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Molecular Docking Studies of Putative Inhibitors of *Acinetobacter Baumannii* Biofilms against CsuC-CsuA/B Chaperone Usher Complex

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ABSTRACT

Acinetobactor baumannii is Gram negative bacillus is an opportunistic pathogen responsible for worldwide nosocomial infections. Certain strains of this multidrug resistant bacteria are resistant to available antibiotics and the development of new active antimicrobial and antibiofilm agents are the need of the present. The biofilm formation depends on the filamentous organelle pili which are assembled by the CsuA/BABCDE chaperon usher system. TheCsu C protein of the chaperone usher system in *Acenitobacter baumannii* is a potential target for the design of drugs and antimicrobials. Plant obtained natural products have proven to be effective compounds with unique properties, thus making them safer drug candidates and efficient antibiotic adjuvants. This paper demonstrates the molecular interaction of Csu C with five phytochemical compounds namely berberine, ellagic acid, eugenol, carvacrol and curcumin. The molecular docking and virtual screening results revealed all compounds to exhibit good docking scores. Among the five compounds tested, ellagic acid showed the most effective binding activity -8.8 kJ with Csu C indicating its ability to inhibit the formation of Acenitobacter biofilms. The high docking score of ellagic acid suggested that this compound may bind the active site of Csu C effectively and the important amino acid residues in the active region of the protein should be considered while designing drugs that can effectively inhibit the biofilm formation in multidrug resistant strains of *Acinetobacter baumannii*.

KEY WORDS: CsuC-CsuA/B chaperone usher complex, *Acinetobacter baumannii*, biofilm, plant active compounds, molecular docking.

1. INTRODUCTION

Acinetobactor baumannii is gram negative bacillus which is aerobic, pleomorphic, non-motile in nature. (Howard *et.al*, 2013) There are numerous multidrug resistant bacillus, *Acinetobactor baumannii*, is one of the opportunistic pathogen which lead to difficulty in treatment, due to this invasion nosocomial infections are increasing which is affecting ventilator associated pneumonia patients along with urinary tract infections, and meningitis. (Berogogne and Towner, 1996). It has been shown to have an ability to survive for a long time under a very dehydrated conditions on abiotic surfaces like glass and equipment used in intensive care units in the form biofilms and even on biotic surfaces such as epithelial cells (Jawad, 1998; Wendt, 1997).

Generally the gram-negative bacteria target to sites of infection through the expressions of fibrous adhesive organelles. This adhesive pili (or fimbriae) is assembled through various the classical, alternative and archaic chaperone-usher (CU) pathways (Nuccio and Baumler, 2007) the archaic Csu pili mediate in the formation of *Acinetobacter baumannii* biofilms which is formed with the help four subunits, namely CsuA/B, CsuA, CsuB, and CsuE, and they are assembled using the CsuC-CsuD chaperone-usher secretion machinery (Tomaras, 2003; Tomaras, 2008) workers proved the expression of of CsuA/BABCDE -dependent pili , it is a part of gene cluster which is related to bacterial loci encoding secretion and pili assembly functions. It is found that the production of pili are required in the early steps of the process that leads to biofilm formation. (Luo, 2015)

In due course of time *Acinetobacter* species has acquired resistance against several antibiotics so for providing effective treatment, several alternate therapy is being searched which has given a pathway for the use of phytochemicals. Since the past decade these products have played an important role as major sources of new drugs (Chastre, 2003). The naturally occurring molecules may prove to become a new source of antibacterial and anti-biofilm drug for clinical purpose. Amongst the various groups polyphenols represent a class of plant natural products which are important as well as in plant defense against microbial pathogens (Slobodnikova, 2015). Based on the existing knowledge the present work has been taken on selected five plant active compounds i.e. berberine (CID 2353), ellagic acid (CID 5281855), eugenol (CID 3314), carvacrol (CID 10364) and curcumin (CID 969516) as ligands and performed molecular docking studies against CsuC protein of the CsuA/B chaperone usher complex of *Acinetobacter baumannii* biofilm mediating pili in order to observe the binding affinity of these compounds.

2. METHOD

Hardware and software: The present study was carried out on Apple Macbook Air, with 1.6 GHz Intel Core i5 processor, 8 GB 1600 MHz DDR3 memory, Intel HD Graphics 600 1536 MB running on macOS Sierra version 10.12.6. Schrodinger Maestro 11 (for academic use), PyRx 0.8, AutodockVina, Deepsite, online resources like NCBI, PDB, were used.

Sequence retrieval and Protein crystal structure: The amino acid sequence of CsuC-CsuA/B chaperone-major subunit pre-assembly complex from Csu biofilm-mediating pili of *Acinetobacter baumannii* was retrieved from NCBI database (NCBI Resource Coordinators, 2017). 3D crystal structure of the protein was obtained from PDB (Berman, 2000) [pdb id: 5D6H].

Protein preparation: The typical structure file may consist of co-crystallised ligands, water molecules, hetero atoms, missing or incomplete residues and atoms which is not suitable for immediate use for molecular modelling studies. Hence, the protein structure PDB entry 5D6H had to be prepared for further analysis. Protein pK predictions were performed using PROPKA (Olsson, 2011). Assigning bond orders, addition of hydrogen, deletion of waters beyond 5A from het groups, optimizing H-bonds were done using the protein preparation wizard of Maestro 11 A Graphical User Interface for Schrodinger Suite of products (Schrodinger Suite, 2012).

Binding site predictions: The potential binding pocket centres of the protein with the highest score was determined by Deep site (Jimenez, 2017).

Ligand preparation: Chemical structures of ligands berberine (CID 2353), ellagic acid (CID 5281855), eugenol (CID 3314), carvacrol (CID 10364) and curcumin (CID 969516) used in this study were obtained from PubChem database (Kim, 2015). Lead optimization in PyRx which include Open Babel, ligand energy minimization interface with UFF (United Force Field) with a limit of 500 iterations for each ligand was performed (Dallakyan and Olsson, 2015).

Conversion of raw files of ligand structures to PyRx supported format pdbqt was done using iBabel.

Molecular docking: Docking studies were performed using AutodockVina wizard in Pyrx.

Autodockvina is a widely used open source molecular docking program offering multi-core capability, high performance and enhanced accuracy and ease of use (Trott and Olson, 2010). The active drug compounds were docked with CsuC-CsuA/B chaperone-major subunit pre-assembly complex protein around its important binding site residues with an optimal grid area of 39x33x49Å.

Ligand receptor interactions: The interactions of the protein CsuC of the CsuC-CsuA/B chaperone-major subunit pre-assembly complex and the five ligands in the docked complex were analysed by Ligand Interaction Diagram (LID script) in Maestro 11 by Schrodinger.

3. RESULTS AND DISCUSSIONS

The binding efficacies of the five compounds berberine (CID 2353), ellagic acid (CID 5281855), eugenol (CID 3314), carvacrol (CID 10364) and curcumin (CID 969516) against Csu C protein (PDB: 5D6H) have been evaluated and the docking interactions studied (Table.1).

Table.1. Docking result of five active compounds against Csu C protein of the CsuC-CsuA/B chaperone-major subunit pre-assembly complex

Ligands	Pub chem id	Molecular weight (g/mol)	Docking score (kJ/mol)	Non bonded interaction	H bond interaction
Berberine	2353	336.367	-8.6	LYS204,VAL203,ALA193,PHE194,GLY195,TYR196,ASP47,ASN151,PHE152,THR69	-
Ellagic acid	5281855	302.194	-8.8	VAL66,ASP67,PHE68,THR89,PHE113,PR0110,GLN109,SER117,PHE151,PRO119,TRP40,GLN42,ASP47,TYR49	GLN112,ASP47
Eugenol	3314	164.204	-6.2	TYR196,PHE194,ALA193,PHE113,ARG174,SER176,GLY195,PHE194,PHE68,THR69,ASP67,VAL224,ASP225,SER226	GLN112
Carvacrol	10364	150.221	-6.1	SER227,ILE229,LEU131,SER226,ASP64,TYR9,ILE8,PRO7,SER10,THR11,HIS8	-
Curcumin	969516	368.385	-7.9	LEU175,TYR196,GLY195,PHE194,ALA193,ASP67,PHE68,THR69,PHE152,ASN151,ASP47,TYR49,SER117,ARG89	GLN112

Docking interaction of Berberine: Berberine (CID: 2353) exhibited a docking score of -8.6kJ/mol with Csu C protein of the CsuC-CsuA/B chaperone-major subunit pre-assembly complex from biofilm mediating pili of *A. baumannii*. The binding pocket was occupied and the residues that favoured the interaction were LYS204, VAL203, LA193, PHE194, GLY195, TYR196, ASP47, ASN151, PHE152, THR69. The docked complex, the ligand interaction is shown in fig.1.

Figure.1. Molecular docking complex and binding interaction of berberine (CID 2353) with CsuC-CsuA/B chaperone usher complex protein. The residues of binding pocket are shown as wires while ligand is represented as ball and stick style in green colour

Docking interaction of Ellagic acid: Ellagic acid (CID: 5281855) exhibited a docking score of -8.8kJ/mol with Csu C protein of the CsuC-CsuA/B chaperone-major subunit pre-assembly complex from biofilm mediating pili of *A. baumannii*. The binding pocket was occupied and H-bond interactions between GLN112, ASP 47 and oxygen atom favoured the binding. The residues that favoured the interaction were VAL66, ASP67, PHE68, THR89, PHE113, PR0110, GLN109, SER117, PHE151, PRO119, TRP40, GLN42, ASP47 and TYR49. The docked complex, the ligand interaction is shown in fig.2.

Figure.2. Molecular docking complex and binding interaction of ellagic acid (CID: 5281855) with CsuC-CsuA/B chaperone usher complex protein. The residues of binding pocket are shown as wires while ligand is represented as ball and stick style in green colour

Docking interaction of Eugenol: Eugenol (CID: 3314) exhibited a docking score of-6.2kJ/mol with Csu C protein of the CsuC-CsuA/B chaperone-major subunit pre-assembly complex from biofilm mediating pili of *A. baumannii*. The binding pocket was occupied and H-bond interactions between oxygen atom and GLN112 favoured the binding. The residues that favoured the interaction were TYR196, PHE194, ALA193, PHE113, ARG174, SER176, GLY195, PHE194, PHE68, THR69, ASP67, VAL224, ASP225 and SER226. The docked complex, the ligand interaction is shown in fig.3.

Figure.3. Molecular docking complex and binding interaction of Eugenol (CID: 3314) with CsuC-CsuA/B chaperone usher complex protein. The residues of binding pocket are shown as wires while ligand is represented as ball and stick style in green colour

Docking interaction of Carvacrol: Carvacrol (CID: 10364) exhibited a docking score of -6.1 kJ/mol with Csu C protein of the CsuC-CsuA/B chaperone-major subunit pre-assembly complex from biofilm mediating pili of *A. baumannii*. The binding pocket was occupied and the residues that favoured the interaction were SER227, ILE229, LEU131, SER226, ASP64, TYR9, ILE8, PRO7, SER10, THR11 and HIS8. The docked complex, the ligand interaction is shown in fig.4.

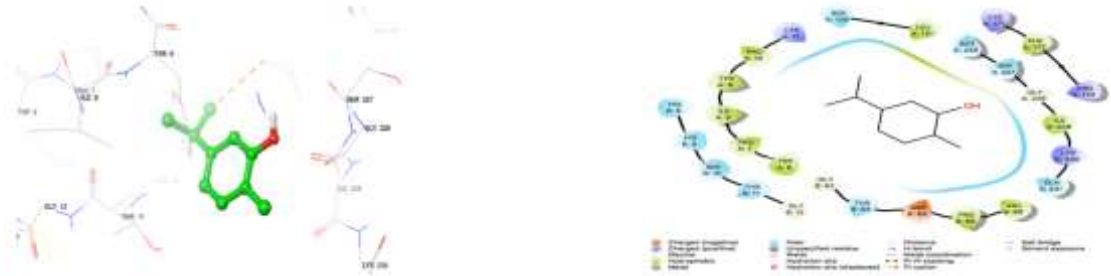

Figure.4. Molecular docking complex and binding interaction of carvacrol (CID: 10364) with CsuC-CsuA/B chaperone usher complex protein. The residues of binding pocket are shown as wires while ligand is represented as ball and stick style in green colour

Docking interaction of Curcumin: Curcumin (CID: 969516) exhibited a docking score of -7.9 kJ/mol with CsuC protein of the CsuC-CsuA/B chaperone-major subunit pre-assembly complex from biofilm mediating pili of *A. baumannii*. The binding pocket was occupied and H-bond interactions between oxygen atom and GLN112 favoured the binding. The residues that favoured the interaction were LEU175, TYR196, GLY195, PHE194, ALA193, ASP67, PHE68, THR69, PHE152, ASN151, ASP47, TYR49, SER117 and ARG89. The docked complex, the ligand interaction is shown in fig.5.

Figure.5. Molecular docking complex and binding interaction of curcumin (CID: 969516) with CsuC-CsuA/B chaperone usher complex protein. The residues of binding pocket are shown as wires while ligand is represented as ball and stick style in green colour

Formation of biofilm in *A. baumannii* has been shown to have positive correlation with the expression of extracellular polysaccharide poly-β-(1,6)-N-acetyl glucosamine (PNAG), outer membrane protein Omp A, and chaperone usher pili assembly system (Zarrilli, 2016). It has been previously reported that the proteins in the Csu chaperone usher system are an attractive target for the design of drugs and antimicrobials (Pakharukova, 2015). Thus, the consideration of CsuC protein of the Csu-CsuA/B chaperone usher system as a target to design anti-biofilm agents have been made.

Berberine has been shown to act as an adjuvant therapeutic agent for the prevention of biofilm-related infections in *S. epidermidis* (Wang, 2009). Another study showed that ellagic acid from plants where it acts as anti-biofilm agents. This substance fights against bacterial infections caused by *E.coli* and inhibit biofilm formation (Hancock, 2010). In a Transcriptional analysis study, it was found that Eugenoldown-regulated 17 out of 28 genes responsible for biofilm formation and attachment in *E.coli* (Yong-Guy, 2016). Works also revealed carvacrol to interfere with the Quorum Sensing (QS) signalling mechanism in *E.coli* cells, which leads to reduction of biofilm formation (Burt, 2014). Similarly, curcumin was found to be a potential inhibitor of genes responsible for biofilm initiation as well as an inhibitor of 31 quorum sensing genes thus strengthening its possibility of being an antibiofilm agent in *P.aeruginosa* infections (Soheil, 2014). These findings support the present work of antibiofilm activity of the selected five natural active compounds i.e. berberine, ellagic acid, eugenol, carvacrol and curcumin against *A.baumannii*. The present work, revealed that all have effectively occupied the binding pockets with ellagic acid, eugenol and curcumin forming H-bond interactions.

4. CONCLUSION

The virtual screening results of the five phytochemical compounds was evaluated by their binding affinities against CsuC protein. The molecular docking studies suggested that all the compounds were effective in occupying the binding regions and can cause inhibition of biofilm formation. The amino acid Glutamine (GLN112) in the active site favours the H-bond interactions in ellagic acid, eugenol and curcumin. This further suggests that the compounds ellagic acid, followed by eugenol and curcumin could be a potent inhibitor for Acinetobacter biofilms and throws light on the possibility to design novel anti-pathogenic drugs for combating Acinetobacter biofilms.

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