Volume 65, Issue 5, 2021
Journal of Scientific Research
of
The Banaras Hindu University

Insilico study of some natural quorum sensing inhibitors with AgrA proteins: molecular docking study and normal mode analysis

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Abstract: **Use of antibiotics for a long time has resulted in the evolution of multiple drug resistance pathogenic bacterial strains, leading to difficulties to treat bacterial infections. Nowadays, the problem of bacterial resistance can be overcome by targeting the virulence mechanism of the bacterial infection process which has been established as the molecular target for the discovery of antibacterial agents. One of the extensively studied gram positive bacterial QS mechanisms is accessory gene regulator (*agr*) QS mechanism responsible for the secretion of different virulence factors. Expression of genes associated with the virulence factors secretion of *agr* quorum sensing system can be attenuated by the inhibition of AgrA-DNA binding interactions. In this study we docked four natural compounds (actinomycin D, obacunone, ursolic acid and ansamitocin P-3) with five homology modelled AgrA proteins of five pathogen, *Streptococcus pyogenes*, *Listeria monocytogenes*, *Enterococcus faecalis*, *Chlamydia trachomatis* and *Macrococcus canis* and also with their template protein (PDB ID: 4G4K) of *Staphylococcus aureus*. Normal mode analyses of the docked complexes were performed in order to obtain different parameters like deformability and B-factor related to stability of the complexes. Drug likeness of the compounds was also studied.**

Index Terms: **AgrA protein, Bacterial resistance, docking, normal mode, quorum sensing**

I. INTRODUCTION

Antibiotics have been widely used around the world to treat life-threatening fatal infectious diseases based on inhibition of growth of pathogenic microorganisms since the last many decades (Jiang, Chen, Yang, Yin, & Yao, 2019). Use of antibiotics for a long time has resulted in the evolution of multiple drug resistance pathogenic bacterial strains, leading to difficulties to treat bacterial infections (Mellini et al., 2019; Ventola, 2015). Nowadays, the problem of bacterial resistance can be overcome by targeting the virulence mechanism of the bacterial infection process which has been established as the molecular target for the discovery of antibacterial agents (Rasko & Sperandio, 2010). One such target mechanism which has gained interest in the discovery of novel anti-virulence drugs is bacterial quorum sensing (QS) (Bodede, Shaik, Chenia, Singh, & Moodley, 2018). QS is a bacterial cell-cell communication process in which bacteria produce chemical signaling molecules in response to fluctuations in cell population density to regulate expression of various genes associated with different bacterial functions. Different physiological processes like bioluminescence, biofilm generation and virulence factor production are controlled by QS (Rampioni, Leoni, & Williams, 2014). QS mechanism offers multiple molecular targets for the action of compounds which interfere with QS-mediated cell-to-cell communication (Bodede et al., 2018). One of the extensively studied gram positive bacterial QS mechanisms is accessory gene regulator (*agr) QS* mechanism responsible for the secretion of different virulence factors. Two promoters, P2 and P3 drive two divergent transcription units respectively at the *agr* locus. P2 synthesises RNA II encoding structural components (AgrB, AgrD, AgrC and AgrA) of *agr* QS

mechanism (Tan, Li, Jiang, Hu, & Li, 2018). The precursor of the autoinducing peptide (AIP) encoded by *agr*D is processed to the final thiolactone AIP and is transported in to the environment by transmembrane protein AgrB (Bezar, Mashruwala, Boyd, & Stock, 2019). When the extracellular concentration of AIP reaches a certain threshold value, it activates the receptor histidine kinase AgrC (Novick et al., 1995). Subsequently activated AgrC auto phosphorylates and transfers a phosphoryl group to Asp59 on the N- terminal domain of response regulator protein AgrA (Lina et al., 1998). Upon phosphorylation AgrA undergoes a conformational change to form a dimer enabling its C-terminal DNA binding domain to bind P2 promoter which in turn activates AIP transcription in a autocatalytic fashion (Srivastava, Rajasree, Fasim, Arakere, & Gopal, 2014). Apart from P2 promoter, P3 promoter which is positively regulated by AgrA controls expression of RNAIII that serves as the transcript for the *hld* gene encoding δ-hemolysin as well as functions as a regulatory RNA (Janzon, Löfdahl, & Arvidson, 1989; Rutherford & Bassler, 2012; Wang & Muir, 2016). RNA III post transcriptionally activates the expression of genes encoding virulence factor secretion and represses the expression of genes encoding surface proteins (Koenig, Ray, Maleki, Smeltzer, & Hurlburt, 2004).

Response regulator, AgrA contains DNA binding LytTR domain (residues 138-238) which is rare among bacteria but not found in higher organisms (Gao, Mack, & Stock, 2007). Transcription factors with the LytTR domains regulate virulence gene expression (Koenig et al., 2004; Miller, Frederick, Sarkar, & Marconi, 2014; Nikolskaya & Galperin, 2002; Ween, Gaustad, & Håvarstein, 1999). Hence LytTR domain has gained interest as a molecular target for the designing of antibacterial agents. It is reported that AgrA on binding with DNA undergoes a conformational change at the residues that interact with DNA back bone (Ser168, Arg170, Tyr183, Lys187, Ser202, Arg218, Asn234) and also at the residues that make direct base-specific contacts with DNA (His169, Asn201 and Arg233) (Leonard, Bezar, Sidote, & Stock, 2012). In this study, four natural compounds, actinomycin D, obacunone, ursolic acid and ansamitocin P-3 were docked with five homology modelled AgrA proteins of five pathogen, *Streptococcus pyogenes*, *Listeria monocytogenes*, *Enterococcus faecalis*, *Chlamydia trachomatis* and *Macrococcus canis* (Basak, Chatterjee, & Pal, 2020) and also with their template protein (PDB ID: 4G4K) of *Staphylococcus aureus* in order to identify potential inhibitors of these proteins. Actinomycin D and ansamitocin P-3 are secondary metabolites derived from marine organisms and their associated bacteria have the ability to inhibit the bacterial quorum sensing (Borges & Simões, 2019). Obacunone and ursolic acid are natural compounds having strong quorum sensing inhibiting activity and therapeutic potential for neurodegenerative diseases (Asfour, 2018; "Leaf-nosed bat," 2009). Homology modelled proteins were taken from our previous study (Basak et al., 2020). Molecular docking approach is helpful in studying drug-receptor interactions to a greater detail. The study of receptor-ligand interactions by molecular docking has been gaining importance as it is a fundamental approach for rational drug design (Shoichet, McGovern, Wei, & Irwin, 2002 Normal mode analysis (NMA) of the docked complexes was performed in order to obtain different stability determining parameters of the complexes like deformability, B-factor etc. ADMET properties of the four natural compounds were also calculated to identify their drug likeness. Our study reveals that these compounds have drug likeness and very good pharmacokinetic properties with the high binding affinity with AgrA proteins under study.

II. MATERIALS AND METHODS

A. Receptors and ligands selection

Homology modelled 3 D structures of AgrA proteins of five pathogens, *Streptococcus pyogenes*, *Listeria monocytogenes*, *Enterococcus faecalis*, *Chlamydia trachomatis* and *Macrococcus canis* were taken from of our previous study (Basak et al., 2020). Template (Protein Data Bank ID: 4G4K) of the modelled proteins was downloaded from RCSB Protein Data Bank (https://www.rcsb.org/) (Bernstein et al., 2008). Four natural compounds, actinomycin D, obacunone, ursolic acid and ansamitocin P-3 having quorum sensing activity were selected from the literature (Asfour, 2018; Borges & Simões, 2019; "Leaf-nosed bat," 2009). 2D structures of these compounds were drawn using the chemical drawing package, Chem Sketch (http://www.acdlabs.com/resources/freeware/chemsketch/). Geometry optimizations for all molecules were done at M06-2X/ def2-TZVP level of theory. Geometry optimized structures were then subjected to docking studies.

B. Molecular docking simulations

The above compounds were docked with all the proteins under study using AutoDock 4.2 programme in order to study the protein-ligand interactions and to identify the potent inhibitor against each of the AgrA proteins. Proteins were prepared by checking, repairing missing atoms, and adding all hydrogen with method no bond order with the aid of graphical user freely accessible interface (GUI) for AutoDock 4.2 programme (Morris et al., 2009) . As the template protein, 4G4K consists of two identical chains (A & B) and the ligands will bind either of the chains, hence chain B and all the heteroatoms were deleted from the pdb file of 4G4K. With the help of ADT a Grid box covering the DNA interacting residues (residues 138-238) were prepared and the grid size was 82,72,88 xyz points with grid spacing of 0.375 Å and grid centre was designated at (x, y, and z): 32.489 30.909 and 39.574 Å. During the docking simulation both the protein and ligand were considered as rigid and Lamarckian genetic Algorithm 4.2 was employed. Keeping the number of genetic algorithm runs at 10 all other docking parameters were kept at default values. Ten different conformations of the docked complex for each of the ligands were obtained on the basis of AutoDock 4.2 scoring functions and were ranked depending upon their binding free energies.

C. Normal mode analysis

Normal mode analysis (NMA) of the docked complexes was performed using iMods online server

(http://imods.chaconlab.org/) (Bhattacharya et al., 2020; Kumar, Sood, Sharma, & Chandra, 2020; Patra et al., 2020; Sarkar, Ullah, Johora, Taniya, & Araf, 2020). Structural dynamics of the protein complexes was investigated efficiently by this fast and user friendly server. Different parameters like Deformability and B-factor were obtained from the output of the server. Protein flexibility was also determined with this fast server (Awan, Obaid, Ikram, & Janjua, 2017; Kovacs, Chacón, & Abagyan, 2004; López-Blanco, Aliaga, Quintana-Ortí, & Chacón, 2014; Lopéz-Blanco, Garzón, & Chacón, 2011; Prabhakar, Srivastava, Rao, & Balaji, 2016). Pdb files of the docked complexes were provided to the iMODS online server in order to perform molecular dynamics simulation.

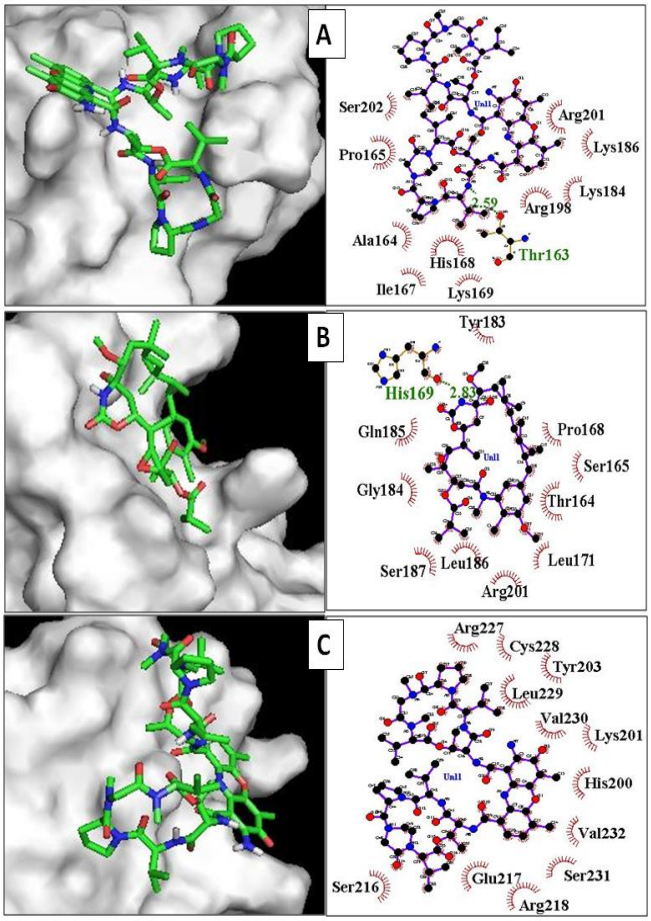
D. ADMET properties

logP, solubility, drug likeness, polar surface area, molecular weight, number of atoms, number of rotatable bonds, volume and drug score of these compounds were calculated using Molinspiration (http://www.molinspiration.com) and OSIRIS Property explorer (http://www.organic-chemistry.org/prog/peo/). PreADMET (http://preadmet.bmdrc.org/) server was also used to study the drug-like character and ADMET (Absorption, Distribution, Metabolism, Excretion and Toxicity) properties.

III. RESULTS AND DISCUSSION

A. Molecular docking analysis

Molecular docking is an extensively used tool for the computer-aided structure-based rational drug design process. DNA binding domain (LytTR domain) of AgrA proteins could serve a primary target for the development of antimicrobial drugs. This DNA binding of AgrA consists of 101 residues extending from residue 138 to 238 (Rajasree, Fasim, & Gopal, 2016). RMSD calculation of apo state and DNA bound state of LytTR domain suggested that a conformational change takes place upon DNA binding for the following residues: the residues that interact with DNA back bone (Ser168, Arg170, Tyr183, Lys187, Ser202, Arg218, Asn234) and the residues that make direct base-specific contacts with DNA (His169, Asn201 and Arg233) (Leonard et al., 2012). Molecular docking studies of actinomycin D, obacunone, ursolic acid and ansamitocin P-3 were performed with LytTR domain of five modelled AgrA proteins associated with the bacterial quorum sensing(QS) of five pathogen, *Streptococcus pyogenes*, *Listeria monocytogenes*, *Enterococcus faecalis*, *Chlamydia trachomatis* and *Macrococcus canis*, and also with their template protein (Protein Data Bank ID: 4G4K) of *Staphylococcus aureus*. Docking results showed that the binding free energies of actinomycin D with all the proteins were found to range from -6.76 to -9.26 kcal/mol. Binding free energies of obacunone, ursolic acid and ansamitocin P-3 were found to range from -7.63 to -8.47 kcal/mol, from -6.23 to -7.37 kcal/mol and from -6.49 to -7.97 kcal/mol respectively. Binding strength of the ligands with the receptor proteins is measured by binding free energy. Lower the free energy of binding, the higher is the strength of binding. The values of binding free energy are shown in Table I. Docking result showed that actinomycin D effectively bound with *Listeria monocytogenes*, *Enterococcus faecalis* and *Streptococcus pyogenes* with a binding free energy of -9.26, -8.13 and -7.29 kcal/mol respectively. Obacunone bound with *Listeria monocytogenes*, *Chlamydia trachomatis*, *Streptococcus pyogenes* and 4G4K with a binding free energy of -8.47, -7.63, -7.63 and -7.47 kcal/mol respectively indicating the effective binding. Ursolic acid is bound effectively with *Enterococcus faecalis* and *Streptococcus pyogenes* with a binding free energy of -7.37 and -7.12 kcal/mol respectively. Ansamitocin P-3 docked with *Chlamydia trachomatis*, *Streptococcus pyogenes* and *Enterococcus faecalis* with a binding free energy of -7.97, -7.38 and -7.01 kcal/mol respectively indicating the strong receptor-ligand interactions. On the other hand actinomycin D was the best inhibitor for *Listeria monocytogenes* and *Enterococcus faecalis*, ansamitocin P-3 was the most effective inhibitor for *Chlamydia trachomatis*, and obacunone was the best inhibitor for *Streptococcus pyogenes* and 4G4K. So, the best complex of each of the proteins was extensively studied here (Fig. 1).



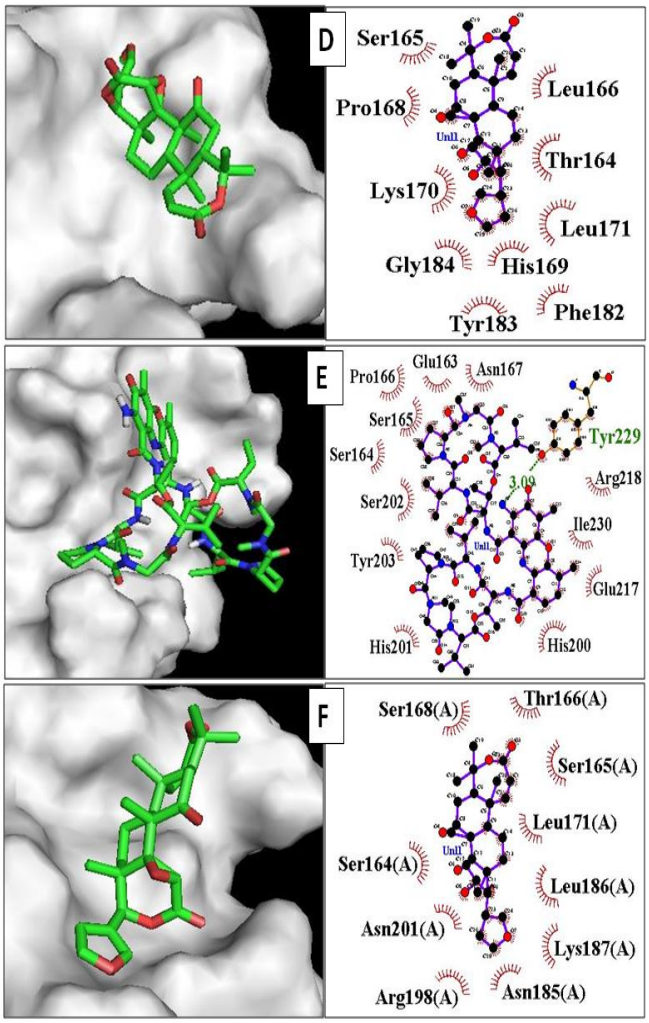


Fig. 1. Lig plots of the docked complexes: (A) *Listeria monocytogenes*- actinomycin D, (B) *Chlamydia trachomatis*- ansamitocin P-3, (C) *Enterococcus faecalis*- actinomycin D, (D) *Streptococcus pyogenes*- obacunone, (E) *Macrococcus canis*- actinomycin D and(F) 4G4K- obacunone

Table I. Binding free energy of the molecules with modelled and template AgrA proteins in kcal/mol

	Molecule			
Protein	Actinomycin D	Obacunone	Ursolic acid	Ansamitocin P-3
Listeria monocytogenes	-9.26	-8.47	-6.7	-6.61
Chlamydia trachomatis	-6.77	-7.63	-6.54	-7.97
Enterococcus faecalis	-8.13	-6.65	-7.37	-7.01
Streptococcus pyogenes	-7.29	-7.63	-7.12	-7.38
Macrococcus canis	-6.76	-6.72	-6.23	-6.62
4G4K (template)	-6.94	-7.47	-6.83	-6.49

In the docked complex of *Listeria monocytogenes* and actinomycin D, the N9 atom of actinomycin D formed a hydrogen bond with Thr163 with a distance of 2.59 Å. Besides this hydrogen bond, a large number of hydrophobic interactions with the residues Ala164, Pro165, Ile167, His168, Lys169, Lys184, Lys186, Arg198, Arg201 and Ser202 at the DNA binding site were also found. Ansamitocin P-3 on docking with *Chlamydia trachomatis* formed a hydrogen bond with His169 with distance of 2.83 Å and made hydrophobic interactions with Thr164, Ser165, Pro168, Leu171, Tyr183, Gly184, Gln185, Leu186, Ser187 and Arg201. In the docked complex of *Enterococcus faecalis*- actinomycin D, there was no hydrogen bonds but was a large number of hydrophobic interactions with His200, Lys201, Tyr203, Ser216, Glu217, Arg218, Arg227, Cys228, Leu229, Val230 and Ser231. Obacunone on binding with *Streptococcus pyogenes* made a large number of hydrophobic interactions with Thr164, Ser165, Leu166, Pro168, His169, Lys170, Leu171, Phe182, Tyr183 and Gly184. In the docked complex of *Macrococcus canis* and actinomycin D, N7 atom of actinomycin D formed a hydrogen bond with Tyr220 with distance of 3.09 Å. A large number of hydrophobic interactions with Gln163, Ser164, Ser165, Pro166, Asn167, His200, His201, Ser202, Tyr203, Glu217, Arg218 and Ile230 were also found in this complex. Obacunone docked with 4G4K with large number of hydrophobic interactions with Ser164, Ser165, Thr166, Ser168, Leu171, Asn185, Leu186, Lys187, Arg198 and Asn201 (Fig. 1).

B. Normal mode analysis

Normal mode analyses (NMA) of the docked complexes were carried out to predict their stability and mobility. Deformability plots of the best docked complexes are illustrated in Fig. 2. Ability of deformation of each amino acid residue of a docked complex is determined by the deformability. Deformability curve suggests that the amino acids corresponding to the peaks are deformable. From thedeformability curve it is clear that *Listeria monocytogenes*- actinomycin D, *Enterococcus faecalis*- actinomycin D, *Macrococcus canis*- actinomycin D and 4G4K-obacunone complexes had greater degree of deformability than *Chlamydia trachomatis*- ansamitocin P-3 and *Streptococcus pyogenes*- obacunone. B-factor plots of the docked complexes provide the comparative understanding of normal mode analysis with reference to the PDB field. In the B-factor test, best results are obtained for *Enterococcus faecalis*- actinomycin D and *Macrococcus canis*- actinomycin D complex as their NMA deviate a little with respect to the PDB fields (Fig. 3). The eigenvalues of the complexes are illustrated in Fig. 2. The energy needed to deform a structure is measured by eigenvalue. A complex with lower eigenvalue is considered to be more deformable. Moreover, the motion stiffness of the protein-ligand

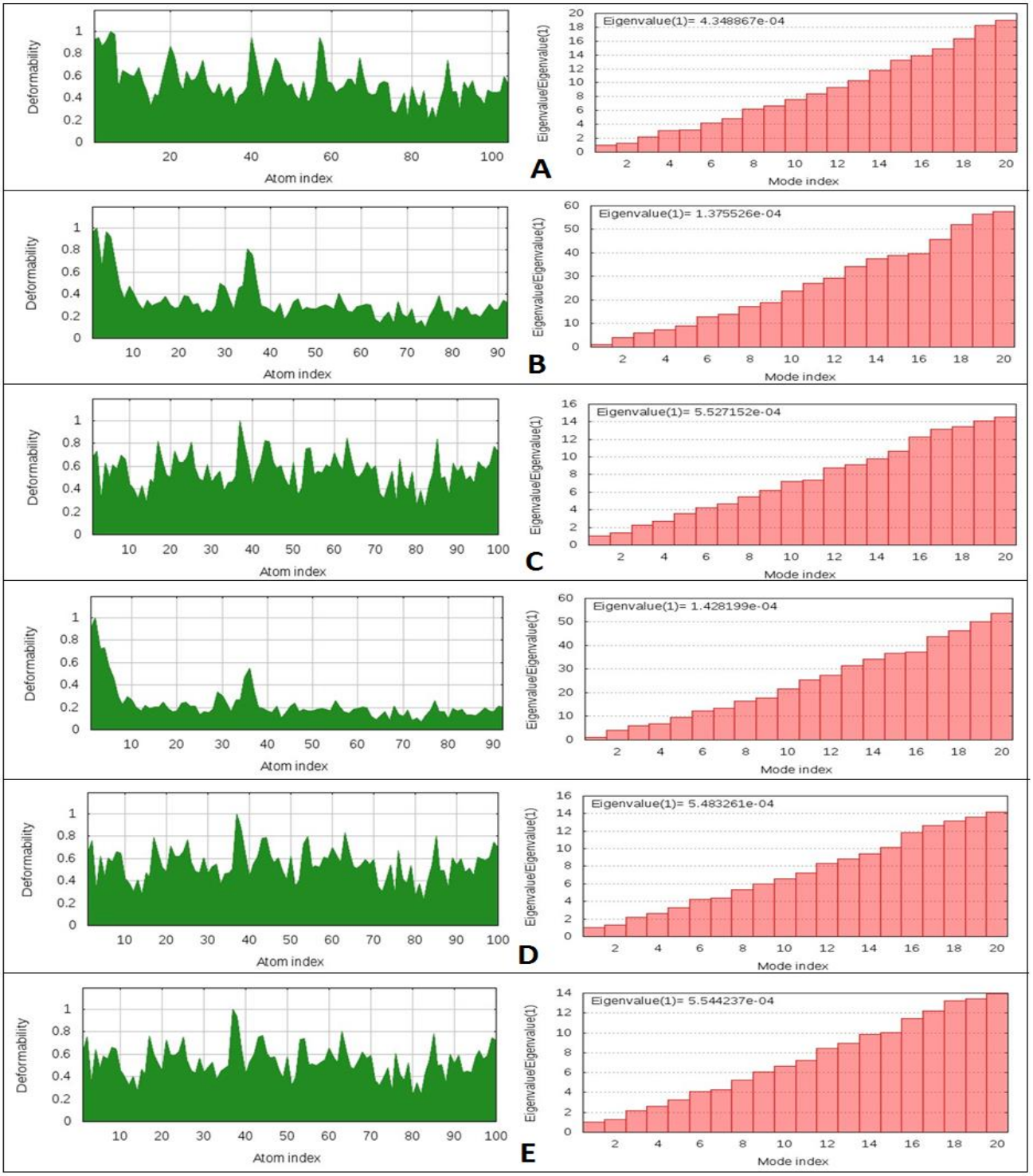


Fig. 2. Deformability and eigenvalue graphs of the docked complexes: (A*) Listeria monocytogenes*- actinomycin D, (B) *Chlamydia trachomatis*- ansamitocin P-3, (C) *Enterococcus faecalis*- actinomycin D, (D) *Streptococcus pyogenes*- obacunone, (E) *Macrococcus canis*- actinomycin D and(F) 4G4K- obacunone

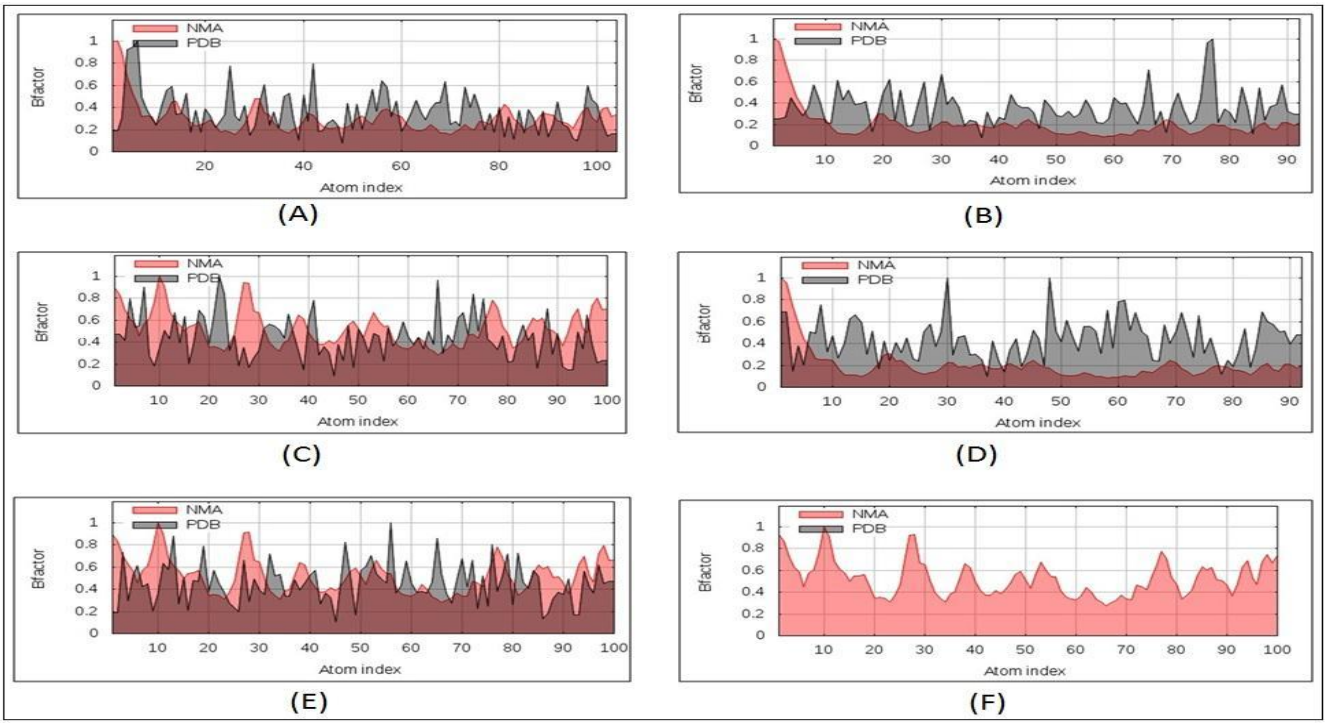


Fig. 3. B-factor graphs of the docked complexes: (A*) Listeria monocytogenes*- actinomycin D, (B) *Chlamydia trachomatis*- ansamitocin P-3, (C) *Enterococcus faecalis*- actinomycin D, (D) *Streptococcus pyogenes*- obacunone, (E) *Macrococcus canis*- actinomycin D and(F) 4G4K- obacunone

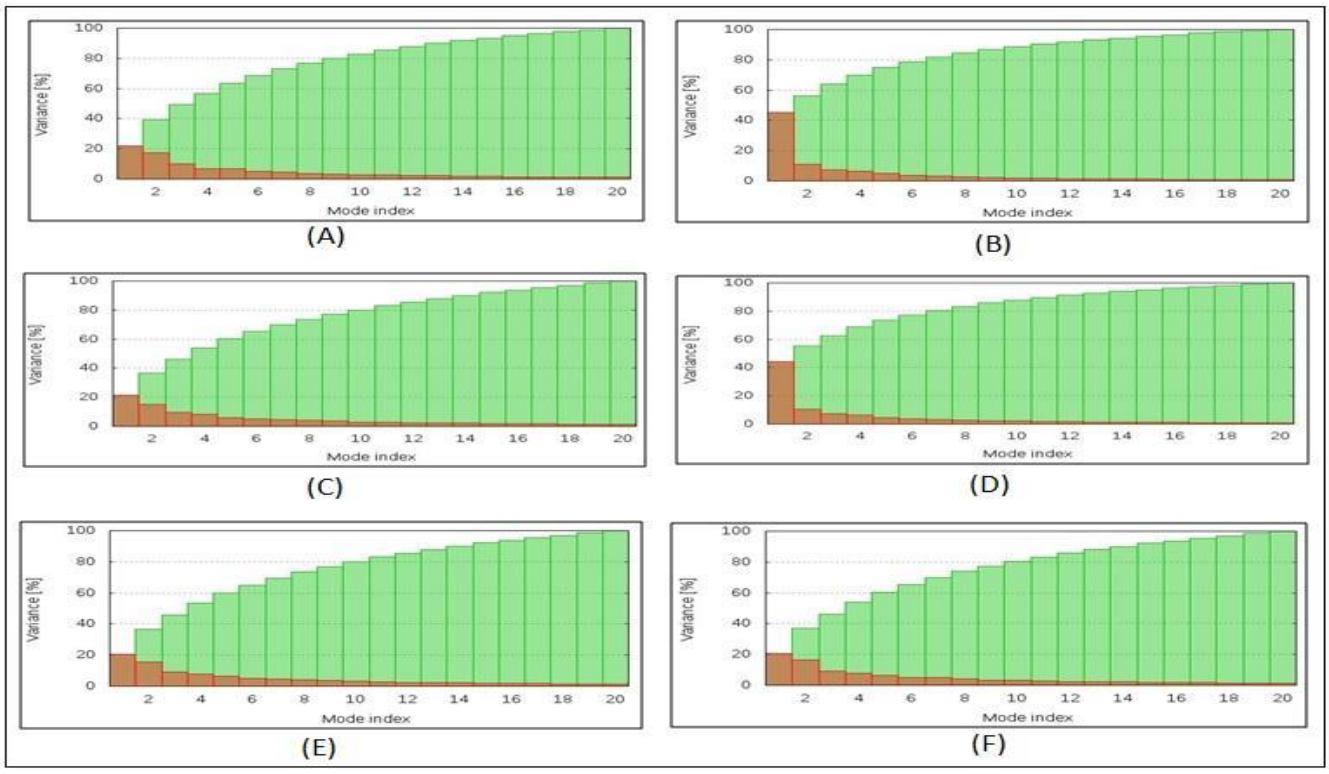


Fig. 4. Variance graphs of the docked complexes: (A*) Listeria monocytogenes*- actinomycin D, (B) *Chlamydia trachomatis*- ansamitocin P-3, (C) *Enterococcus faecalis*- actinomycin D, (D) *Streptococcus pyogenes*- obacunone, (E) *Macrococcus canis*- actinomycin D and(F) 4G4K- obacunone

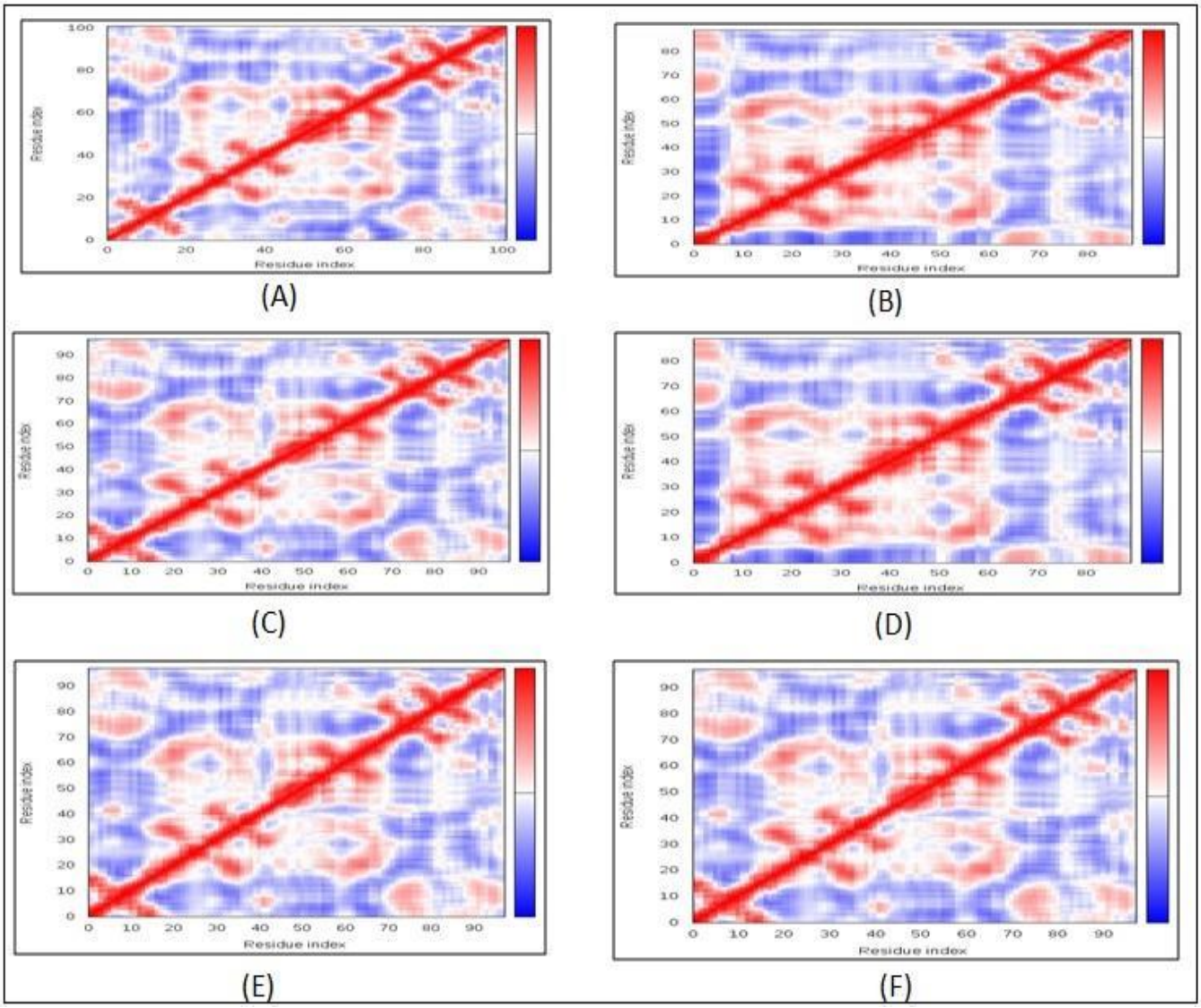


Fig. 5. Co-variance maps of the docked complexes: (A*) Listeria monocytogenes*- actinomycin D, (B) *Chlamydia trachomatis*- ansamitocin P-3, (C) *Enterococcus faecalis*- actinomycin D, (D) *Streptococcus pyogenes*- obacunone, (E) *Macrococcus canis*- actinomycin D and(F) 4G4K- obacunone

complex is also represented by the eigenvalue. The higher eigenvalue of 4G4K- obacunone (5.544237e-04), *Enterococcus faecalis*- actinomycin D (5.527152e-04), *Macrococcus canis*-actinomycin D (5.483261e-04) and *Listeria monocytogenes*-actinomycin D (4.348867e-04) complexes indicate much harder to deform than the other two complexes. The variance maps of all the docked complexes provide quite similar results. In the variance map individual variance and cumulative variance represented by red coloured and green coloured bars respectively (Fig. 4). In the covariance graph (considering the two residues), red colour shows the correlated motion, white colour represents uncorrelated motion and blue colour represents anti-correlated motion. Fig. 5 gives the nature of the motion between the residues.

C. ADMET properties

Four natural compounds used in this study were evaluated as potential AgrA inhibitors. All the compounds qualified the topological polar surface area (TPSA), number of hydrogen bonds (nON and nOHNH), number of rotatable bonds (nrotb) and molecular weight to be orally bioavailable (Table II). The order of drug likeness of these compounds is actinomycin D> ursolicacid> obacunone> ansamitocin P-3 (Table III). Results revealed that the fragments present in all the compounds are similar to existing good drugs and hence the compounds can be used as potent drugs. Higher the drug score of a compound, the better is its drug-like character. The order of drug score of these compounds is as follows: actinomycinD> ursolicacid> obacunone> ansamitocin P-3 (Table III). Modern drug designing process involves the prediction of preADMET properties which includes HIA, Caco-2 cell permeability, MDCK, plasma protein binding and BBB. HIA percentage, plasma protein binding percentage, blood brain barrier, Caco-2 and MDCK cell permeability of all the compounds were found to lie within the

qualified range and hence, the compounds can be used as good drugs (Table IV).

Table II. Molecular descriptor properties of the ligands

	Actinomycin D	Obacunone	Ursolicacid	Ansamitocin P-3
miLogP	0.78	4.07	6.79	6.59
TPSA	359.98	95.35	57.53	136.17
nON	28	7	3	11
nOHNH	6	0	2	2
nrotb	8	1	1	5
volume	1143.41	422.56	471.49	683.48
natoms	90	34	33	50

Table III. Fragment base drug-likeliness of the ligands

	ActinomycinD	Obacunone	Ursolicacid	Ansamitocin P-3
cLogP	-0.49	2.97	6	7.05
Solubility	-4.322	-4.54	-6.11	-7.57
Molweight	1254	468	456	722
TPSA	355.5	95.34	57.53	136.1
Druglikeness	6.48	-7.65	-3.66	-10.78
Drug score	0.41	0.3	0.17	0.03

Table IV. PreADME prediction of the ligands

	Actinomycin D	Obacunone	Ursolicacid	Ansamitocin P-3
HIA (%)	62.78379	98.62233	95.9964	96.44497
Caco2 (nm/sec)	20.9763	29.5879	21.8616	32.8782
MDCK (nm/sec	0.0434155	2.80265	0.0451719	0.0434155
Plasma_Protein_Binding (%)	35.13465	90.30349	100	90.1415
BBB	0.0361849	0.0979989	8.00777	1.26912

IV. CONCLUSION

In the agr quorum sensing system, DNA binding LytTR domain of AgrA is the potential target for the development of antibacterial drugs. In this study, molecular docking study was performed for actinomycin D, obacunone, ursolic acid and ansamitocin P-3 with AgrA proteins of *Listeria monocytogenes*, *Chlamydia trachomatis*, *Enterococcus faecalis*, *Streptococcus pyogenes*, *Macrococcus canis* and also with their template protein (PDB ID: 4G4K) of *Staphylococcus aureus*. It was found that actinomycin D was best inhibitor for *Listeria monocytogenes* and *Enterococcus faecalis*, ansamitocin P-3 was best inhibitor for *Chlamydia trachomatis*, and obacunone was best inhibitor for *Streptococcus pyogenes* and 4G4K of *Staphylococcus aureus*. Normal mode analysis of these complexes suggested that complexes were stable. All the natural compounds possess drug likeness and good pharmacokinetic properties with the high binding affinity with AgrA proteins. Hence, actinomycin D, obacunone, ansamitocin P-3 and ursolicacid can be used as potential inhibitors of the AgrA proteins. However, experimental research should be carried out on these compounds to verify their QS inhibiting potentiality.

ACKNOWLEDGMENT

The authors are deeply indebted to Late Prof. A. K. Bothra for his unending support and encouragement during the course of this work.

CONFLICT OF INTEREST

No conflict of interest exists among the authors.

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