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Comparative modeling and molecular docking studies of quorum sensing transcriptional regulating factor SdiA from *Klebsiella pneumoniae*

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ABSTRACT: *Euphorbia hirta* a traditional medicinal plant which is endowed with curative properties including anti-bacterial, anti-fungal, anti-viral and analgesic properties. Bacteria make use of cell to cell signalling system known as Quorum sensing (QS) and respond to their own population. In most of the gram negative bacteria, the transcriptional regulators belonging to LuxR protein plays a crucial role in QS mechanism by detecting the presence of signaling molecules known as N-acyl homoserine lactones (AHLs). Certain bacteria do not produce any of the quorum-sensing signaling molecules including AHL on its own, while they are capable to sense the AHLs of other bacterial species to regulate the pathogenicity. In such cases they encodes a LuxR homolog, SdiA (Suppressor of cell division inhibition), that can recognise the AHLs produced by other bacteria. Thus SdiA is one of the potential drug target as its AHL binding induces the transcription of many virulent genes. In this present work, anti-quorum sensing activity of *Euphorbia hirta* was evaluated against *Klebsiella pneumoniae*. Anti-quorum sensing efficacy of *Euphorbia hirta* was estimated with reference to QS Bio-monitoring strain *Chromobacterium violaceum*. The binding efficacy of the phytochemicals of *Euphorbia hirta* against CviR Protein from *Chromobacterium violaceum* and SdiA Protein from *Euphorbia hirta* were studied..

Keywords: *Quorum sensing, Euphorbia hirta, Klebsiella pneumoniae, Chromobacterium violaceum;*

1. INTRODUCTION

Globally, in last few decades the emergence and wide spread of antimicrobial resistant antimicrobial drug resistant strains of *Pseudomonas spp.*, *Klebsiella* spp. Proteus spp., *Enterococcus spp.* and *Staphylococcus* spp. become the alarming situation of greater public health concern [1]. *Klebsiella pneumoniae* and *Staphylococcus aureus* mainly accounts for post-operative wound infections, endocarditis, toxic shock syndrome and more prevalent in most of the hospital-acquired infections. While *Proteus spp and Enterococcus spp* are the causative agents of urinary tract infections [2].

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Competing interests
The authors have declared that no competing interests exist.

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In general, antibiotics are used to control these microbial infections by inhibiting their growth. However, the continuous usage and misuse of antibiotic therapy has led to the appearance of multi-drug resistant strains to the tolerance against broad spectrum of available antibiotics [3]. The continuous emergence of these multiple drug resistant bacteria has forced the scientists to search for new antibacterial agents have become the main concern. Though the search for new antimicrobial substances has resulted novel antimicrobial chemotherapeutic agents as synthetic drugs from various sources, the higher cost production and its adverse effects has limited its usages when compared to plant derived drugs [4]. Thus the search for novel anti-pathogenic agents has increased the focus on the potential compounds from plant sources that are wide spread 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 has gained attentions of many pharmaceutical industries [5].

Bacteria make use of cell to cell signalling system known as Quorum sensing (QS) and respond to their own population. Mostly in gram-negative bacteria, the transcriptional regulators belonging to LuxR protein plays a

crucial role in QS mechanism by detecting the presence of signaling molecules known as N-acylhomoserine lactones (AHLs) [6] Certain bacteria does not produce any of the quorumsensing signalling molecules AHL on its own and can detect AHLs produced by other bacterial species and regulates the pathogenicity. In such cases they encodes a LuxR homolog, SdiA (Suppressor of cell division inhibition), that can recognize the AHLs produced by other bacteria. SdiA is considered as the potential drug target as its AHL binding induces the transcription of many virulent genes [7].

In this present work, anti-quorum sensing activity of *Euphorbia hirta* was evaluated against *Klebsiella pneumonia*. Even though majority of the isolates were sensitive to most of the antibiotics, the lactamase which means that they quickly become resistant to standard antibiotics during treatment, requiring change in antibiotic to avoid worsening of the sepsis [8]. Anti-quorum sensing efficacy of *Euphorbia hirta* was estimated with reference to QS bio-monitoring strain *Chromobacterium violaceum*. The binding efficacy of the phytochemicals against CviR Protein from *Chromobacterium violaceum* and SdiA Protein from *Klebsiella pneumonia* were studied.

2. METHODOLOGY

2.1 Target -Template Alignment

The protein sequence of SdiA from *Klebsiella pneumonia* sequences was retrieved from the UniprotKB (A6TB66) [9]. Using NCBI-BlastP (Basic Local Alignment Search Tool) [10] against PDB, the crystal structure LuxR-type transcriptional factor, CviR from *Chromobacterium violaceum* (PDB ID: 3QP5) was obtained as most homologous sequence and its atomic co-ordinate file was retrieved from the protein databank [11].

2.2 Comparative modeling of SdiA

The 3D models of *Klebsiella pneumonia* SdiA was generated by using CviR from *Chromobacterium violaceum* (A-chain) (PDB ID: 3QP5) as template structure. The structure of SdiA, was generated by using Swiss Model Webserver [12] and validated.

2.3 Conserved LuxR family proteins

Multiple sequence alignment was used to establish the homology and conserved residues among the transcriptional regulators LasR from *Pseudomonas aeruginosa* (PDB ID: 2UV0), and CviR from *Chromobactereum Violaceum* (PDB ID: 3QP5) and the developed model of SdiA from *Klebsiella pneumonia* by using ClustalW at the EBI server [13].

2.4 Binding Site determination

The amino acids in the binding site of the model was predicted through Pocket finder at What-If server [14]. The binding sites of the CviR from *Chromobactereum Violaceum* and SdiA *Klebsiella pneumonia* was compared with that of the LuxR family proteins which were the experimentally

2.5 Ligands

The principle compounds of *Euphorbia hirta* was retrieved from Duke Ethanobotanical database and their respective structures were obtained from Pubchem Database. The structures were retrieved in SDF format.

2.6 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 SdiA using the default parameters such as triangle matching (in base placement, maximum allowed overlap volume of 2.9 A^{03} and 200 solutions per iteration and solutions in FlexX [15] and visualized by pose view [16].

3. RESULT AND DISCUSSION

In most of the gram-negative bacteria, LuxI homologs generates Acyl homoserine lactones as signal molecules,. These AHL signals are detected by the LuxR homolog receptors. Where as in *Klebsiella pneumonia* 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. However, they can responds to the AHL signals produced by the other pathogenic bacteria. However, it encodes a LuxR homolog, SdiA which can sense the signal molecules produced by the mixed community genera [17,18]. Thus SdiA a transcriptional regulator was considered as a potential targets.

The 3D structure of the target protein SdiA from *Klebsiella pneumonia* were not available in any of the structural database, it was developed by using 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 *Klebsiella pneumonia* transcriptional regulator SdiA is homologous with the structure CviR, LuxR- type transcriptional factor from *Chromobacterium violaceum* (PDB ID: 3QP5) over 40%. As all these sequences belong to the same family, the structure of 3QP5 (Figure 1a) was considered as a template structure for comparative modeling. The model was generated by using swissmodel webserver.

The alignment of the sequences of the target proteins SdiA and CviR (template) obtained from BLASTP was further refined using ClustalW with default parameters. With this sequence alignment and the atomic coordinate file of the template structure 3QP5, the structure of SdiA, was generated by using Swiss Model Webserver. Three models are generated and the best models was selected for further analysis (Figure 1c). The multiple sequence alignment (Figure2) of LuxR family proteins LasR from *Pseudomonas aeruginosa*, CviR from *Chromobacterium violaceum* and SdiA from *Escherichia coli, Klebsiella pneumonia and Enterobacter aerogens* showed that amino acids are conserved in LuxR family proteins. These alignment enlights that SdiA from

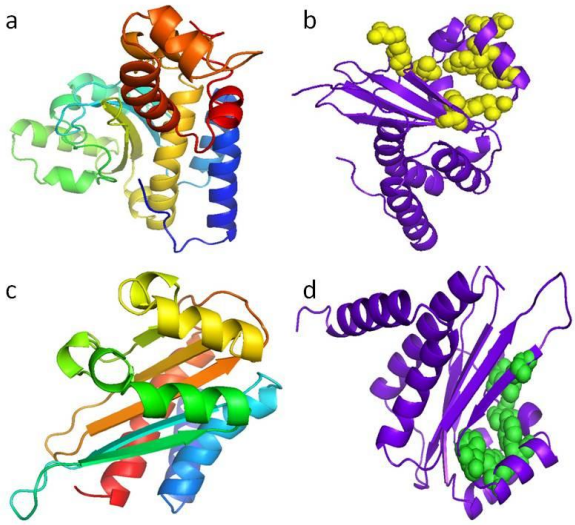


Fig. 1: Modelled Structure of Quorum sensing Transcriptional activator shown in cartoons representation and the active site is highlighted as spheres. (a) CviR Protein from *Chromobacterium violaceum* (b) Active site of CviR Protein from Chromobacterium violaceum (c) SdiA Protein from *Klebsiella pneumonia* (d) Active site of SdiA Protein from *Klebsiella pnuemonia*

Klebsiella pneumonia was considered for further docking studies. The binding pocket for the model SdiA (Figure 1d) was predicted by Pocket finder at What – If server and the homologous proteins CviR were analysed (Figure 1 b).

The Docking program FlexX, from LeadIT was used to dock *Euphorbia hirta* compounds with the binding pocket of the LasR, CviR and the developed model, SdiA. The docking was carried out with the radius of 6.5 A^0 at the site of docking. The interactions between the binding site residues of CviR and the modeled protein SdiA with the compounds as ligand molecules in the docked complexes were given in Table 1 and Table 1. A keen observation of these interacting residues of the LuxR family proteins, the modeled SdiA and the ligand molecules revealed the most important functional groups of the ligand molecules and the amino acids LuxR family proteins favoring the interactions. The best docked ligand molecules and their interactions with the amino acids in the active site of CviR and the modeled protein SdiA is given in Figure 3 and 4.

A total of 19 compounds were found as the principle compounds of the *Euphorbia hirta*. 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 *Chromobacterium violaceum and* SdiA from *Klebsiella pneumonia* by using FlexX. Out of these 19 compounds, 17 compounds formed docking complex with all both CviR and SdiA and its binding energies were analyzed by LeadIT. Considering the binding energy score, the 3 best docked compounds for each protein CviR and SdiA were selected and their docking interaction with the active site residues were analyzed by using pose view of LeadIT.

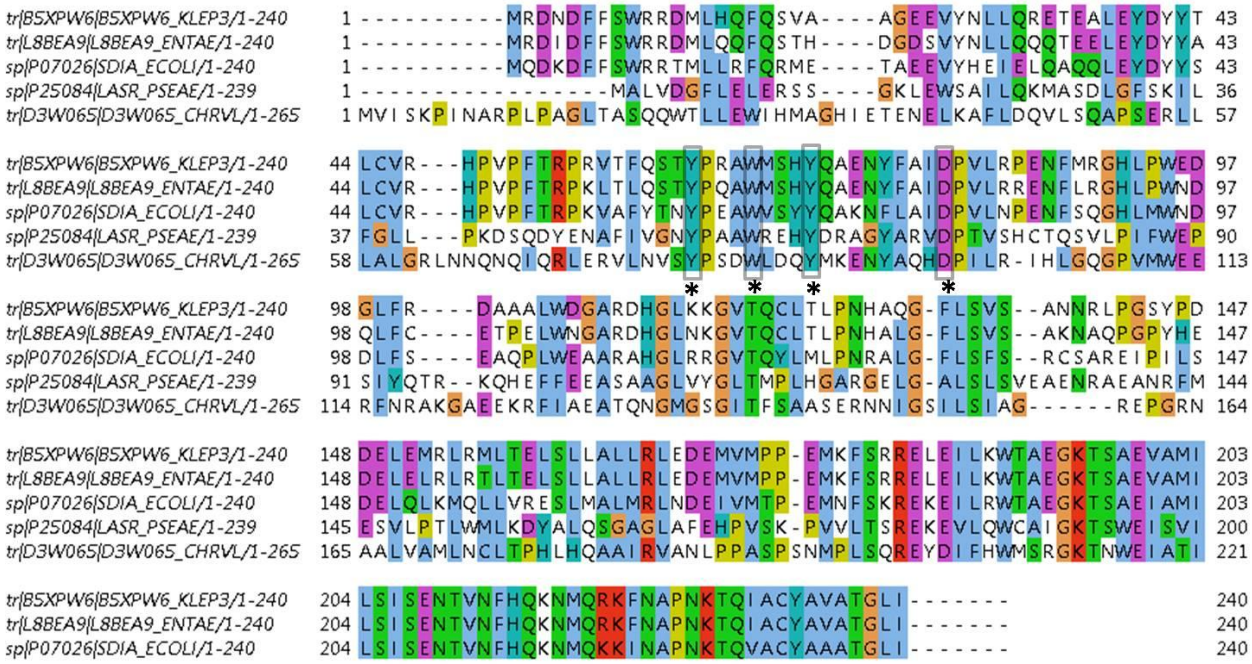


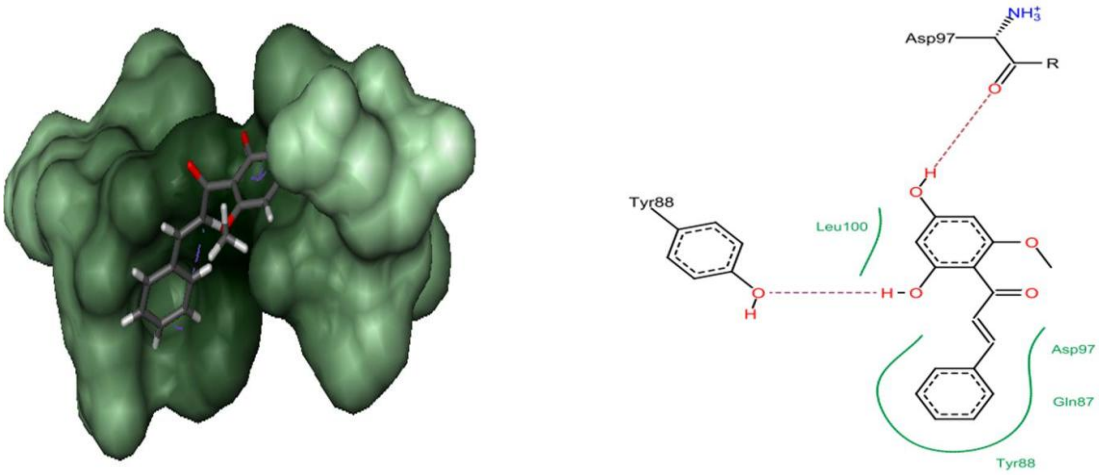
Fig. 2: The Multiple sequence alignment among *Klebsiella pneumonia, Enterobacter aerogens, Escherichia coli, Pseudomonas aeruginosa* and Chromobacterium violaceum. The conserved regions were shown in clustal X colr format and the conserved active site is highlighted with rectangle box and also marked with Astrik (*)

Table 1: Docking Interactions of plant compounds with the active site amino acids of CviR from *Chromobacterium Violaceum* and their binding scores.

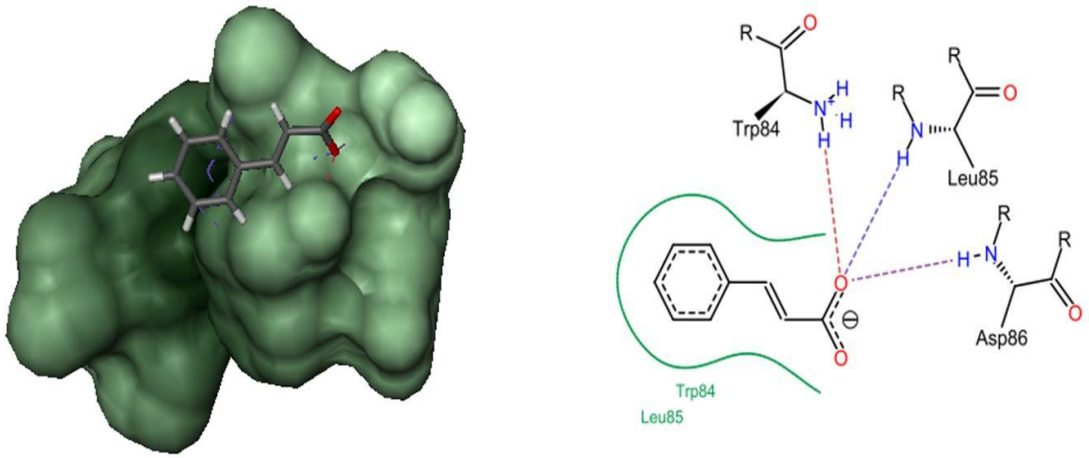
Compound ID	Compounds	Type of Interaction with active site residues		Docking Score (kJ/mol)
		Bonded	Non bonded	
57124935	Norpatchoulenol	Asp86,Trp84	Trp84,Asp86,Leu85	-2.8686
6437979	Callicarpenal	-	Pro98,Asp97,Trp84,Tyr88	-0.4569
5481240	Phytol	Trp84,Asp97	Trp84,Ile99.Asp97,Leu100,Tyr88	-8.2647
5366074	Retusin	Trp84,Leu85,Asp86	Asp86,Leu85	-8.3853
5281126	Damascenone	Trp84,Leu85,Asp86	Leu85,Tyr88,Trp84,Leu100	-0.0456
5280934	Punicic acid	Leu85,Trp84,Asp86	Trp84,Asp86,Tyr88,Leu85	-1.4330
5280442	linolenic acid	Gln87,Asp97	Gln87,Tyr88,Trp84,Leu100,Asp97	-9.7346
641785	acacetin	Asp97,Tyr88	Asp97,Gln87,Tyr88,Leu100	-12.1467
444539	Cardamonin	Trp84,Leu85,Asp86	Trp84,Leu85	-11.5130
348962	Cinnamic acid	-	Asp97,Trp84,Tyr88,Pro98	-4.5239
301798	butyl trityl	Asp97,Gln87	Gln87,Tyr88,Pro98,Trp84,Asp97	-7.7922
10465	Poriol	Asp86,Trp84,Leu85	Leu85,Tyr88,Asp86	-4.4680
10416	Margaric acid	Trp84,Asp86,Leu85	Leu85,Asp86	-3.4816
10212	Malvalic acid	Trp84	Trp84,Asp97,Tyr88,Ile99	-6.9059
1135	Imperatorin	Trp84,Leu85,Asp86	Leu85	-8.0549
985	Quinic acid	Asp86,Leu85,Trp84	Leu85,Asp86,Tyr88	-3.1656
323	Thymine	Asp86,Trp84,Leu85	Trp84,Leu85	-8.6772

Table 2: Docking Interactions of plant compounds with the active site amino acids of SdiA from *Klebsiella pneumonia* and their binding scores.

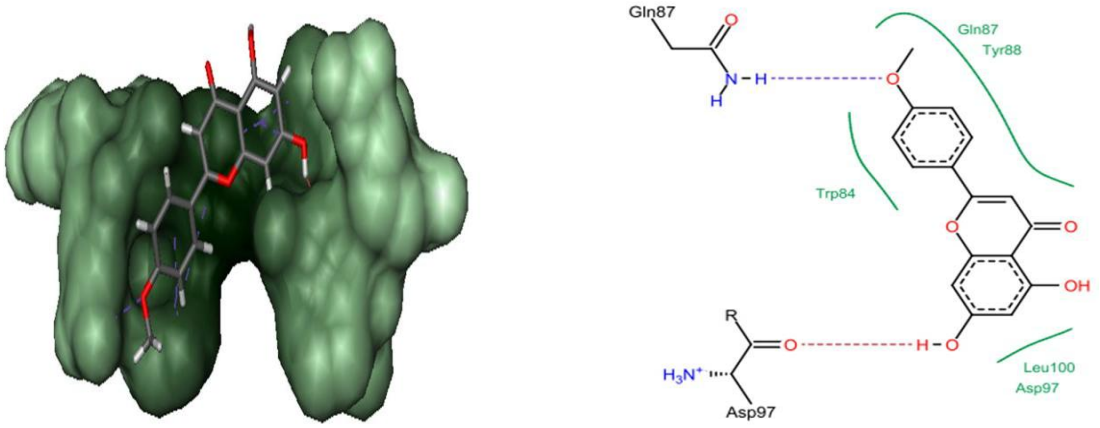
Compound ID	Compounds	Type of Interaction with active site residues		Docking Score (kJ/mol)
		Bonded	Non bonded	
57124935	Norpatchoulenol	Trp67	Tyr71,Val82,Asp80,Tyr63,Trp67	-2.6933
6437979	Callicarpenal	Asp80	Tyr63,Asp80,Trp67,Tyr71	-1.2080
5481240	Phytol	Asp80,Trp67	Trp67,Tyr63,Tyr71,Val82,Asp80	-13.5553
5366074	Retusin	Trp67	Trp67,Val82,Tyr71,Tyr63	-7.9594
5281126	Damascenone	Trp67	Trp67,Tyr63,Tyr70	-4.1555
5280934	Punicic acid	Trp67	Tyr70,Asp80,Tyr71,Trp67	-5.1023
5280442	linolenic acid	Trp67	Trp67,Val82,Tyr63,Tyr71	-12.9562
641785	acacetin	Trp67,Asp80	Asp80,Val82,Trp67,Tyr71,Tyr63	-14.8740
444539	Cardamonin	Asp80	Tyr71,Tyr70,Trp67	-9.3555
348962	Cinnamic acid	Trp67	Tyr63,Tyr71,Val82,Trp67,Asp80	-9.6899
301798	butyl trityl	Asp80	Tyr63,Tyr71,Trp67	-9.9831
10465	Poriol	Trp67	Tyr63,Tyr70,Trp67	-7.7286
10416	Margaric acid	Trp67	Tyr71,Asp80,Tyr70,Trp67	-7.3933
10212	Malvalic acid	Trp67	Tyr71,Val82,Tyr63,Trp67	-13.2575
1135	Imperatorin	Asp80,Tyr63	Trp67	-8.3390
985	Quinic acid	-	Tyr63,Tyr71,Val82,Trp67	-8.1672
323	Thymine	Trp67	Trp67,Tyr71,Val82,Tyr63	-9.7149



Docking complex and interaction of CID_641785

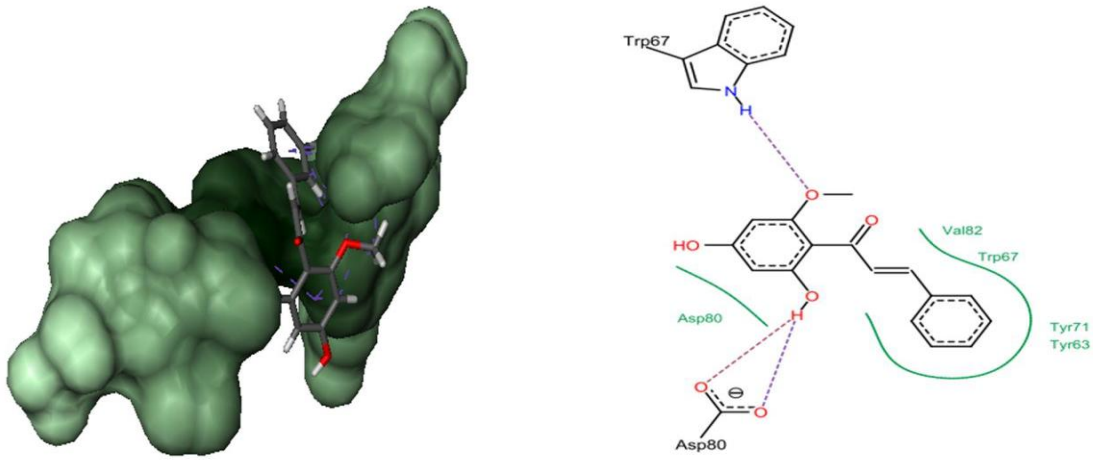


Docking complex and interaction of CID_444539

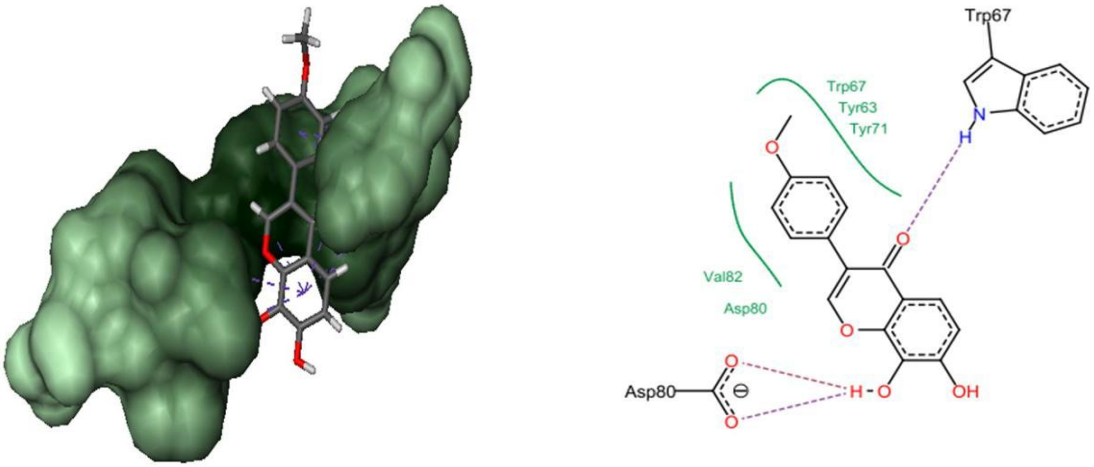


Docking complex and interaction of CID_5280442

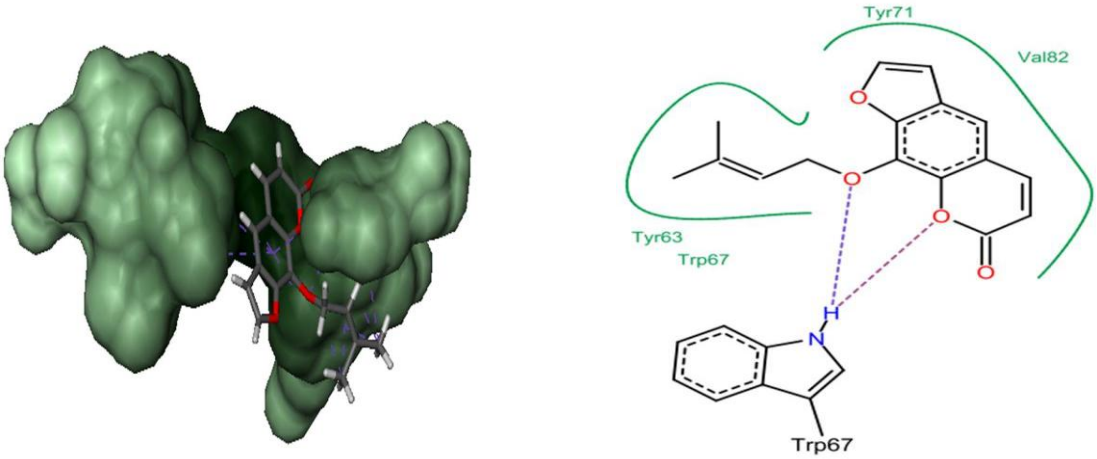
Fig.3 : Docking complex and the interactions of the best three compounds with the active site amino acids of CviR from *Chromobacterium Violaceum*



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 SdiA from *Klebsiella pneumonia*

The binding interactions in the docking studies of *Chromobacterium violaceum* CviR and *Klebsiella pneumonia* SdiA with the 3 best docked compounds of the *Euphorbia hirta* exposed the similar binding of AHL residues, that are responsible for Quorum sensing activity. This result indicates that in *Chromobacterium violaceum* CviR, it is found that Trptophan (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 *Klebsiella pneumonia* SdiA, it is observed that Trptophan (Trp67) and 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 *Chromobacterium violaceum.* It is observed that natural ligand 3-oxo-C6-HSL exhibited the docking score of -8.3776kJ/mol. Thus among the docked compounds it is revealed that the compound all the three compounds CID_641785, CID_444539 and CID_5280442 is having highest docking score when compared to that of the natural ligand. Thus these compound can be used to inhibit the quorum sensing mechanism in *Chromobacterium violaceum.*

Soule're et al,. [19] reported the structure –activity relationships of AHLs derived from carboxamides, sulphonamides and urease. They explored that Tyr61 is very crucial in interactions, which is found to be a conserved in the LuxR proteins. 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 SdiA transcriptional regulator from *Klebsiella pneumonia.* It is observed that natural ligand 3-oxo-octanoic acid exhibited the docking score of -8.3989 kJ/mol. Thus among the docked compounds it is revealed that the compound CID_641785 is having highest docking score when compared to that of the natural ligand. Thus this compound can be used to inhibit the quorum sensing mechanism in *Klebsiella pneumonia.*

Ahumedo et al.[20] reported that the residues (Y53, Y61, W57, D70, W85 to TraR, Y56, Y64, W60, D73, W88 to LasR) serves as site-specific targets for the development of potential antagonists. They have evaluated AHLs molecular analogs and found that interactions with the conserved amino acids (D73, W60, Y56, S129 to LasR and D70, W57, Y53 to TraR) of the LuxR type protein family are crucial for their docking intercations. The overall docking results of principle compounds with CviR and SdiA 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 *Euphorbia hirta*, with highest binding score and might have a better inhibition activity against the quorum sensing regulation of *Klebsiella pneumonia*.

4. CONCLUSION

Klebsiella pneumonia, 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 QS mechanism by detecting the presence of signaling molecules known as N-acylhomoserine lactones (AHLs) and regulates the pathogenicity. *Klebsiella pneumonia* habors a transcriptional regulator SdiA (Suppressor of cell division inhibition), that can recognise the AHLs to enhance the pathogenecity. Hence, SdiA from *Klebsiella pneumonia* is considered as a valid drug target. Thus in the present study, anti-quorum sensing activity of *Euphorbia hirta* was evaluated against *Klebsiella pneumonia*. Anti-quorum sensing efficacy of *Euphorbia hirta* was estimated with reference to QS Bio-monitoring strain Chromobacterium violaceum. The binding efficacy of the phytochemicals of *Euphorbia hirta* was docked with the SdiA from *Klebsiella pneumonia* and also with CviR Protein from Chromobacterium 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 highest binding score might be an effective inhibitor of *Klebsiella pneumonia* pathogenisis.

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