic structural motifs (zingerone (**4**), [6]- [6]-shogaol (**5**), [6]-gingerol (**6**)) and present in *Zingiber officinale* Roscoe (ginger rhizome) has been identified as inhibitors of QS by Kumar *et al.* [9] This research group further derivatized [6]-gingerol and [6]-shogaol and obtained [6]-Azashogaol, and isoxazolin derivative of [6]-gingerol which are tested against *P. aeruginosa* and *C. violaceum* and found to exhibit fairly good inhibitory activities. The structures of the compounds are provided in Figure 2.

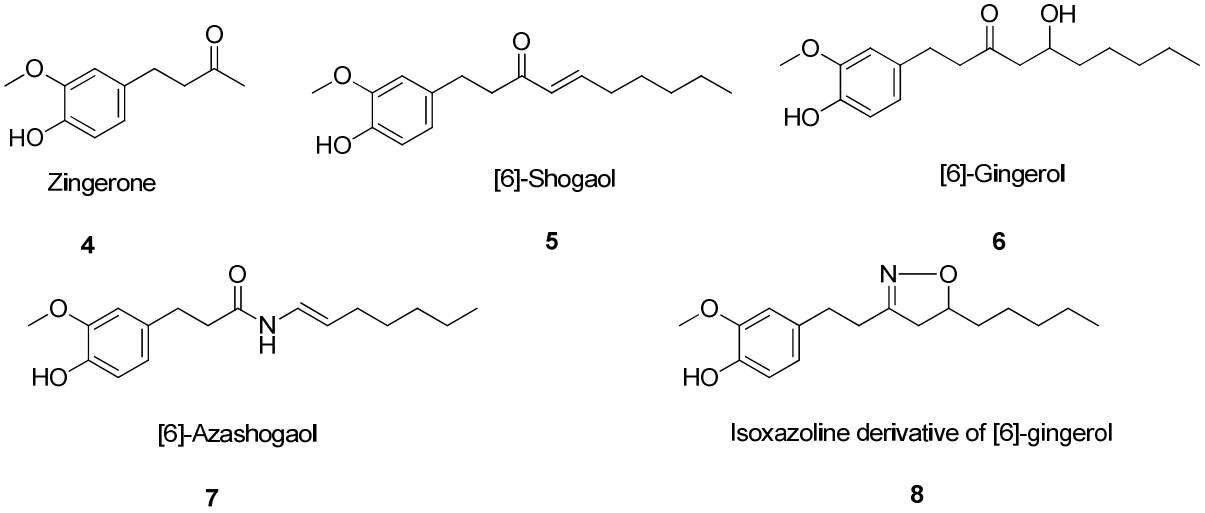
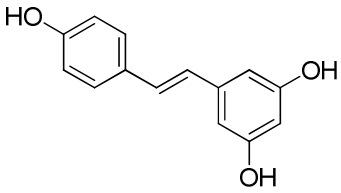


Figure 2. Structural representation of phenolic compounds of ginger and their derivatives

The polyphenolic compounds are present as secondary metabolites in many plants and compounds like resveratrol, quercetin, rutin, catechin, proanthocyanidins which belongs to polyphenols class have been reported to possess multiple biological activities,[10] including cardio-protective, anti-inflammatory, anti-carcinogenic, ant-iviral and anti-bacterial property. The polyphenols are versatile pharmacophore in relation to their physico-chemical properties as they have well balanced hydrophilicity and lipophilicity parameter which is helpful in imparting efficient pharmacokinetic profiling.

On this template QS inhibitory activity of *Vitis vinifera* (common grape vine) conserve was tested against *P. aeruginosa* and found to show fairly good inhibitory activity as this biological source is rich in diverse type of polyphenolic compounds.[11] The structures representation of polyphenols (**9**-**17**) which are present in *Vitis vinifera* is provided in Figure 3.

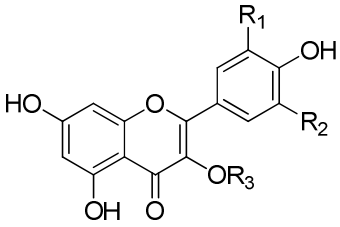
Stilbenes



trans-resveratrol

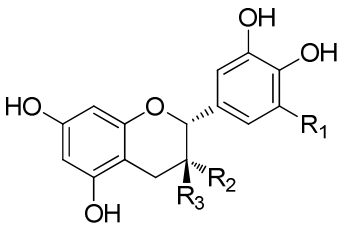
9

Flavonols



		R_1	R_2	R_3
10	Kaempferol	H	H	H
11	quercetin	OH	H	H
12	myricetin	OH	OH	H
13	isorhamnetin	OCH_3	H	H

Flavan-3-ols



		R_1	R_2	R_3
14	catechin	H	H	OH
15	epicatechin	H	OH	H
16	gallocatechin	OH	H	OH
17	epigallocatechin	OH	OH	H

Figure 3. Various class of polyphenols present in the *Vitis vinifera* (common grape vine).

Under *in silico* studies molecular docking analysis of phenolic compounds of *Zingiber officinale* Roscoe (ginger rhizome) and *Vitis vinifera* was done in the active site pocket of CviR and LasR receptor protein. It was found that the binding affinity of polyphenolic derivatives of *Vitis vinifera* was much better in comparison with phenolic compounds of ginger as they are binding with greater affinity with QS receptor protein. Further MESP (molecular electrostatic surface potential) of two best docked compounds based on docking score and docking pose was developed and plotted using Argus lab. [12]

Computational Details

Molecular docking

Molecular docking is a vital tool used for studying drug-macromolecule interaction at the active site.[13-15] The ranking of various ligands can be done on the basis of binding affinity and docking pose in the active site. This method is highly useful in finding out the binding conformation of the docked ligands and key amino acids which are responsible for stabilizing the drug-macromolecular interaction. Following three steps were carried out for molecular docking analysis: protein preparation, grid generation and ligand docking.

Protein structure preparation
The structure of CviR (PDB ID code: 3QP5)[16] and LasR receptors (PDB ID code: 2UV0)[17] co-crystallized with chlorolactone ($C_{14}H_{16}ClNO_4$) and N-3-oxo-dodecanoyl-L-homoserine lactone ($C_{16}H_{27}NO_4$) were taken from RCSB protein data bank depository. The proteins were prepared by adding hydrogens, Kollman charges, assigning AD4 type, and repairing missing atoms and converted to pdbqt format.

Ligand Preparation
The ligands were drawn in ChemBioDraw Ultra 12.0 followed by MM2 minimization of ligands (using ChemBio3D Ultra 12.0) by keeping a check on the connection error in the bonds. Ligands and Grid preparation was done using the open source software AutoDock Vina 1.1.2 [18] in order to carry out molecular docking analysis. The torsions for the ligands were set by detecting the roots in AutoDock Vina 1.1.2 followed by setting aromaticity criteria of 7.5.

Ligand docking
For the validation of docking protocol, bound ligand was extracted and then re-docked to generate the same docking pose as found in its co-crystallized form. Finally, a set of optimized ligands were docked on CviR and LasR receptor protein using AutoDock Vina 1.1.2, and they were analysed based on their docking score and inter-molecular interactions.

Visualization
The results obtained from AutoDock Vina 1.1.2 was visualized using academic version of Pymol software.

RESULTS AND DISCUSSION

Molecular docking analysis of phenolic compounds of *Zingiber officinale* Roscoe
Molecular docking analysis was carried out using Autodock vina 1.1.2 program to study the binding potential of the phenolic compounds (**4**-**8**) in the active site of CviR and LasR protein. The docking calculations were carried out using X-ray crystal structure of CviR protein (PDB code:3QP5) bound to its antagonist chlorolactone which is reported by Chen *et al.* Figure 4 represents a 3D ribbon representation of CviR protein monomer. This protein contains two binding domains (Ligand binding domain (LBD) and DNA-binding domain (DBD)) placed in a ‘‘crossed-domain’’ conformation connected to each other with a short flexible coil. The LBD is made of α-helices and β-sheets while in contrast DBD is made up of few α-helices. The antagonist chlorolactone (HLC) binds in the LBD as shown in the solid surface in Figure 4 and stabilizes the closed conformation of CviR in comparison to agonist (C_6-HSL) and thus prevents the QS activity.

Similarly, LasR protein which is present in the tetramer form as shown in 3D ribbon representation is activated by N-3-oxo-dodecanoyl-L-homoserine lactone (OHN). This autoinducer may serve as lead structure for designing new molecules which can inhibit its domain.

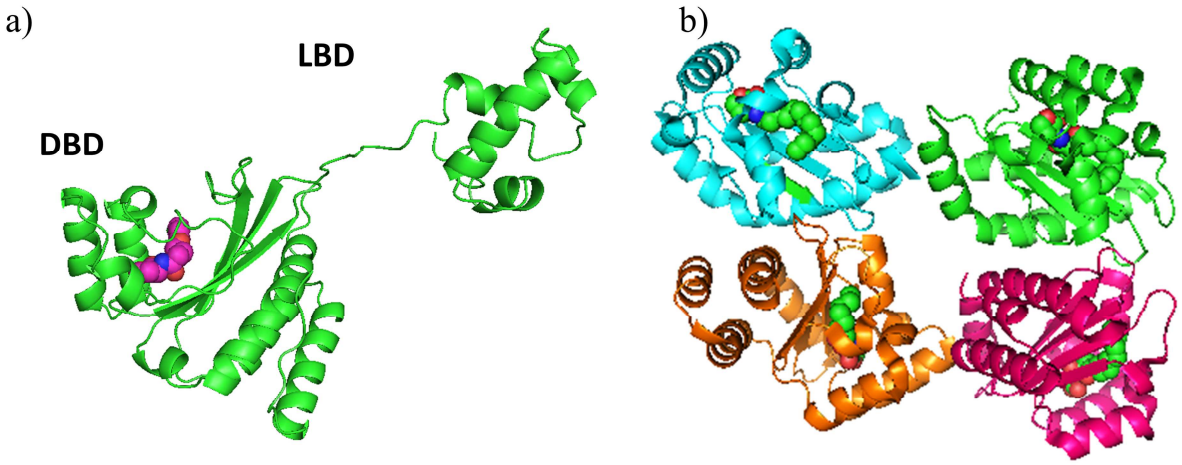


Figure 4. The 3D-ribbon representation of CviR (PDB Id: 3QP5) and LasR (PDB Id: 2UVO) protein

The docking protocol was standardized by performing the re-docking of bound ligand HLC, this ligand is able to dock inside the active site of protein with almost identical binding pose as compared to its co-crystallized structure (Figure 5) with the docking Score of -8.2. The lactone head group of HLC forms H-bond with nearby Trp84 residue, while the acyl group shows hydrogen-bonding with Asp97 and Ser155 residues. The tail part is buried inside the

hydrophobic pocket forming π– π stacking interaction with Tyr88. Similarly, for ligand OHN, the formed interactions after performing docking analysis resembles the interaction represented in co-crystallized form and found suitable for performing molecular docking of different set of investigated compounds.

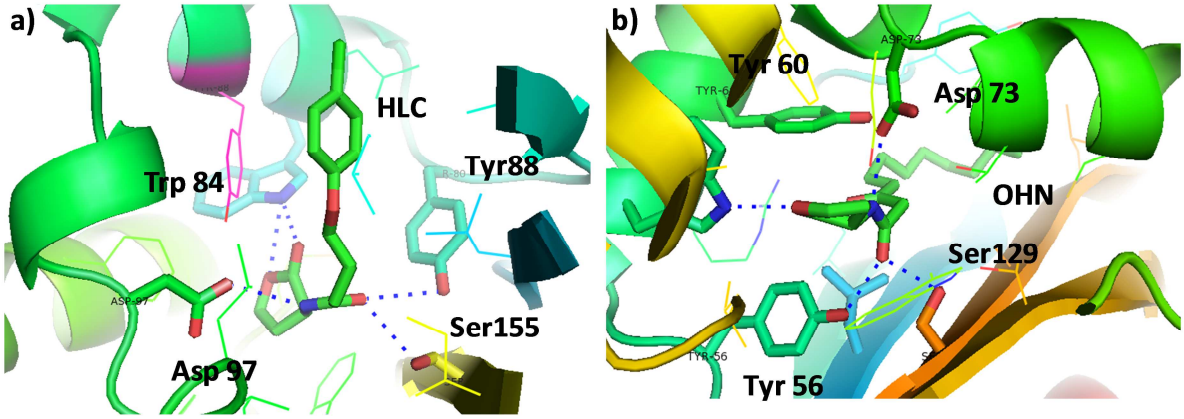


Figure 5. The docked pose of co-crystallized ligand a) HLC in the active site pocket of CviR protein, b) OHN in the active site pocket of LasR protein. The blue dotted line indicates hydrogen bonding interaction

Further, the molecular docking analysis of phenolic compounds as shown in Figure 2 (**4**-**8**) was carried out and the results are mentioned in Table 1. From this study it is observed that out of 5 different compounds, shogaol (**5**) and isoxazoline derivative of gingerol (**8**) is showing maximum binding affinity against CviR and LasR receptor proteins. The result is equally supported by the experimental results which show that compound **5** and **8** exhibit good inhibitory activity against *P. aeruginosa* and *C. violaceum* organism in comparison with the rest of the phenolic isolates of ginger.[9]

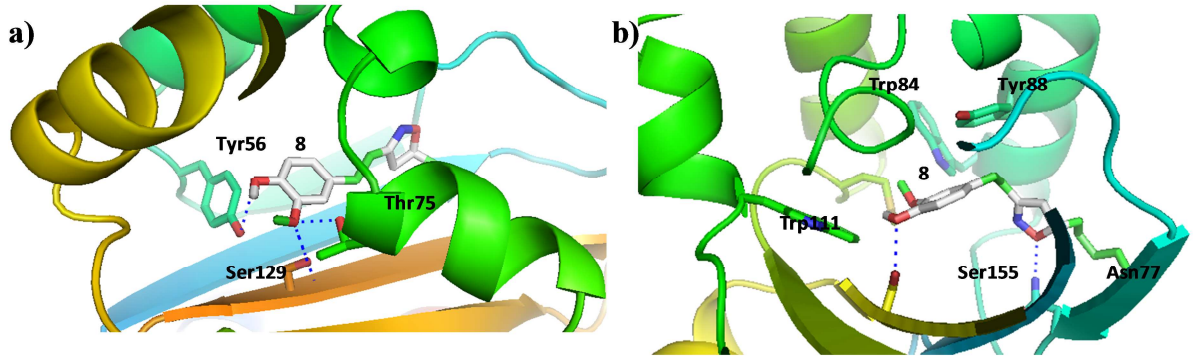


Figure 6. The docked pose of compound 8 (isoxazoline derivative of [6]-gingerol) a) in the active site pocket of LasR protein, b) in the active site pocket of CviR protein. The blue dotted line indicates hydrogen bonding interaction

List of binding scores and key interactions formed are provided in Table 1 for the autoinducer (OHN), antagonist (HLC) and the docked phenolic compounds (**4**-**8**) under study. The results of molecular docking analysis indicated that some of the phenolic compounds show higher docking scores than that of the co-crystallized ligand OHN in the active pocket of LasR protein (Table 1, coulomb 1). Hence, it is clear that phenolic compounds can bind with greater affinity and form interaction with key amino acid residues as shown in Figure 6. Mainly the interaction is van der Waal force of attraction along with the formation of hydrogen bond primarily with Ser129, Ty56 and Thr75. However, in CviR receptor protein the docking score of HLC (synthesized antagonist of CviR) was found to be -8.2 kcal/mol. The phenolic compounds show less docking score against CviR receptor protein in comparison to HLC. Compound **5** and **8** again found to be among top 2 ranked compounds of the series which displays maximum docking score and biological activity and form hydrogen bond mainly with Ser155 and Trp84.

Table 1. The list of docking scores of phenolic derivatives of ginger in the active site pockets of LasR and CviR QS receptor protein

Compounds	LasR	Interactions	CviR	Interactions
OHN	-8.7	Ser129, Tyr56, Asp73	--	--
HLC	--	--	-8.2	Trp84, Asp97, Ser155, Tyr88
4	-8.6	Ser129, Tyr56, Leu36, Ile52	-6.8	Tyr88, Trp111,leu57
5	-9.1	Ile86, Phe87, Ser129	-7.3	Ser155, Tyr88
6	-5.2	Ser77, Ile86, Phe87	-7.3	Asp97, Ser155, Tyr88
7	-9.2	Ser129, Tyr64, Tyr56	-7.0	ASN77, Trp84
8	-9.6	Ser129, Tyr56	-7.9	Trp84, Tyr88, Met135, Trp111

Molecular docking analysis of polyphenolic compounds of *Vitis vinifera*

Taking this study as template the screening of polyphenolic compounds of *Vitis vinifera* (**9**-**17**) was carried out on the LasR and CviR QS receptor proteins. The studied compound as shown in Figure 3 belongs to three different class *viz.* stilbenes, flavanols, flavon-3-ols. The polyphenols provide a kind of framework which is suitable both for forming hydrophilic and hydrophobic interactions. The list of docking scores of compounds on LasR receptor protein (Table 2) and CviR receptor protein is shown in Table 3.

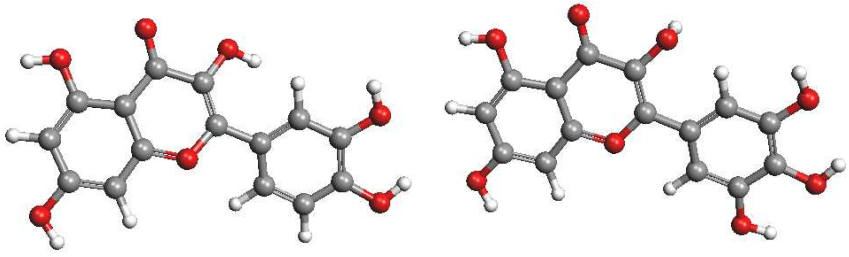
Table 2. The list of docking score of polyphenolic derivatives of *Vitis vinifera* in the active site pocket of LasR protein

Compound	Docking score	Interactions
OHN	-8.7	Ser129, Tyr56, Asp73
9	-9.5	Leu110, Tyr56, Trp60
10	-8.9	Ser129, Thr76, Tyr56
11	-10.5	Ser129, Tyr56, Tyr47, Arg61, Thr75, Thr115
12	-11.4	Tyr56, Ser129, Tyr64, Trp40, Tyr47, Thr75, Gly126
13	-6.8	--
14	-9.9	Ser129, Arg61, Tyr56, Tyr64
15	-10.4	Ser129, Leu125, Tyr56, Leu36
16	-10.2	Ser129, Tyr64, Tyr56
17	-8.7	Ser129, Trp60, THR115

Table 3. The list of docking score of polyphenolic derivatives of *Vitis vinifera* in the active site pocket of CviR protein

Compound	Docking score	Interactions
HLC	-8.2	Trp84, Asp97, Ser155
9	-7.8	Met135, Trp84
10	-7.9	Trp84, Trp111
11	-7.9	Trp84, Tyr88
12	-8.0	Asp97,Trp84, Tyr88
13	-7.9	Trp84, Trp111
14	-7.5	Trp84, Leu57, Ile90
15	-7.2	Trp84, Asn77
16	-7.1	Met135, Trp84
17	-7.1	Trp84, Asn77

From Table 2 it is interpreted that mostly all the investigated polyphenolic compounds (**9**-**17**) have potential to stabilize the ligand-receptor domain of LasR protein. The 3D structure of two best docked compounds (quercetin (**11**) and myricetin (**12**)) is shown in Figure 7.



11 **12**

Figure 7. The optimized 3D geometry of compound 11 and 12 which display highest docking score

The docking pose of these compounds in the active site pocket of LasR protein is displayed in Figure 8. The amino acid residues present around the ligands in the active site pocket of proteins were Tyr56, Ser129, Thr75, Leu36, Ala127, Tyr64, Ile 52, Val76, Cys79, Ala50, Tyr47, Leu40, Gly126, Leu125. The compounds (**11** and **12**) which is

showing docking score (-10.5 and -11.4 kcal/mol) forms hydrogen bonding interaction with Ser129 and Tyr56. These polyphenolics have overall good quality parameters so this framework can act as a better lead molecule for the development of protein inhibitor of the QS mechanism of *P. aeruginosa*.

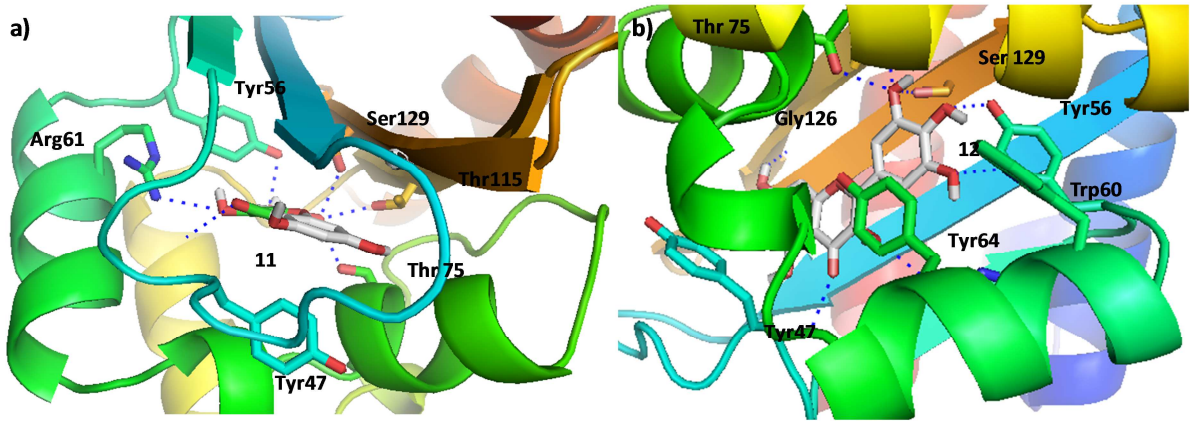


Figure 8. Molecular docking of pose of a) compound 11 and, b) compound 12 in the active site of LasR protein

Similarly the same set of compounds was docked in the active site pocket of CviR protein, compound **12** proves to be the best docked compound of the series with the docking score of -8.0 kcal/mol and forming interaction with Tyr88, Trp84, Met135, Leu85, Asp97, Tyr80, Leu72, Leu100 amino acid residues. The hydrogen bonding interaction is formed by Trp84 where oxygen of phenolic functionality is acting as hydrogen bond acceptor and polar hydrogen of NH of tryptophan is acting as hydrogen bond donor with the hydrogen bond distance of 2.26 Å. Similarly compound **11** also show fairly good docking results. The ligand interaction diagrams of these compounds are given in Figure 9.

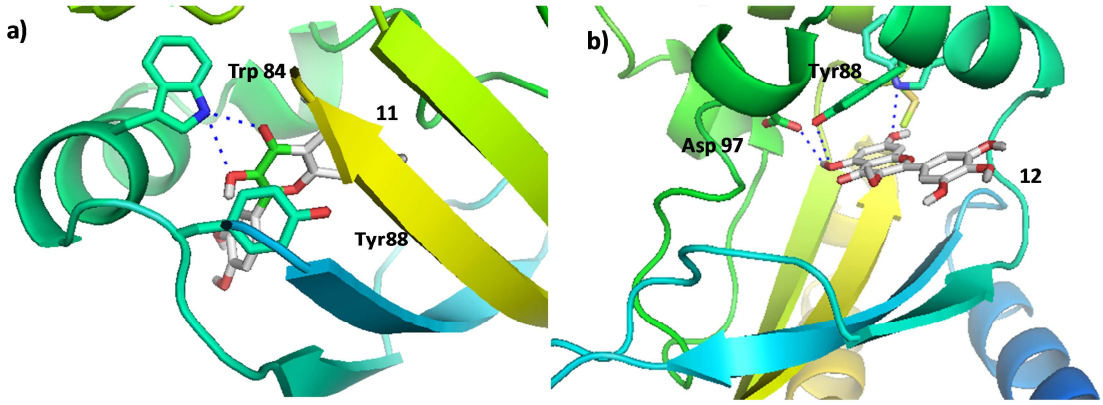
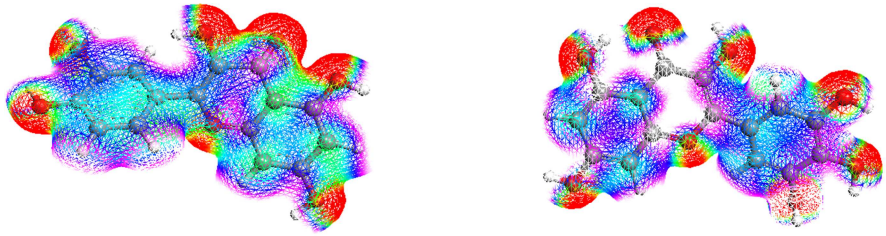


Figure 9. Molecular docking of pose of a) compound 11 and, b) compound 12 in the active site of CviR protein

Molecular Electrostatic Surface Potential (MESP)

The molecular electrostatic potential (MESP) is an important parameter which is in general used to predict the behaviour and reactivity of the molecule. It is very useful in understanding the potential sites for electrophilic (negative region) and nucleophilic (positive region) reactions. MESP surface plays a crucial role in drug-receptor recognition of one molecule (macromolecule) by another (small molecule/ligand), as commonly observed in drug-receptor, and enzyme-substrate interactions, because it is through their potentials that the two species recognize each other for bonding. To predict reactive sites (electrophilic and nucleophilic) in order to find out hydrogen bond donor and hydrogen bond acceptor sites in the investigated molecule, the MESP surface is calculated for the geometries of compound **11** and **12** which are optimized at PM3 level and shown in Figure 10. The different values of the electrostatic potential at the surface are represented by different colours and potential increases in the order red<green<cyan<blue<violet. The colour code of these maps is in the range between −0.0500 a.u. (deepest red) and 0.0500 a.u. (violet) in the compound, where violet indicates the most electropositive i.e. electron poor region and red indicates the most electronegative region, i.e. electron rich region. These contour plots were generated using Argus lab software. From the Figure 10, it is evident that the most electronegative region is located over oxygen atom attached by carbon atom which effectively acts as electron donor or hydrogen bond acceptor in the molecular framework. This is also supported by ligand interaction diagram where phenolic oxygen moiety is getting involved in forming hydrogen bond by acting as hydrogen bond acceptor.



11 **12**

Figure 10. MESP surfaces of 11 and 12; plotted onto a surface of constant electron density (0.002 e/au^3)

CONCLUSION

The present study is based on the identification of binding pose and key amino acid residue interactions made by phenolic compounds of *Zingiber officinale* Roscoe (ginger rhizome) in the QS receptor proteins of *P. aeruginosa* and *C. violaceum*. These compounds have potential of forming hydrogen bonding interaction with Ser129 (PDB Id: 2UV0) and Asp97, Ser155 and Trp84 (PDB Id 3QP5). Besides these interactions hydrophobic stabilization formed by π-π sandwich type aromatic stabilization is also one of the important criteria for the compound to act as QSI. Using this study as template the virtual prediction of QSI activity of polyphenolics which are mainly present in *Vitis vinifera* (common grape vine) was undertaken. The results prove out to be highly favourable indicating that the polyphenolic structural framework is capable of stabilizing macromolecular domain of CviR and LasR receptor protein. Among the three studied classes, flavonols proved to be the best scored compounds in which quercetin (**11**) and myricetin (**12**) have shown the high binding affinity towards QS receptor proteins.

Further the MESP contour plots of compounds (**11** and **12**) indicated that these compounds have functional units which can act as hydrogen acceptor and get involved in the hydrogen bonding. Similarly, the aromatic moieties can form π-π aromatic stabilization. Thus, the polyphenolic class of compounds can serve as a lead moiety which has structural features which are responsible for the drug-receptor recognition of the target proteins which are involved in process of QS. As these compounds are not associated with toxicity so they can be exploited by the drug design scientist for the lead development to quench the QS mechanism.

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