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ORIGINAL ARTICLE

Quenching of quorum sensing regulated proteins of nosocomial *P. aeruginosa* by *In silico* approach

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ABSTRACT
In microorganisms, quorum sensing is a cell-to-cell communication process that uses signalling molecules. Pseudomonas aeruginosa is an opportunistic pathogenand well known for its nosocomial infection and multi-drug resistance (MDR).P. aeroginosa uses quorum sensing system to regulate their co-operative phenotypic behaviours like biofilm formation, virulence factor etc.,Las and Rhl are acylhomoserine lactone(AHL) based QS systems present in P.aerginosa and the Las system comprised of the transcriptional factor called LasR which can activate the expression of target virulence gene. Thus the pathogenic activity of in P.aerginosa can be repressed by restraining the target protein, LasR with suitable bioactive molecules. In the present study, bioactive compounds from various natural food sources have been selected and tested against LasR protein using bioinformatics tools for its quorum quenching activity. In silico analysis gave prominent results for thirteen compounds out of 25 compounds tested with best docking score. These selected for compounds can be suggested for in vitro and in vivo studies and could be used for QSI based drugs against P.aerginosa infections.

Keywords*: Quorum Sensing Inhibitors (QSI), P. aerginosa, Nosocomial infection, Molecular Docking, Bioactive compounds.*

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INTRODUCTION

Quorum sensing (QS) is a type of cell-to-cell communication that requires the creation of a signal, detection and response towards the Auto inducers (AIs) which are the extracellular signalling molecules. As the bacterial population increases these auto inducers accumulate and are responsible to monitor and track changes in cell numbers and can collectively alter gene expression to regulate their co-operative phenotypic behaviours like biofilm formation, virulence factor etc., *Pseudomonas aeruginosa* is a Gram negative bacteria which is a causative for various infections like urinary tract infections, pneumonia, diarrhoea and also an important nosocomial infecting pathogen. It is considered a medical nuisance because it causes obstructions in stents, filtration devices, and other medical devices. *P.aeruginosa* was found to be resistant to many of the antibiotics such as cephalosporin, amino glycosides etc., In recent years, the multidrug resistant has played an important role in wide variety of public health. In Worldwide, the most emerging challenges of related to nosocomial infections are related to significant morbidity and mortality, due to developing and developed countries [1]. Because MDR medications are recognised for a variety of reasons, including major difficulties. Antimicrobial medicinal medicines have spawned so much study that it may be possible to control drug resistance [2].

Pseudomonas aeruginosa is one of the leading causes of nosocomial infections, because of capacity of inflict extremely severe infections. The quorum sensing systems of *P. aeroginosa* includes Las and Rhl which are AHL-based.Because MDR medicines have been identified for a variety of reasons, including significant challenges. Antimicrobial medicinal treatments have spurred so much research that drug resistance may be controlled. *P.aeruginosa* uses these signalling molecules to regulate its virulence activity and biofilm formation.

Recent studies witnessed the evolution of multidrug resistance in bacteria, and thus need of a novel antimicrobial strategy which can interrupt with bacterial communication by Quorum Sensing inhibitors (QSI). Research evidences has shown that blocking quorum signalling mechanism can help in proper action of the immune system and can lead to removal of infecting microbequorum-quenching mechanisms that efficiently interfere with microbial quorum sensing have been reported in both prokaryotes and eukaryotes, and function by preventing crucial phases of quorum sensing, such as signal creation, signal accumulation, and signal receipt [2-8].

Researches had documented health promoting and diseases resistant properties as well as importance of various food components to reduce various diseases and the role of various probiotics content in dairy products in improving and preventing gastrointestinal and other diseases [9]. Various studies relieved the importance of personalized diets according to risk group of an individual and its health benefits. Many studies had relieved the presence of active Quorum sensing Inhibitor components in various food sources and found that various crude extracts from food products helps in rapid clearing of microbes [10-18]. According to studies conducted by Zimmer *et al*, blueberry fruit extracts showed marked activity against *S. epidermidis* biofilm without inhibiting bacterial growth [30-33]. Various studies reported the presence of potential substances that can control or inhibit quorum sensing, however these studies are limited there are still many substances that can be used for quorum quenching. Studying these food derived components can be helpful for the well-being of people and thus there comes a need to explore this field [19, 20]. In the present study natural food components were screened for their Quorum sensing inhibiting activity against Las1R target of *P.aeruginosa* using computer aid programmes like virtual screening and molecular docking. These techniques may enable us to identify potential anti QSI molecules against LasR of *P.aeruginosa* thereby can be tested in-vitro and in-vivo and could be used as food-based drug against drug resistant *P.aeruginosa* infections. The goal of this study was to see how effective edible bioactive chemicals were at inhibiting quorum sensing by *In silico* method against nosocomial infection causing drug resistant *P. aeruginosa.*

MATERIAL AND METHODS

Selection of bioactive compounds

The molecular docking analysis was performed using SwissDock to understand docking interactions between edible bioactive compounds against quorum sensing regulated proteins of *P.aeroginosa*. Bioactive compounds from certain foods were selected based on the previous research records and are listed in the table 1.

Table 1: Food Source and bioactive compounds

FOOD SOURCE	BIOACTIVE COMPOUNDS
Milk	Bovine Lactoferrin
Green tea	(-) - epicatechin (EC)
	(-) – epicatechin-3-gallate(ECG)
	(-)-epigallocatechin(EGC)
	(-)-epigallocatechin-3-gallate(EGCG)
Zingiber officinale	6-gingerol
	8-gingerol
	10-gingerol
Curcuma amada	Cinnamic acid
	Ferulic acid
	P-coumaric acid
	Protocatechic acid
	Gentisic acid
	Gallic acid
Orange	Naringin
	Neohesperidin
	Hesperidin
Grapes	Rasveratrol
Lemon	D-Limonene
	Limonexic acid
Mango	Mangiferin(2-β-D-glucopyranosyl-1,3,6,7-tetrahydroxy-9H-Xanthen-9-one)
Pumpkin, Chilli, Eggplant	Zeaxanthin
Fish	Docohexaenoic acid
	Eicosapentanoic acid
Cauliflower	Quercetin

Retrieval of 3D Structures of Target and food compounds

Protein Data Bank (PDB)

The Protein Data Bank (PDB; http://www.rcsb.org/pdb/) is a global database that contains three-dimensional structural data for biological macromolecules such as proteins and nucleic acids. These structures are freely accessible and are submitted by the researchers from the biological methods like X-ray crystallography, NMR spectroscopy, or cryo-electron microscopy. The high resolution three dimensional structures of the target protein (PDB ID: 6MVN) used in this paper are from the PDB database and also we used the structure of known anti-quorum sensing compound bounded to our preferred targets [21].

PubChem

Millions of compound structures and descriptive datasets can be freely downloaded through FTP from PubChem (https://pubchem.ncbi.nlm.nih.gov), a public chemical information database at the National Center for Biotechnology Information (NCBI). Substance, Compound, and Bioassay are three interconnected databases created by PubChem. Here we are taking structures of 25 food derived compound from PubChem database. The downloaded structures are in SDF format, it can be converted to MOL2 format using chimera for docking process [22].

Molecular Docking of Bioactive Compounds to Target by SwissDock

Most of the biological processes are based on the interaction between molecules. These molecules maybe proteins, enzymes or drugs, and a convenient interaction between them gives the best result. Prediction of these interaction by a software is called Docking. In simple definition, docking is a molecular modelling method helps to predict how well a protein interacts with a ligand molecule. Docking methods are extremely useful in various areas of biological research, and obviously we need some of the docking software's to do these processes. The Swiss Dock web service is devoted to small molecule docking on target proteins. It offers simple docking algorithms that allow the target protein and ligand structures to be automatically prepared for docking. Furthermore, the docking runs do not require any computational resources from the user; the server handles all of the calculations. The UCSF Chimera molecular viewer can be used to analyse docking results, integrate them, and visualize docking predictions; it can be launched immediately from the web browser [25].

3D visualization and the structural analysis of molecular entities and the analysis of Fitness score by UCSF Chimera

It's a molecular modeling software for visualisation and study of molecular structures that enables for 3-D visualisation and structural analysis of molecular entities, as well as innovative extension features that improve researcher workflow. The Resource for Biocomputing, Visualization, and Informatics created Chimera (RBVI). This software includes a large number of capabilities that can be accessed by both command and graphical interfaces, and it allows you to create high-quality photographs and movies for publication and presentation. It has been widely used by researchers, instructors, and students in academia and industry.Chimera is an extensible visualisation system that allows third-party developers to add new features, such as Multiscale modes, which allows users to visualise large-scale molecular assemblies such as viral coats, and Multalign Viewer, which allows users to see multiple sequence alignments and associated structures [24].

RESULTS

Twenty five bioactive compounds from different food based molecules are collected from PubChem database and are subjected to molecular docking studies using SwissDock. The crystallographic structures of LasR protein with PDB ID: 6MVN is used as target. The molecular docking data were obtained from different edible bioactive compounds and its interactions between the secondary metabolites and the target receptors such as LasR protein. These results of in silico molecular dockings studies evidenced the possible regulatory role of the phytochemicals present in the natural resources.

Table 2: Docking scores of known inhibitor

Target name	Ligand	Cluster Number	No of members in the cluster	FullFitness (kcal/mol)	Estimated ΔG (kcal/mol)
LasR protein (PDB ID: 6mvn)	3-oxo-N-[(3S)-2-oxotetrahydrofuran-3-yl] decanamide (C_{14} H_{23} N O_4)	0	10	-1022.15	-10.05
		1	5	-1004.27	-8.28
		2	7	-997.07	-8.3
		3	8	-992.14	-6.78

Table 3: Docking scores of those compounds that had given best score similar to score of known inhibitor

Target Name	Ligand	Cluster Number	No of members in the cluster	FullFitness (kcal/mol)	Estimated ΔG (kcal/mol)
LasR protein (PDB ID: 6mvn)	Oleic acid	0	8	-1007.16	-6.93
		1	8	-1006.77	-7.06
		2	8	-1006.54	-6.88
		3	8	-1006.5	-6.89
	6-Gingerol	0	7	-978.66	-9.01
		1	4	-978.6	-8.52
		2	8	-967.97	-6.41
		3	8	-967.74	-6.67
	8-Gingerol	0	8	-973.62	-6.74
		1	8	-972.34	-6.88
		2	8	-972.22	-6.86
		3	8	-971.2	-7.12
	Reseratrol	0	4	-982.2	-7.31
		1	0	-981.14	-7.23
		2	10	-979.84	-7.09
		3	3	-979.06	-7.02
	Gentisic acid	0	17	-978	-6.29
		1	8	-977.97	-6.51
		2	8	-977.53	-6.46
		3	12	-977.48	-6.45
	Eicosapentanoic acid	0	8	-978.54	-7.38
		1	2	-978.54	-7.38
		2	8	-976.72	-7.09
		3	8	-976.59	-6.59
	Docosahexaenoic acid	0	8	-979.41	-7.62
		1	8	-978.47	-7.24
		2	8	-978.1	-7.13
		3	8	-977.08	-7.27
	Coumaric acid	0	8	-987.18	-6.98
		1	6	-986.53	-6.71
		2	8	-985.47	-6.63
		3	8	-983.13	-6.52
	Caffeic acid	0	8	-984.55	-7.11
		1	11	-983.75	-6.8
		2	14	-982.85	-6.82
		3	8	-978.64	-7.25
	Ferulic acid	0	11	-975.75	-7.12
		1	16	-974.18	-7.15
		2	3	-969.7	-6.83
		3	12	-952.48	-5.9
	Protocatechuic acid	0	2	-963.27	-6.31
		1	13	-963.19	-6.49
		2	4	-962.44	-6.47
		3	4	-962.43	-6.44
	Limonene	0	13	-969.12	-6.71
		1	8	-968.09	-6.66
		2	2	-966.73	-6.53
		3	10	-963.47	-6.32
	Quercetin	0	8	-949.04	-8.09
		1	8	-943.44	-7.53
		2	8	-938.28	-7.41
		3	8	-937.95	-6.55

The compound that is extensively used for inhibiting the biofilm formation of *Pseudomonas aeruginosa* was docked for the comparison purpose. The results obtained from Swiss Dock had been analyzed using UCSF chimera and full fitness and ΔG Kcal/mol are noted in the table 2.

From the molecular docking studies, 10 food components, 6-gingerol, 8-gingerol,Gentisic acid, Caffeic, Ferulic, Coumaric acid, Protocatechuic acid, Reseratrol, Oleic acid, Eicosapentaenoic acid, Docosahexaenoic acid, Limonene, Quercetin have similar docking scores with the know inhibitor. The compounds that given the best docking scores are given in Table 3. The ribbon representation with interaction of selected compounds were given in Fig.1.

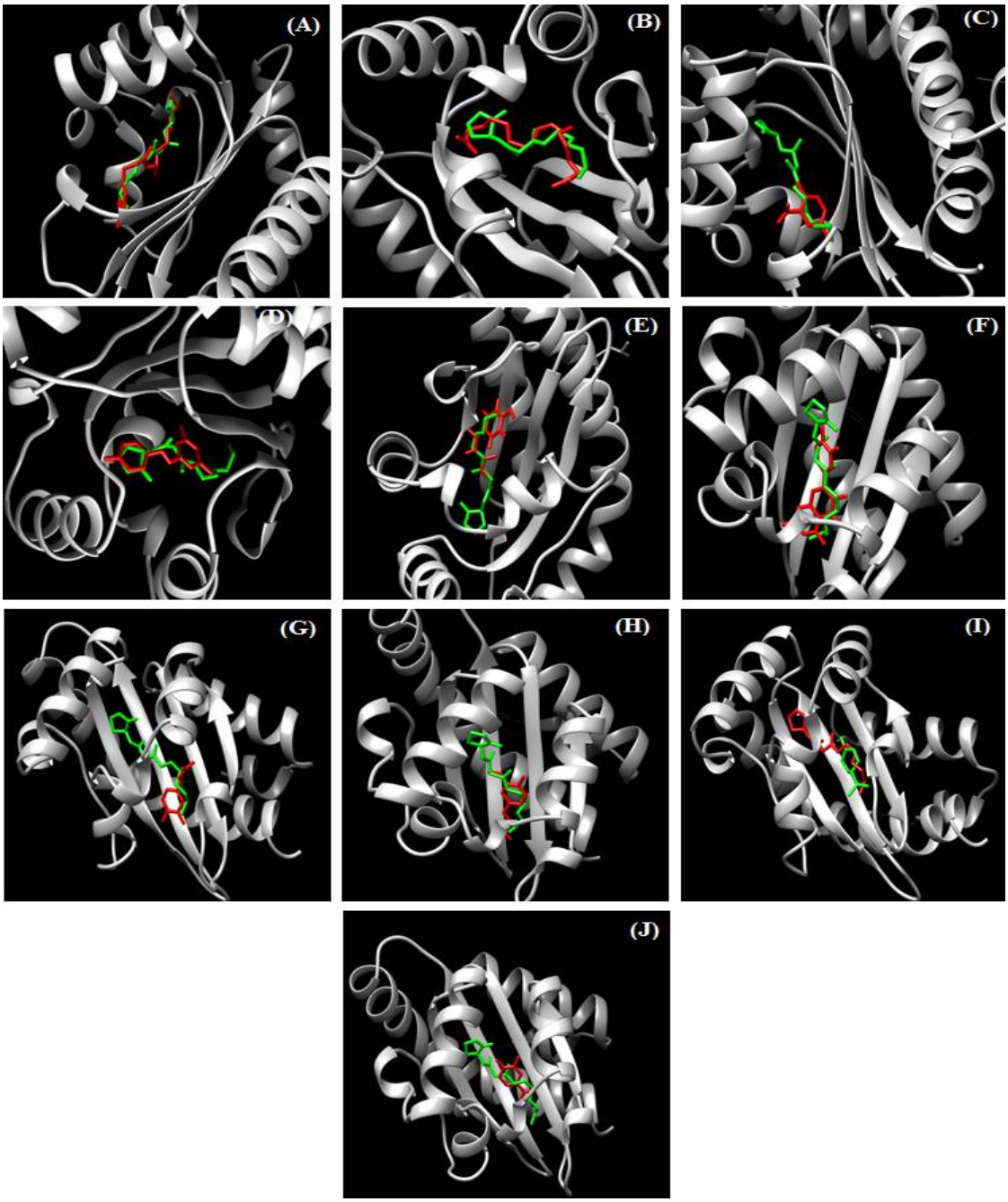

Fig.1: Ribbon representation of LasR protein docked with 8-Gingerol (A), Oleic acid (B), Reseratrol (C), Gentisic acid (D),6-Gingerol (E) , Quercetin (F), Caffeic acid (G), Ferulic acid(H),. Limonene(I), Protocatechuic acid (J). The red coloured ligand is the food compounds and green coloured ligand is the original inhibitor.

DISCUSSION

Pseudomonas aeruginosa is a common opportunistic bacteria that causes nosocomial infections that are associated with greater morbidity and death, especially in immunocompromised and intensive care unit patients. [36]. As many of the microorganisms are evolving against various currently available drugs, the

need for new therapeutics which can be used as an alternative is increasing day by day. Instead of drugs that kill the bacteria or disrupt pathogen, the modern approaches are focusing to interfere and inhibit the virulence of pathogens. For bacteria that forms biofilm, interfering in the QS activity might be an efficient way as it's needed for the cell-cell communication. In the present study Twenty-five bio active compounds from different natural food sources are collected from PubChem database and are subjected to molecular docking studies using SwissDock to find their anti-quorum sensing activity on LasR target of *P.aeruginosa*. The crystallographic structures of LasR protein with PDB ID: 6MVN is used as target for the study. For comparison the compound 3-oxo-N-[(3S)-2-oxotetrahydrofuran-3-yl] decanamide (C_{14} H_{23} N O_4), which is extensively used for inhibiting the *P.aeruginosa* biofilm was docked and the results obtained from Swiss Dock had been analyzed using UCSF chimera and full fitness and ΔG Kcal/mol are noted (Table: 2). The scores of the compounds subjected to current study are compared with 3-oxo-N-[(3S)-2-oxotetrahydrofuran-3-yl] decanamide (C_{14} H_{23} N O_4),and Table: 3 shows the 13 compounds having the best similar scores. Quorum sensing inhibition in *Pseudomonas aeruginosa* using gram negative bacteria *Delftia tsuruhatensis* 11304 wast studied by Malešević et al. 2019 and they demonstrated a novel AHL species, dihydroxy-N-octadecanoylhomoserine lactone. Quorum sensing activity of *P. aeruginosa* enables the formation of a biofilm that confers the bacteria with certain properties that make it even more lethal and resistant to antibiotics. It is relatively easier to prevent biofilm formation, than to disrupt it once already established [34-37]. As antibiotic resistance is an evolving trait in microorganisms, there is a need for strategies which help to find effective drug against these microorganisms. Screening natural compound against potential targets is a useful approach for designing drugs. The sequencing of specific target genes may useful to detect the molecular mechanism through *in silico* studies and also public health threat of antimicrobial resistance.

CONCLUSION

The current in silico study of twenty-five different bioactive compounds commonly found in various food sources against *P. aeruginosa* receptor protein LasR found that ten molecules had similar high docking scores with the known inhibitor and thus can be used as potential inhibitors of *P. aeruginosa* biofilm formation. The compounds that showed the high fitness scores have to be suggested for further in vitro and in vivo studies to ensure its potential. Further gene expression and molecular interaction studies are also needed for understanding more about these quorum quenching molecules and their mode of action in depth. The potential quorum sensing inhibitors can be developed as new effective drug candidates and may help in effective treatment along with the current antibiotics.

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AUTHOR CONTRIBUTIONS

All the authors contributed equally for this research work.

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CONFLICT OF INTEREST

The authors have no conflict of interest.

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