Original Research Article

EFFECT OF OCIMUM SANCTUM BIO COMPOUNDS AGAINST csuE GENE PROTEIN OF *ACINETOBACTER BAUMANII*

Running title: Drug ligand interactions of *O.sanctum* with csuE of *A.baumannii*

ABSTRACT

Background:

Acinetobacter baumannii is a Gram-negative bacillus that is aerobic, pleomorphic and non-motile. Multi-drug resistance and biofilm formation contributes to the virulence and pathogenicity of the bacterium. Among many virulence factors, csuE is critical for initiation and assembly, showing much homology to type 1 and P pili. With much propensity of drug resistance, in recent years alternative medications have spurred renewed interest in targeting potent pathogens. *Ocimum sanctum*, also known as holy basil or tulsi possess various bio-active properties and can be used as alternative medicine to treat systemic ailments.

Aim:

This study was aimed to analyze the drug-ligand interactions between csuE protein of *A.baumann*ii and the bio-compounds from *O.sanctum* using in-silico docking analysis.

Material and Methods:

csuE protein was retrieved and optimisation of protein was done. Ligands were selected and were assessed for drug likeness using molinspiration parameters. Further the compounds were subjected for docking analysis and the interacted molecules were visualized for binding energy and hydrogen bonds.

Results:

Out of the 9 compounds of *Ocimum sanctum*, benzofuran showed good interaction with csuE protein of *Acinetobacter baumannii* with a least docking energy of -5.31Kcal/Mol.

Conclusion:

The present study recommends benzofuran as the potent candidate for novel drug design to treat the infections caused by *A.baumannii* upon further evaluations for its safety and immunological response.

Keywords: *A.baumannii; O.sanctum*; novel benzofuran; Drug ligand; innovative interactions; Environmental strains

Introduction

Acinetobacter baumannii is a Gram-negative bacterium that is normally small, almost oval, and rod-shaped. It may be an opportunistic pathogen in humans, targeting patients with weakened immune systems, and it is becoming more common as a hospital-acquired infection (1). These are non-motile but possess a typical motile behaviour through type IV pili, which are pole-like structures that can stretch and retract (2). Organisms of the genus *Acinetobacter* are often thought to be widespread in nature inhabiting soil and water. *A. baumannii* is a member of the ACB complex (*A. baumannii, A. calcoaceticus*, and Acinetobacter genomic species 13TU). The unique species of ACB complex members are difficult to identify, and they are the most scientifically important members of the genus. *Acinetobacter baumannii* has also been classified as an ESKAPE pathogen *(Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, and Enterobacter* species), which is responsible for the majority of nosocomial infections (3). Because of its apparent abrupt appearance in military treatment facilities during the Iraq War, *A. baumannii* is colloquially known as "Iraqibacter." (4). Extensive knowledge and research experience in the present research area by our team has been translated into high quality publications and has been utilized in the present study (5–15).

Multi-drug resistance and biofilm formation contribute to its virulence and pathogenicity of the bacterium. Among many virulence factors, csuE must be critical for initiation of assembly, showing much homology to type 1 and P pili. csuE is completely abrogated in the pilus assembly and folded into beta barrel domains. The angle between the domains gives the molecule an overall C like shape, bending over domains of csuE. csuE pilus is elaborated from four protein

subunits, csuA/csuB and csuE via archaic chaperone usher pathway. Archaic CU pill constitute the foremost important family of CU systems and along with the choice of CU family form the 'non classical' branch of CU superfamily. csuE associated virulence is documented in many earlier reports portraying its importance in the pathogenesis of *A.baumannii* (16) It is now a challenging task to treat the patients infected with *A.baumannii* due to its severity of multi-drug resistance and biofilm formation.

In this line, alternative medication plays a vital role in the eradication of the pathogen. The aromatic perennial herb *Ocimum sanctum*, also known as holy basil or tulsi, belongs to the *Lamiaceae* family. It is indigenous to the Indian subcontinent and is widely grown in the Southeast Asian tropics. *Ocimum Sanctum* is responsible for its various medicinal properties and their effects at the molecular level need to be investigated in more detail (17). Tulsi is cultivated for its natural oil, as well as for religious and herbal medicine purposes. It is widely used as a herbal tea, is commonly used in Ayurveda, and plays a role in Hinduism's Vaishnava culture, where devotees practise worship using holy basil plants or leaves. Similar in-silico based studies have been referred to design the present study and the expertise as received from previous literatures were also incorporated for the same. With the advent of science and technology, the drug ligand interaction studies are possible with the wide computational platform of bioinformatics databases and tools. The present investigation was thus aimed to target the csuE protein of *A.baumannii* using the bio-compounds from *O.sanctum.*

Materials And Methods

Study setting: This is an observational in-silico study done in the Department of Microbiology, Saveetha Dental College and Hospital. Institution approval for research was obtained.(IHEC/SDC/UG-1942/21/155)

csuE Retrieval And Optimisation Of Protein

The crystal structure of csuE was obtained from RCSB protein data bank. The optimisation of csuE, which is crystal structure, done by addition of hydrogen atoms. Kollman united atoms force field was used to assign electronic charges to the protein atoms which was done in AutoDock tool- 1.5.6 and the RASMOL tool was used for the visualisation of three dimensional

structure of csgA protein. (Citation(s))

Optimisation And Ligand Preparation (Reference(s))

The structures of the bioactive derivatives of *A. baumannii* were obtained from the chemsketch software . The generated 3d structures were then optimised. The selected ligands were then optimised. The selected ligands were subjected to subsequent conversions by open label molecular converter to subsequent conversions by open label molecular converter program. They were then saved in PDB format. The selected ligands were further saved in a molecular file.

Molinspiration Assessment Of The Molecular Properties Of Selected Compounds

The counts of hydrogen bond acceptors and donors in relation to the membrane permeability and bio availability of the compounds, log P for partition coefficient, molecular weight of compounds of basic molecular descriptions were assessed with the help of molinspiration assessment program. The characteristics of absorption, distribution, metabolism and elimination of the selected bio compounds were further evaluated on the basis of "The Lipinski's rule of five". (reference(s))

Docking Stimulation

AUTODOCK tool was used for docking analysis to interpret the affinity between the bio compounds of *A. baumannii* against csuE protein.

Docking Visualisation

Using the discovery studio visualiser, the hydrogen bond interaction between the biocompounds of *A. baumannii* against csuE proteins were visualised. With further docking score assessments, binding affinities, molecular dynamics and energy simulations, the relative stabilities were evaluated. (citation(s))

Results

The sequence of csuE from *A. baumannii* was retrieved from the uniport database and its sequence is Q6XBY2. The structure of csue was not available in the PDB database. Hence it was

modeled using swiss model server using the template 6FJY-B chain. The modeled structure was found to be highly plausible as it had 100% sequence identity with that of the template. Moreover, the Ramachandran plot also showed 94% of residues in the disallowed region.

Structural Retrieval Of csuE Protein From *A. baumannii*

FASTA sequence of csuE from *A. baumannii* was retrieved from UNIPORT database and its sequence ID was Q6XBY2. Using the swiss model server, the homology model was made with 6FJY-B chain as template. The model was highly plausible with 100% sequence identity with the template. Besides, the Ramachandran plot showed 94% of the residues in the disallowed region. The 3d structure of the csuE was visualised using RASMOL with the pink coloured denoting the alpha helix, yellow arrow denoting the beta sheets and white colour denoting the turns.

Molinspiration Assessment Towards Drug Likeness

Based on the calculation of the ion channel modulation, GPCR ligand, nuclear receptor ligand, kinase inhibitor, enzyme inhibition and protease inhibition, the bioactivity score prediction of essential compounds of *A.indica* against *A.baumannii* towards drug likeness was accessed and tabulated.

Table 1 showing the molinspiration results of the *O.sanctum* selected compounds

Compounds	**M.wt**	**Hydrogen Bond Donor**	**Hydrogen Bond Acceptor**	**miLogP**	**Rotatable bonds**	**nViolations**	**TPSA (Å)**	**Volum**
Estragole	148.21	0	1	2.82	3	0	9.23	154.12
Eugenol	164.20	1	2	2.10	3	0	29.46	162.14

Methyleugenol	18.47	0	2	2.41	0	0	18.47	179.67
Benzofuran, 7-(2,4-dinitrophenoxy) -3-ethoxy2,3-dihydro-2,2-dimethyl	374.35	0	9	4.49	6	0	119.35	318.05
Hexahydro-1,6-dimethyl-4- (1-methylethyl)-	220.36	0	1	4.66	1	0	17.07	238.11
Citral	152.24	0	1	3.65	4	0	17.07	169.74
Ceftazidime	546.59	4	13	–5.68	9	2	191.23	439.78

Table 2 showing overall docking energies of the drug-ligand interactions

CsuE docking with compounds	**Number of hydrogen bonds**	**Binding energy**	**Ligand efficiency**	**Intermolecular energy**	**vdW + Hbond + desolv Energy**	**Electrostatic energy**	**Torsional energy**	**Total internal Unbound**
Estragole	1	-4.9	-0.45	-5.79	-5.74	-0.05	0.89	-0.18
Eugenol	2	-4.59	-0.38	-5.78	-5.65	-0.14	1.19	-0.71
Methyleugenol	2	-4.89	-0.38	-6.08	-6.00	-0.09	1.19	-0.49
Benzofuran, 7-(2,4-	4	-5.31	-0.2	-7.1	-6.38	-0.72	1.79	-0.79

dinitrophenoxy)-3-ethoxy2,3-dihydro-2,2-dimethyl								
Hexahydro-1,6-dimethyl-4- (1-methylethyl)-	1	-5.73	-0.36	-6.03	-5.97	-0.06	0.3	-0.28
Citral	1	-4.1	-0.37	-5.29	-5.28	-0.01	1.19	-0.37
Ceftazidime	5	-5.35	-0.14	-8.63	-7.99	-0.65	3.28	-2.41

Table 3 showing overall binding interactions among the csuE and *O.sanctum*

KPSM docking with compounds	**Hydrogen bonds interactions**	**van der Waals interactions**	**π-σ interactions/ π-π T-shaped interactions/ amide-π stacked interactions**	**alkyl/π-alkyl interactions**	**Other interactions**
Estragole	ASN57	SER58, GLY198, GLY59, SER32, SER37	-	LEU200, LEU154, PHE56	THR36 (carbon hydrogen bond)

Eugenol	ASN57 SER32	GLY198, SER58, LEU197, GLY35, SER34	-	LEU154	THR36 SER37 (carbon hydrogen bond)
Methyleugenol	ASN57 SER32	SER61, SER32, GLY59, SER196, LEU197, SER58, GLY198, SER37, GLY35, THR36	-	LEU154	-
Benzofuran, 7-(2,4-dinitrophenoxy)-3-ethoxy2,3-dihydro-2,2-dimethyl	ALA139 (2) ARG136 SER53	GLU51, SER53, ASN52, LEU137, GLY50, GLU48	ASN49	PRO138 ARG136 ASN49	-
Hexahydro-1,6-dimethyl-4- (1-methylethyl)-	VAL215	ASP214	-	LEU147 (2) VAL333 ILE274 PHE312 VAL335(2) VAL233	-
Citral	THR36	LEU197, GLY59, ASN57, GLY35, SER37, GLY198, SER199, SER32	-	LEU200(2) PHE56 LEU154	-
Ceftazidime	ARG236 ILE210 (2) ALA212 (2)	GLU48 GLN213	LEU211	ALA212 ILE210	-

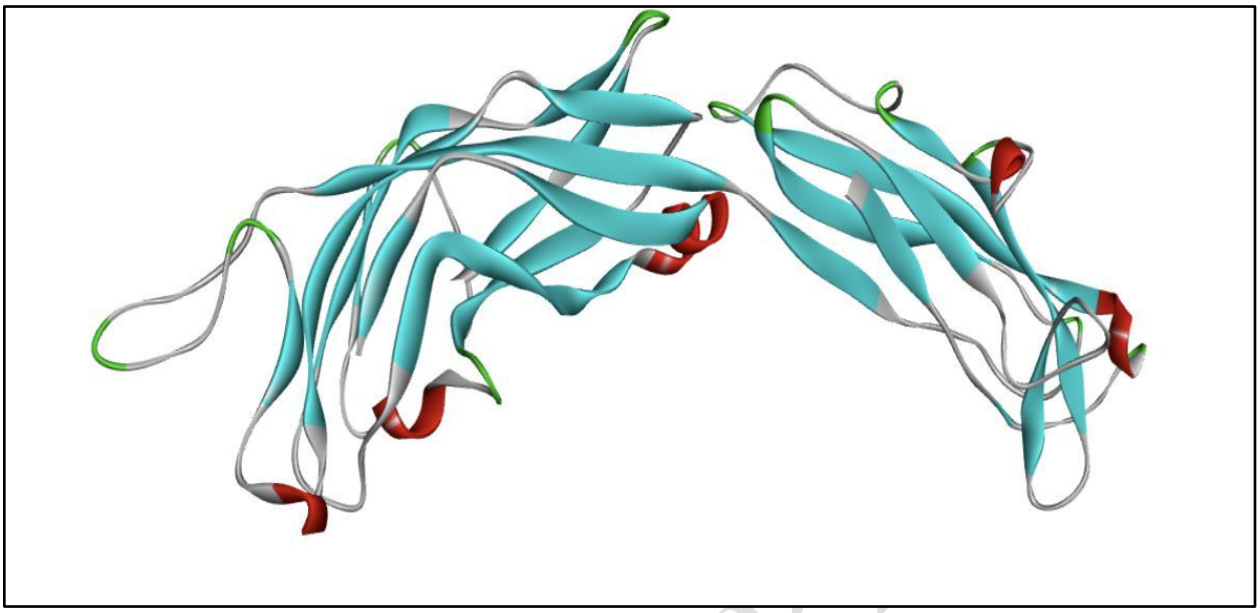

Figure 1: Structure Prediction of CsuE by Homology modeling using Swissmodel server

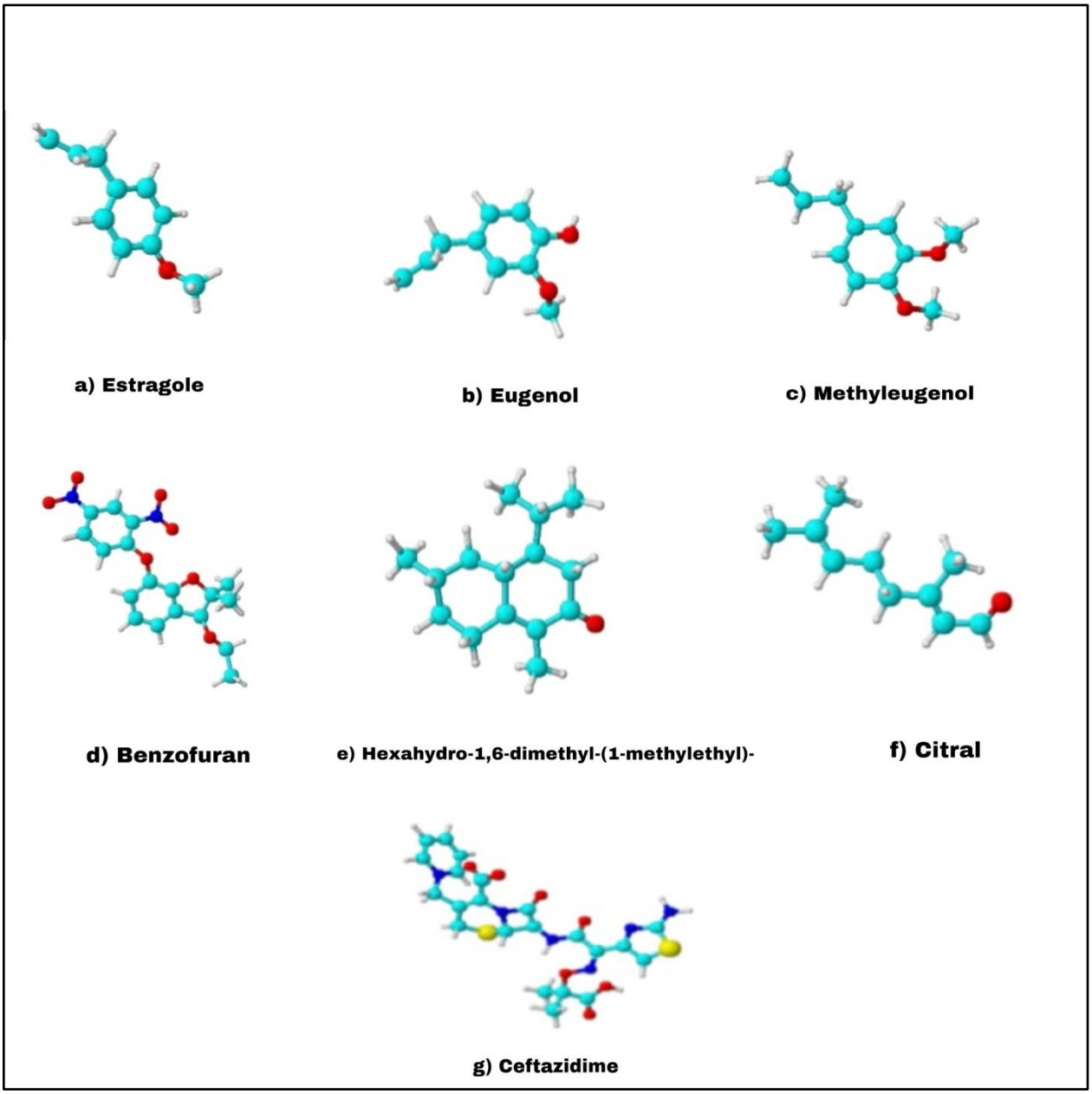

Figure 2: 3D structures of the *O.sanctum* compounds selected for the study

Discussion

The potent virulence factor of *A. baumannii* is its ability to form biofilms in a major step process viz, attachment of bacteria to the surface, formation of micro colony, maturation of biofilms and compounds for the in vitro analysis. (reference(s)) Selection of bioactive compounds from *Ocimum sanctum* was done based on the available earlier literature. (reference(s)) The compounds are vital for transforming into nano-formulations as well due to their hydophilicity. With the aid of computational bioinformatics tools and databases, the drug ligand interactions were analyzed based on the pose and strength (18). Drug likeness was highly promising from the molinspiration results with no violations for all the selected bio compounds except bis(2- propyl pentyl) phthalate ester.

The drug molecules that have low molecular weight (<500) are transported, diffuse band absorbed without difficulty in comparison to large molecules. Molecular weight is one of the critical aspects in corrective drug action; if it seems to increase correspondingly, which affects the efficiency of the drug. Some hydrogen bond donors and a number of hydrogen bond acceptors are natural compounds as established in Lipinski's limit range 1-16 and 1-11 that come out to be less than 10 and 5 (19–23)

The molecules are more flexible when the number of rotatable bonds increases and more susceptible to proficient interactions with a precise binding pocket. Selected five commonly used aromatic profiles of essential oil of ocimum sanctum were estragole, eugenol, methyleugenol, benzofuran, hexahydro-1,6 -dimethyl 1-4, citral, ceftazidime. For benzofuran, there are 4 compounds and the docking energy (Kcal/Mol) was -5.31. For citral, there is only one compound and the docking energy was -4.9. For eugenol the number of compounds were two and the docking energy was -4.59 Kcal/Mol. For Methyleugenol, there were two compounds and docking energy was -4.89. For ceftazidime, there are 5 interactions and docking energy was -5.35. Though ceftazidime is an antibiotic and showed least binding energy, it had violated two

out of 5 Lipinski's rules. The findings of the present study suggest benzofuran as the best candidate against *A. baumannii* on csuE gene as it has -5.31 Kcal/Mol docking energy and has 4 compounds. (to discuss and describe from statistical aspect) The results also correlate with our earlier studies (24). The limitation of the study is that it was done as an computational observational study and the future prospects are set to experimentally evaluate the antimicrobial effects using in-vitro and in-vivo studies (25) (26) (27)

Conclusion

The present study was undertaken as a computational approach to evaluate the drug - ligand interactions of the bio-active compounds from *O.sanctum* with csuE of *A.baumannii*. Out of the 5 compounds selected for the study, benzofuran is considered as the best compound with a docking energy of -5.31 Kcal/mol. However, further experimental validations have to be made *in-vitro* to assess the safety and efficacy of the compounds in the development of novel drugs and to curtail *A.baumannii* associated infections.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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