Insilico Docking of Various Inhibitors of *E.Faecalis* Folate Pathway

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Abstract- Drug resistance to therapeutic antibiotics pose a challenge to the identification of novel targets and drugs for the treatment of infectious diseases. Infections caused by *Enterococcus faecalis* are a major health problem. Moreover, among UTI causing *enterococci*, multi-drug resistant *E. faecalis* such as vancomycin-resistant strains (VRE) have been reported increasingly in many countries. SMX & TMP are the commonly prescribed inhibitors of DHFR & DHPS, the enzymes of the folate biosynthetic pathway. In this study, *insilico* docking of various ligands/inhibitors to the *Enterococcus faecalis* DHFR & DHPS proteins has been performed by using Autodock Suite, version 1.5 6rC2.

Index Terms- Dihydrofolate reductase (DHFR), Dihydropteroate synthase (DHPS), Docking, Folate Pathway Inhibitors.

I. INTRODUCTION

Most clinical isolates from urine samples of UTI patients show presence of *Enterococcus faecalis* and account for 80–90% of clinical strains. *E. faecium* accounts for the remaining 5–10% of such isolates (4). Enterococci currently ranks fourth in frequency among bacteria isolated from hospitalized patients (4). They are nosocomial pathogens and are associated with high mortality. The treatment of these infections pose a great challenge, due to the inherent resistance of Enterococci to many antibiotics (1). In addition, they have the capacity to easily acquire and express new resistance genes and can thus tolerate antibiotic selective pressure (2).

Enterococci are Gram-positive ubiquitous bacteria that are widely found in all types of animals and in the environment. They are typically harmless inhabitants of various body sites—particularly the intestinal tract. However, enterococci are opportunistic pathogens (3). In addition, enterococci are inherently resistant to many antimicrobials, including penicillin, clindamycin, trimethoprim–sulfamethoxazole, and low levels of aminoglycosides, and they are poorly responsive to cephalosporins and fluoroquinolones *in vivo* (3).

They were traditionally regarded as low grade pathogens but have emerged as second leading cause of nosocomial infections and third most common cause of bacteremia. The most frequent infections caused by enterococci are UTI, endocarditis, bacteremia, intra-abdominal and intra-pelvic abscesses (5,6). This is amplified due to their acquired resistance to all currently available antibiotics that leaves the clinicians with limited treatment options and results in the selection and spreading of multidrug-resistant (MDR) strains in hospitals (7).

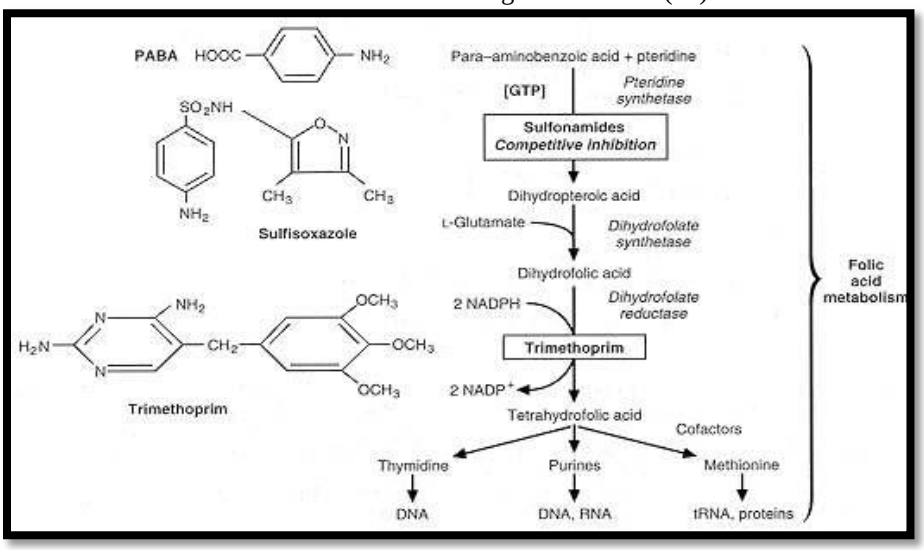
Trimethoprim (TMP) and sulfamethoxazole (SMX) are inhibitors of bacterial enzymes involved in the folate synthesis pathway. Folic acid is necessary to carry out a variety of important cellular functions, including synthesis of nucleic acids, particularly thymidine. Most bacteria are unable to take up exogenous folate from the environment and instead must synthesize it from the *p*-amino benzoic acid precursor (Refer Fig:1). TMP and SMX inhibit successive enzymes in this pathway, limiting the production of dihydrofolate and its subsequent conversion to tetrahydrofolate (8).

Use of computational methods is a cost effective strategy for speeding up the process of drug discovery and development process. Hence, understanding binding interactions between receptor and ligand is very essential for drug discovery scientists (9).

Molecular docking, a computational method of studying binding interactions in terms of binding energies is immensely used in the process of drug discovery to save on cost and time. In this method, computer generated representation of a small molecule or ligand is placed into the active site of the target or protein's computational structure in a variety of positions, conformations and orientations. The position, orientation and conformation of the ligand in the active site of protein is called as a 'pose'. In order to identify the energetically most favorable pose, each pose of the ligand is evaluated for binding energy computationally. The main objective of molecular docking method is to find a pose which has the lowest binding energy (9).

AutoDock abbreviated as AD, is an automated suite of protein-ligand docking tools. It is designed to predict the protein interactions with small molecules such as drug molecule and substrate. The application of this tool is immense, ranging from structure based drug design, lead molecule optimisation, protein-ligand docking, protein-protein docking, analysis and validation of mechanism of action of drug molecules, etc., AutoDock has two versions, namely, AutoDock4 and AutoDock Vina. The prior has been used in this study, AutoDock4 analyzes the interactions of ligand molecules at the specified target site of the protein. The users can define this specific target sites with the use of GridBox. AutoDock4 has two executable key programs, i.e., Autogrid4 and AutoDock4. Autogrid4 prepares a grid map of the amino acids presents within the GridBox defined by the user. AutoDock4

then analyzes the interactions of those amino acids with the ligand molecule (10).



Fig(1): FOLATE PATHWAY IN BACTERIA.

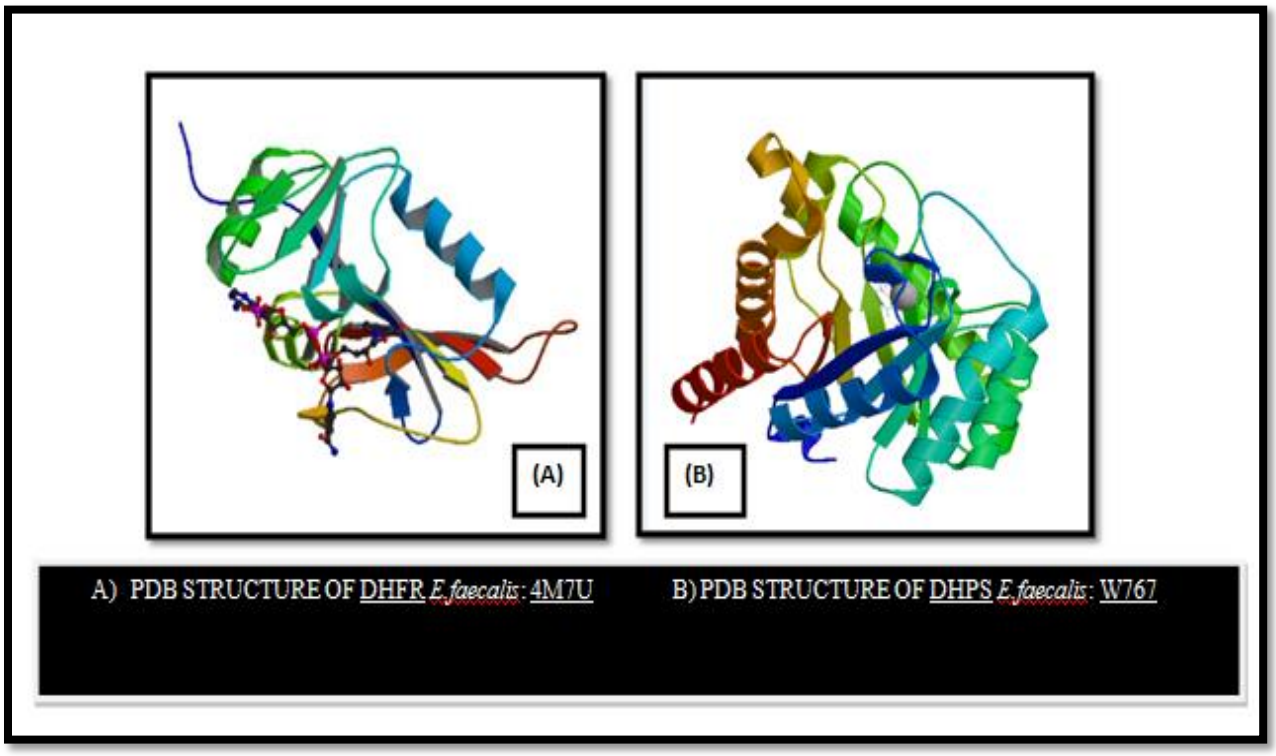
II. MATERIALS & METHODS

In silico studies

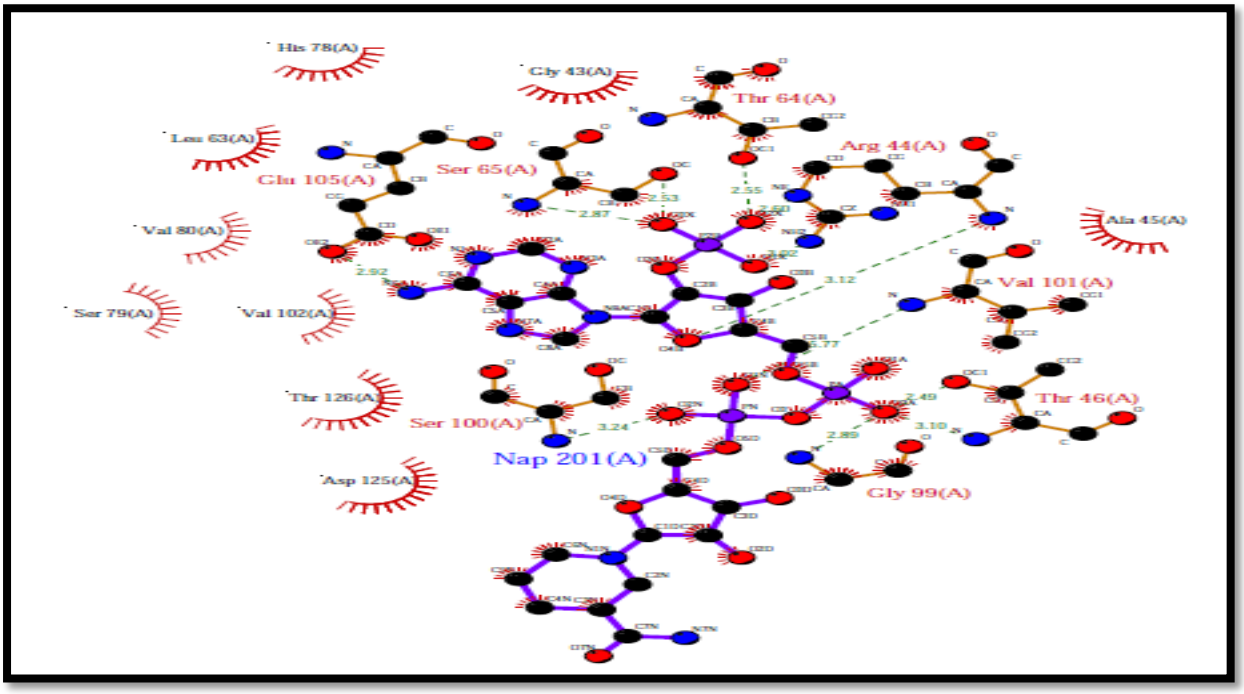
In silico studies utilizing molecular docking is an important tool to study the interaction of ligands with active site residues of the receptor (11, 12). The docking involves the use of sampling algorithm and a scoring function to evaluate the proper orientation and pose of ligand molecule in relation to the binding energy. The correct identification of this binding pose of one or more related ligands is important in establishing a structure-activity relationship in lead optimization. The second use of scoring functions is to rank different ligands to predict their relative experimental activity (12-14).

In silico studies were performed using Autodock 4 suite (version 1.5 6rC2). The ligands viz., Chlorogenic acid, Ellagic acid, Gallic Acid, Hippuric acid, Quercetin and Standard antibiotics viz., Clavulanic acid, Cephalosporin, Cephalosporin C, Penicillin, Sulfamethoxazole and Trimethoprim were docked with DHFR & DHPS enzymes of *E.faecalis*. The ligands and the Standard antibiotics were selected on the basis of reported antibacterial activity and prescribed drugs.

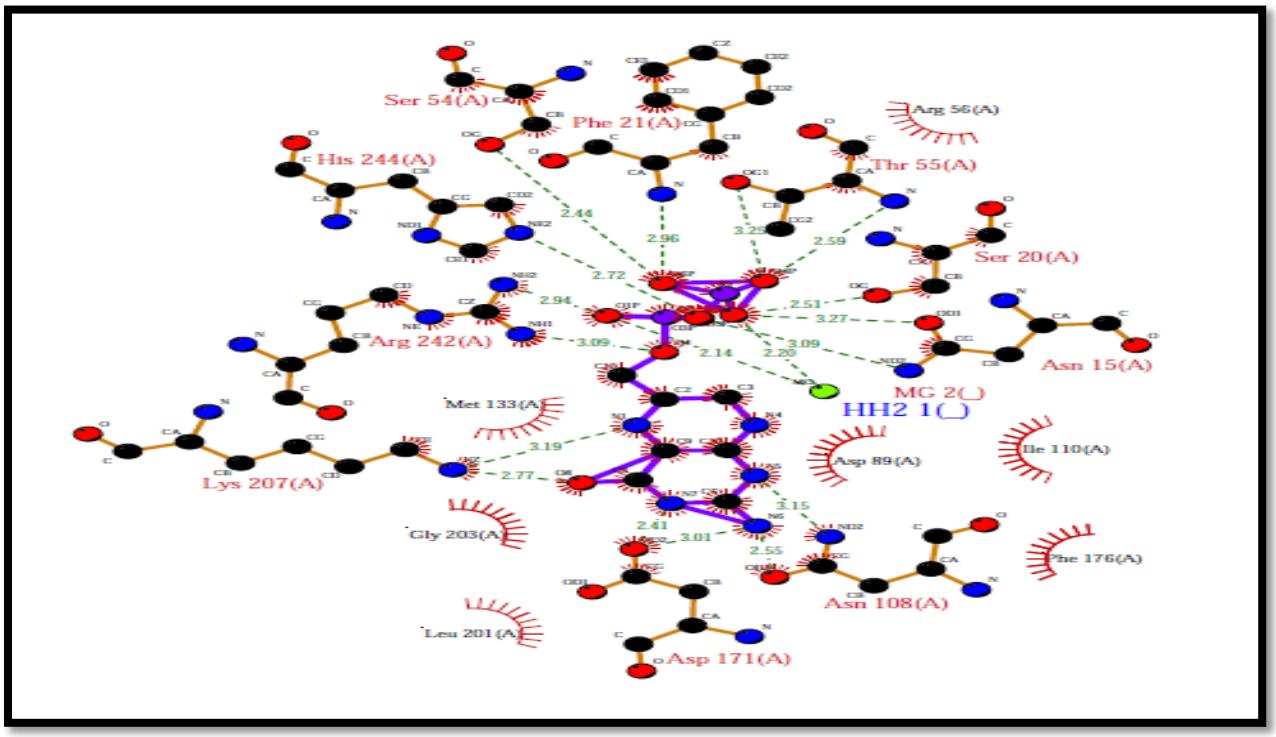
The DHFR (PDB Id 4M7U) protein structure of *E.faecalis* was downloaded from PDB (*X ray diffraction* of 2.1A^0) . The crystal structure of DHPS *E.faecalis* is unavailable in PDB hence, to obtain structural information of DHPS, the homology model was generated using Swiss model and PdbSum. DHPS *E.faecalis* protein was found to have 40.86% sequence identities with PDB ID: W767. This structure was used for the preparation of the model of DHPS of *E. faecalis*. The prepared model was further validated by Ramachandran plot with the help of PROCHECK. This plot verified the DHPS protein (W767) and hence was used for docking studies Fig (2). These models were further used to analyse and compare the effect of binding efficiency of DHPS towards commonly prescribed antibiotics as well as various inhibitors (15). Next, the PubSum database yielded the ligands with their Ligplots. Ligplots give interacting sites of the DHFR Fig (5) & DHPS Fig (4). Fig (5A& B) depicts the Ramachandran Plots of DHFR and DHPS *E.faecalis* respectively. The structure of ligands were downloaded from Pubchem (chemical structure data base) online portal and drawn in Marvin Sketch version 5.8.1.Fig (6,7). After docking, the results were analyzed on the basis of their binding energy and their interactions (15).



Fig(2): PDB STUCTURE OF DHFR AND DHPS PROTEIN OF *E.FAECALIS* .



Fig(3): Ligplot of DHFR *E.faecalis* Ligand: Ligand Nap201(A) –(Ser65, Thr64, Arg44, Glu105, Val101, Ser100, Thr46, Gly99) interaction are shown by green dashed line.



Fig(4): Ligplot of DHPS *E.faecalis* Ligand: Ligand HH2 1(_) –(Ser54, His244, Arg242, Lys207, Asp171, Asn108, Asn15, Ser20, Thr55, Phe21) interaction are shown by green dashed line.

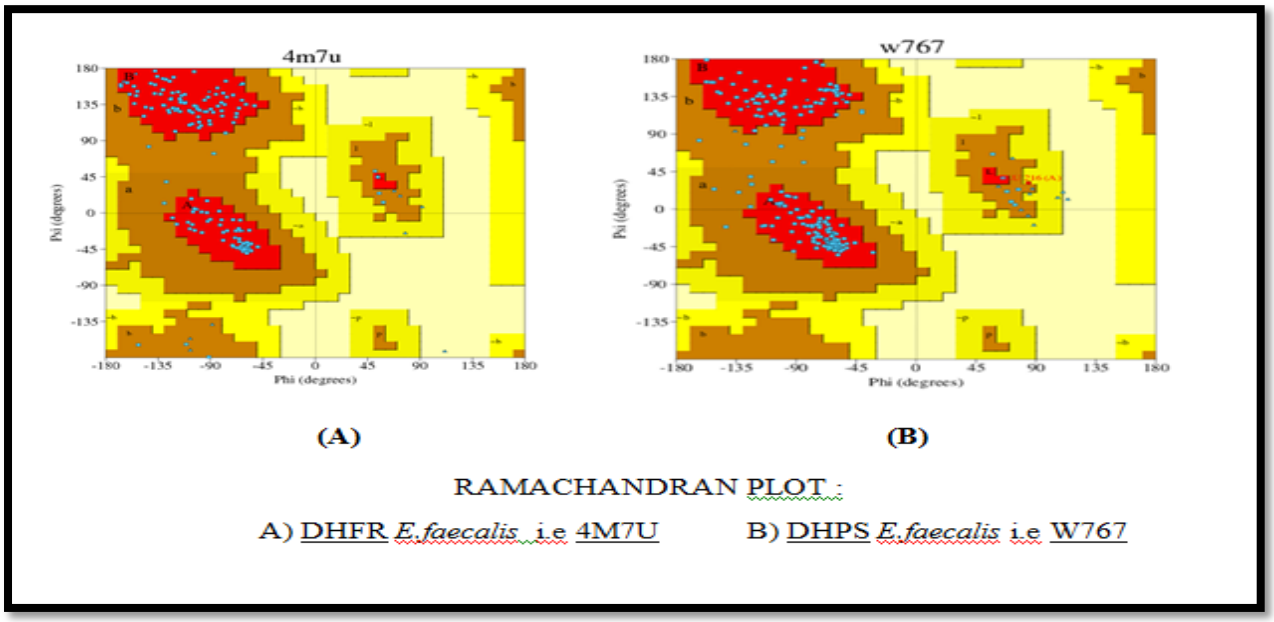
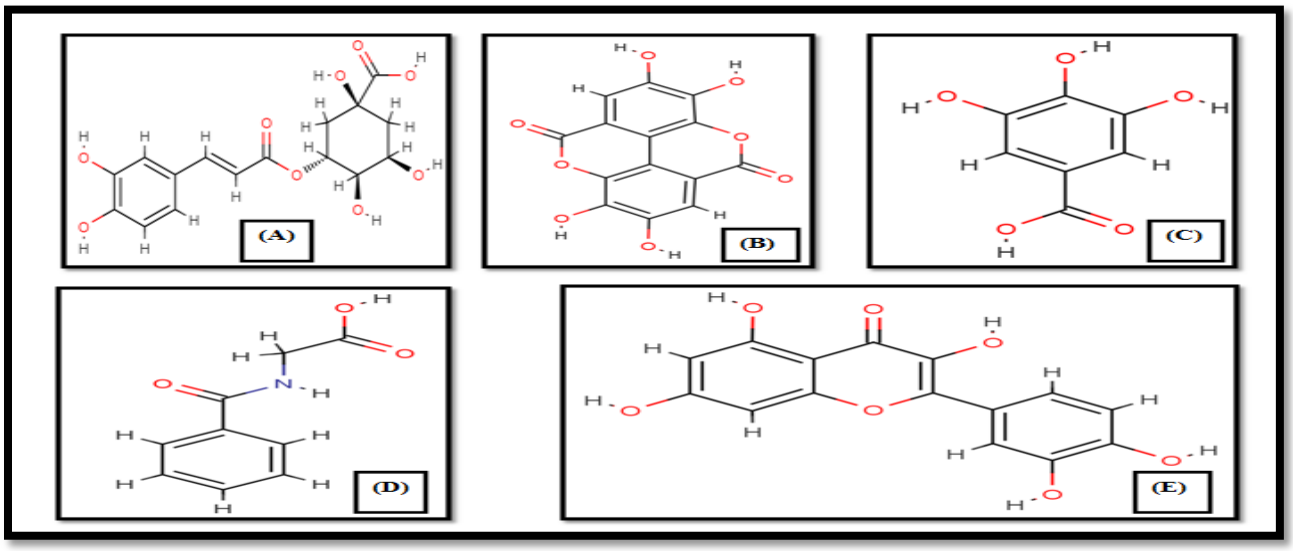
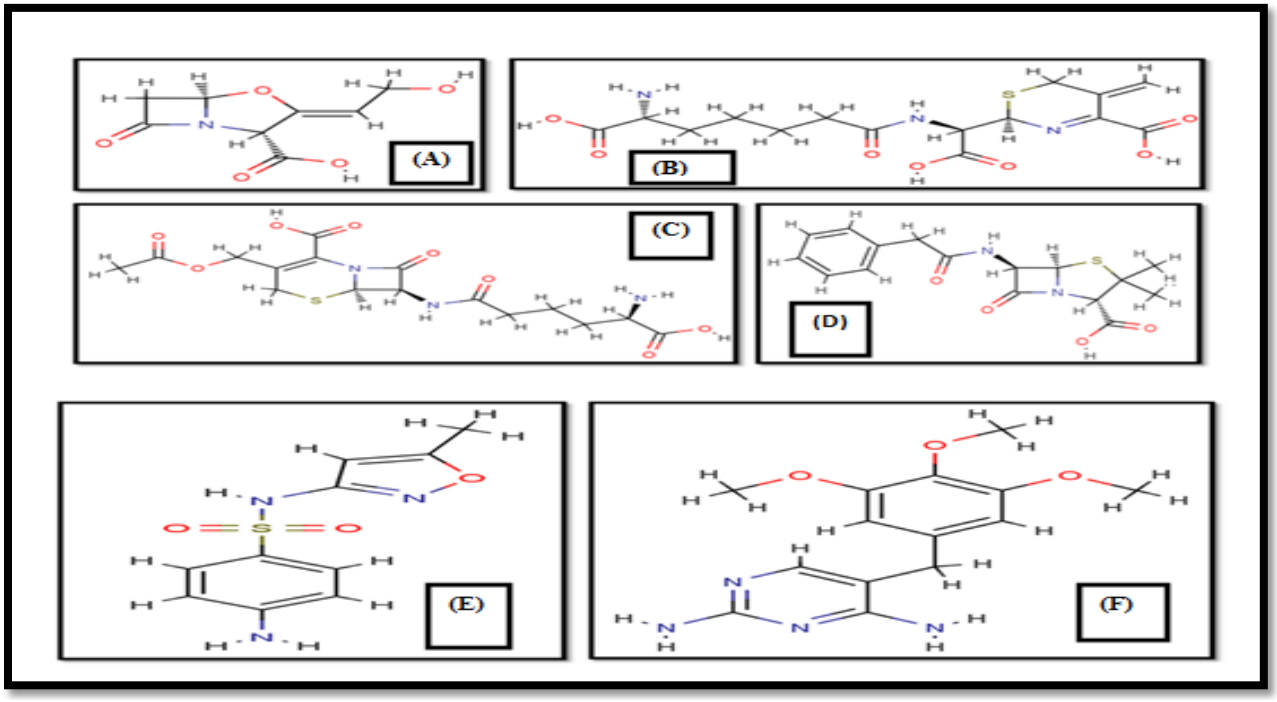


Fig (5): The Ramachandran plot shows the phi-psi torsion angles for all residues in the structure. Glycine residues are separately identified by triangles as these are not restricted to the regions of the plot appropriate to the other sidechain types. The colouring/shading on the plot represents the different regions: the darkest areas (here shown in *red*) correspond to the "core" regions representing the most favourable combinations of phi-psi values.



Fig(6): STRUCTURES OF INHIBITORS- (A) Chlorogenic acid,(B) Ellagic acid, (C) Gallic acid, (D) Hippuric acid and (E) Quercetin.



Fig(7) : STRUCTURES OF ANTIBIOTICS: (A)Clavulanic acid, (B) Cephalosporin,(C) CephalosporinC, (D) Penicillin, (E) Sulfamethoxazole and (F) Trimethoprim.

Preparation of Proteins and Ligands:

The ligands viz., Chlorogenic acid, Ellagic acid, Gallic Acid, Hippuric acid and Quercetin and Standard Antibiotics viz., Clavulanic acid, Cephalosporin, Cephalosporin C, Penicillin, Sulfamethoxazole and Trimethoprim that have exhibited prominent antibacterial activity towards isolated multidrug-resistant bacteria and have been reported were selected for molecular docking analysis (15). The structures of DHFR & DHPS were opened in Biovia Discovery Studio 2016 version 16.1.0.15350. The structure of protein was cleared (i.e. the extra groups which includes water molecules, ligand groups were removed) by deleting the heteroatoms present in the protein (16). Only the protein and active site for docking is required, hence was saved in the PDB format. The structure of ligands were downloaded from Pubchem and drawn in Marvin Sketch view version 5.8.1 and cleaned in 2D and 3D. This cleared the 2 dimensional and 3 dimensional structure of the ligand. For

docking, the protein structure was obtained in PDB format and ligands in tripos-Mol format or PDB format (16).

Grid formation by Autodock

Grid points generate the coordinates or interaction points where the ligand is docked. The grid box was generated at 60x60x60 A^0 to cover all the active site residues, and allowed the flexible rotation of ligands. The GA (genetic algorithm) and number of generation were set to 10 and 27000 for DHFR and DHPS respectively. The Lamarckian genetic algorithm was followed for ligand confirmation. All the above parameters decide the different confirmation of ligand in which the ligand will be docked. Other parameters for example, free energy (after docking is complete we get the value of free energy), rotatable bonds (number of rotatable bonds varies according to the ligand structure), number of torsions (16) etc were used as default (16).

RESULT:

Docking studies revealed the interaction of the protein with the ligands, w.r.t binding energy, type of interaction and amino acids involved in interactions. Binding energy should be ideally negative. More negative the binding energy, better the binding affinity of ligand and protein (16). Table 1 & 2 give the binding energy of ligands with DHFR & DHPS proteins respectively with inhibitors viz., Chlorogenic acid, Ellagic acid, Gallic Acid, Hippuric acid and Quercetin and Standard Antibiotics viz., Clavulanic acid, Cephalosporin, Cephalosporin C, Penicillin, Sulfamethoxazole and Trimethoprim.

TABLE NO.I: BINDING ENERGY OF LIGANDS UPON DOCKING WITH DHFR PROTEIN OF *E.FAECALIS*.

LIGANDS	Binding Energy
INHIBITORS	
Chlorogenic acid	-6.68
Ellagic acid	-7.47
Gallic acid	-5.19
Hippuric acid	-6.03
Quercetin	-7.47
ANTIBIOTICS	
Clavulanic acid	-5.43
Cephalosporin	-8.26
Cephalosporin C	-7.54
Penicillin	-8.35
Sulfamethoxazole	-7.67
Trimethoprim	-6.38

Inhibitors (Chlorogenic acid, Ellagic acid, Gallic Acid, Hippuric acid and Quercetin) and Standard antibiotics (Clavulanic acid, Cephalosporin, Cephalosporin C, Penicillin, Sulfamethoxazole and Trimethoprim) were docked and the results obtained provide a comparative insight into the potency of inhibitors and standard antibiotics through analysis of their binding capacities. The binding energies of DHFR of *E.faecalis* with Penicillin, Cephalosporin, Ellagic acid & Quercetin show highest binding than other ligands. Table 1 clearly shows that the standard antibiotics viz., Penicillin and Cephalosporin are more effective than the inhibitors docked, but *E.faecalis* has emerged resistant to these antibiotics (15).

TABLE NO.II: BINDING ENERGY OF LIGANDS UPON DOCKING WITH DHPS PROTEIN OF *E.FAECALIS*

LIGANDS	Binding Energy
INHIBITORS	
Chlorogenic acid	-7.93
Ellagic acid	-6.84
Gallic acid	-5.81
Hippuric acid	-6.66
Quercetin	-8.22
ANTIBIOTICS	
Clavulanic acid	-6.77
Cephalosporin	-9.31
Cephalosporin C	-7.61
Penicillin	-8.68
Sulfamethoxazole	-7.77
Trimethoprim	-7.58

Inhibitors (Chlorogenic acid, Ellagic acid, Gallic Acid, Hippuric acid and Quercetin) and standard antibiotics (Clavulanic acid, Cephalosporin, Cephalosporin C, Penicillin, Sulfamethoxazole and Trimethoprim) were docked and the results obtained provide a comparative insight into the potency of inhibitors and standard antibiotics through analysis of their binding capacities. The binding energies of DHPS protein of *E.faecalis* with Cephalosporin, Penicillin, Quercetin & Chlorogenic acid are showing highest binding than other ligands. From Table 2, it is clear that standard antibiotics viz., Cephalosporin and Penicillin are more effective than the inhibitors i.e Chlorogenic acid and Quercetin.

Table 3 shows the interaction of various ligands with DHFR & DHPS i.e. hydrogen bond length, hydrogen bond name and amino acid involved in the interaction. As these ligands have proven antibacterial (Sulfamethoxazole and Trimethoprim (20), Clavulanic Acid (21), Penicillin (22) Cephalosporin (25), Cephalosporin C (24)) antimicrobial (Gallic Acid (23)) and anticancer activities (Chlorogenic acid (19), Quercetin (19), Ellagic acid (18) and Gallic acid (23)) these ligands can be further used as lead compounds in treatment of multidrug resistant urinary tract infection caused by *E.faecalis*.

The interacting sites of DHFR & DHPS inhibitors and standard antibiotics matches with Ligplots of both DHFR and DHPS are shown in Fig 8, 9, 10 and 11 respectively.

TABLE NO.III: INTERACTIONS OF LIGANDS WITH DHFR AND DHPS PROTEINS OF *E.FAECALIS*

LIGANDS	**DHFR *E.faecalis***			**DHPS *E.faecalis***		
	Hydrogen Bond Length In A°	Hydrogen Bond Name	Interacting Sites	Hydrogen Bond Length In A°	Hydrogen Bond Name	Interacting Sites
Chlorogenic acid	2.181 2.034 2.146 1.988	- Gly18 Arg44 Gly99	Thr46 Ser100	1.954 2.205 1.797 2.019	- - Asn15 Arg242	Ser54 Asp171 Asn15
Ellagic acid	1.969 1.921 1.646 1.993 2.187	- Arg44 Ser65 Val101 Val102	Ser65 Gly99	1.821	Phe21	Phe21 Asn15
Gallic acid	2.163 1.721 1.934	- - Arg44	Glu105 Ser65 Arg44	1.941 1.897	- Phe21	Lys207 Thr55 Asn15 Phe21
Hippuric acid	1.997 1.967 1.824	Ala45 Thr46 Gly99	Thr46 Ser100	1.736 1.849	Phe21 Thr55	Phe21 Thr55 Asn15 Ser20
Quercetin	1.937 2.024 2.079 1.787	- Ala45 Ser100 Val101	Thr64 Glu105 Ser100	2.238 1.9	- -	Thr55 Phe21 Asn15 Ser20
Clavulanic acid	2.168 2.185	Arg44 Ala45	Ser65 Glu105 Gly99	2.133 2.171 2.011 2.053	Asn15 Lys207 Arg242 Arg242	Thr55 Phe21 Asn15
Cephalosporin	1.961 2.069 2.109 2.172 2.147	- Arg44 Ala45 Val80 Gly99	Glu105 Thr64 Gly99 Thr46	2.028 2.102 1.993	Phe21 Thr55 Thr55	Arg208 Thr55
Cephalosporin C	1.957 2.138	Thr46 Ser65	Glu105 Ser100 Gly99	2.191 2.027	Thr55 Arg208	Arg208 Thr55
Penicillin	2.194	Ser65	Thr64 Ser100 Gly99	2.194	Thr55	Thr55 Lys207
Sulfamethoxazole	2.133 2.024 2.071	Gly99 Ser100 Val102	Thr64 Gly99	2.204 1.861 2.034 2.249	- - Thr55 Lys207	Lys207 Thr55
Trimethoprim	2.063 2.062 2.014	- - Val101	Glu105 Arg44	2.152 2.177 2.17 1.926	- Thr55 Arg242 Arg242	Arg242 Thr55

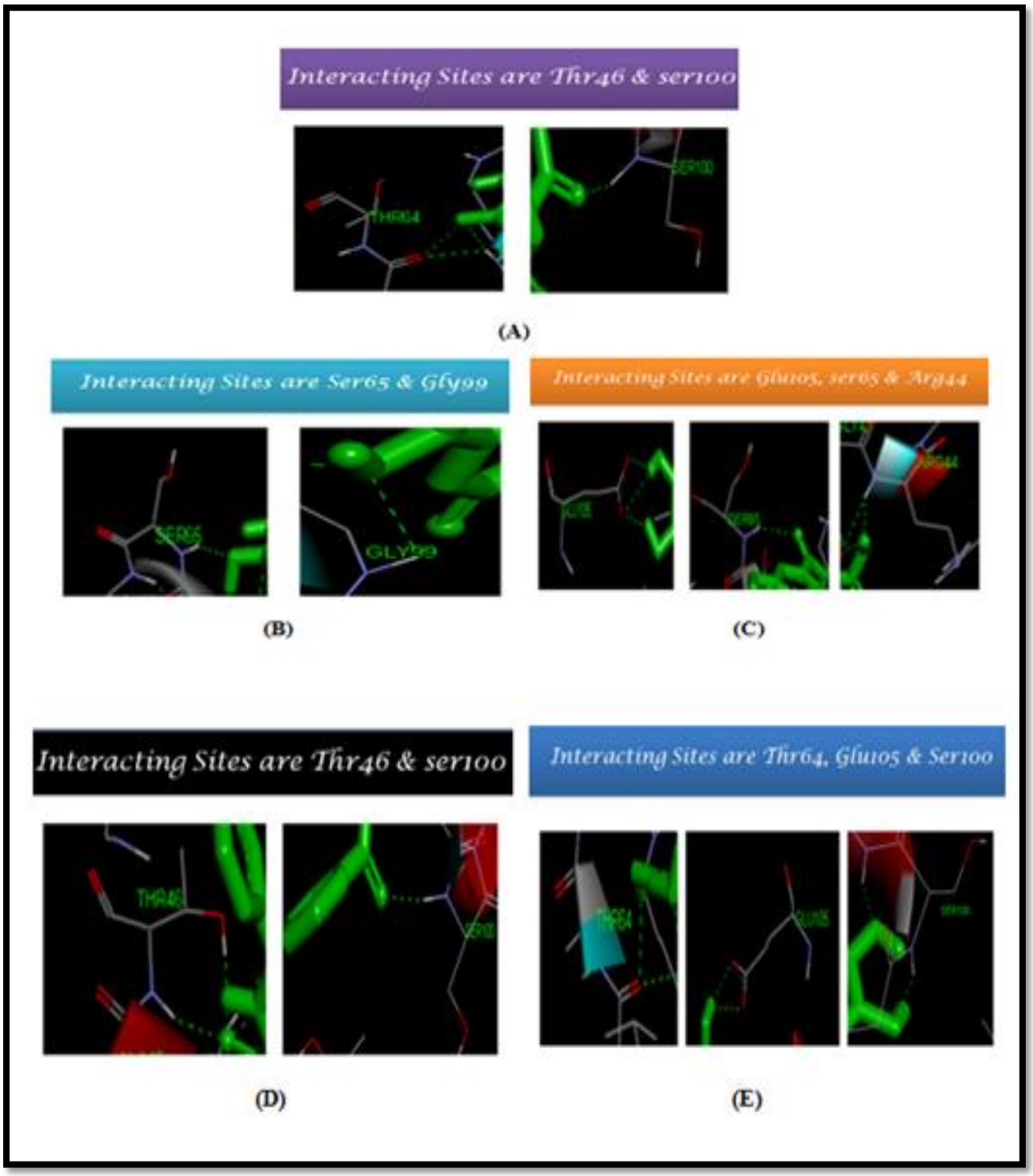
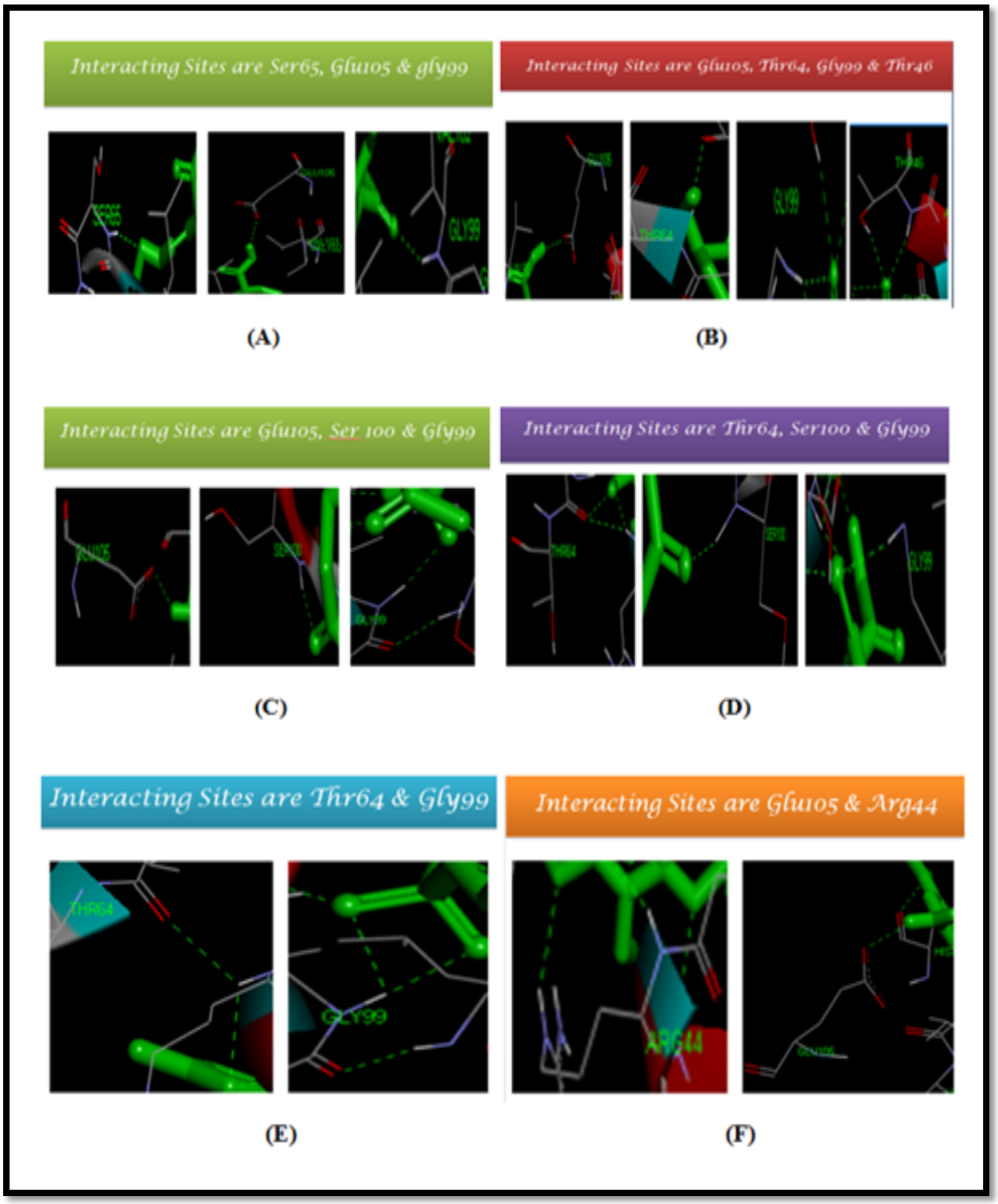
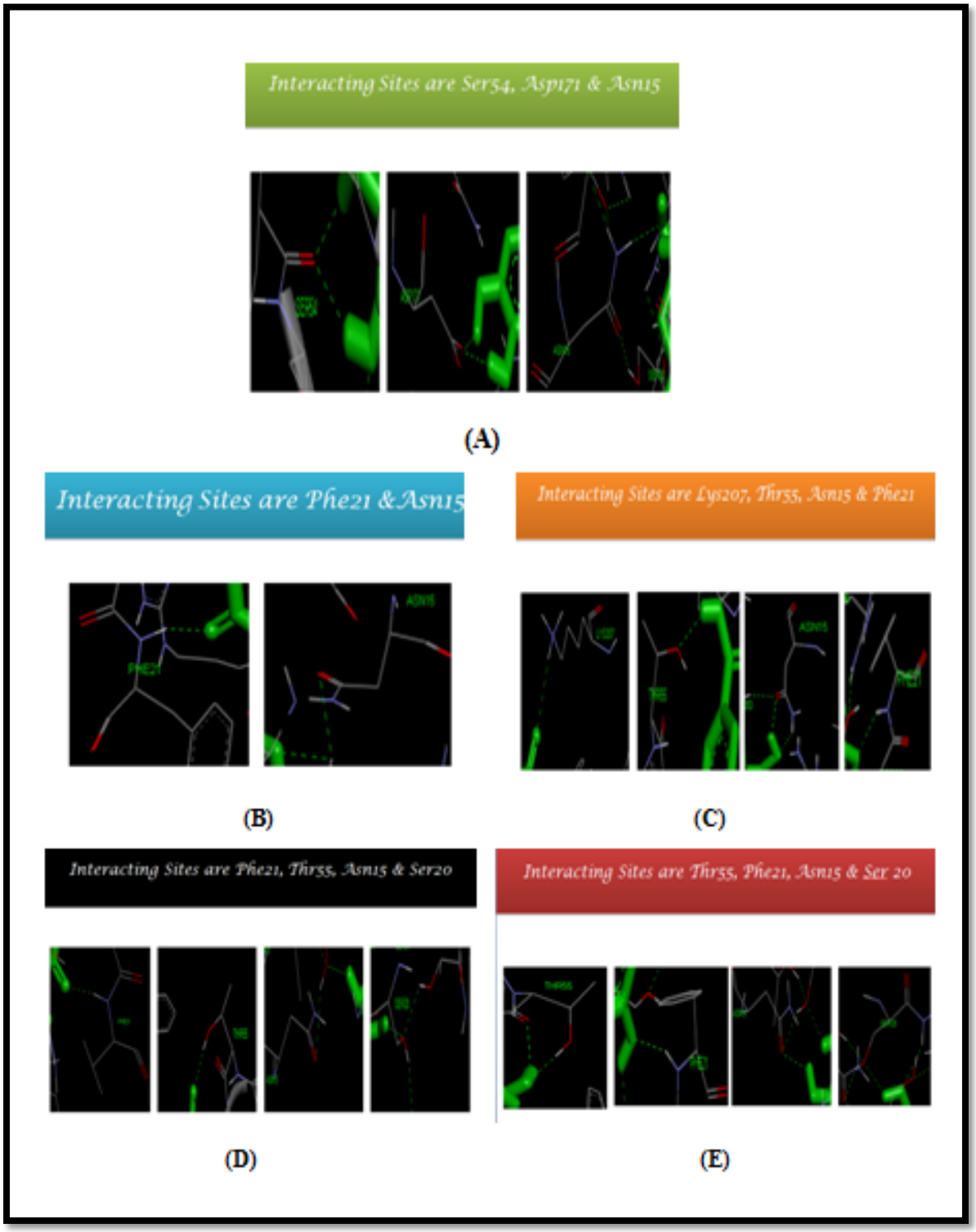


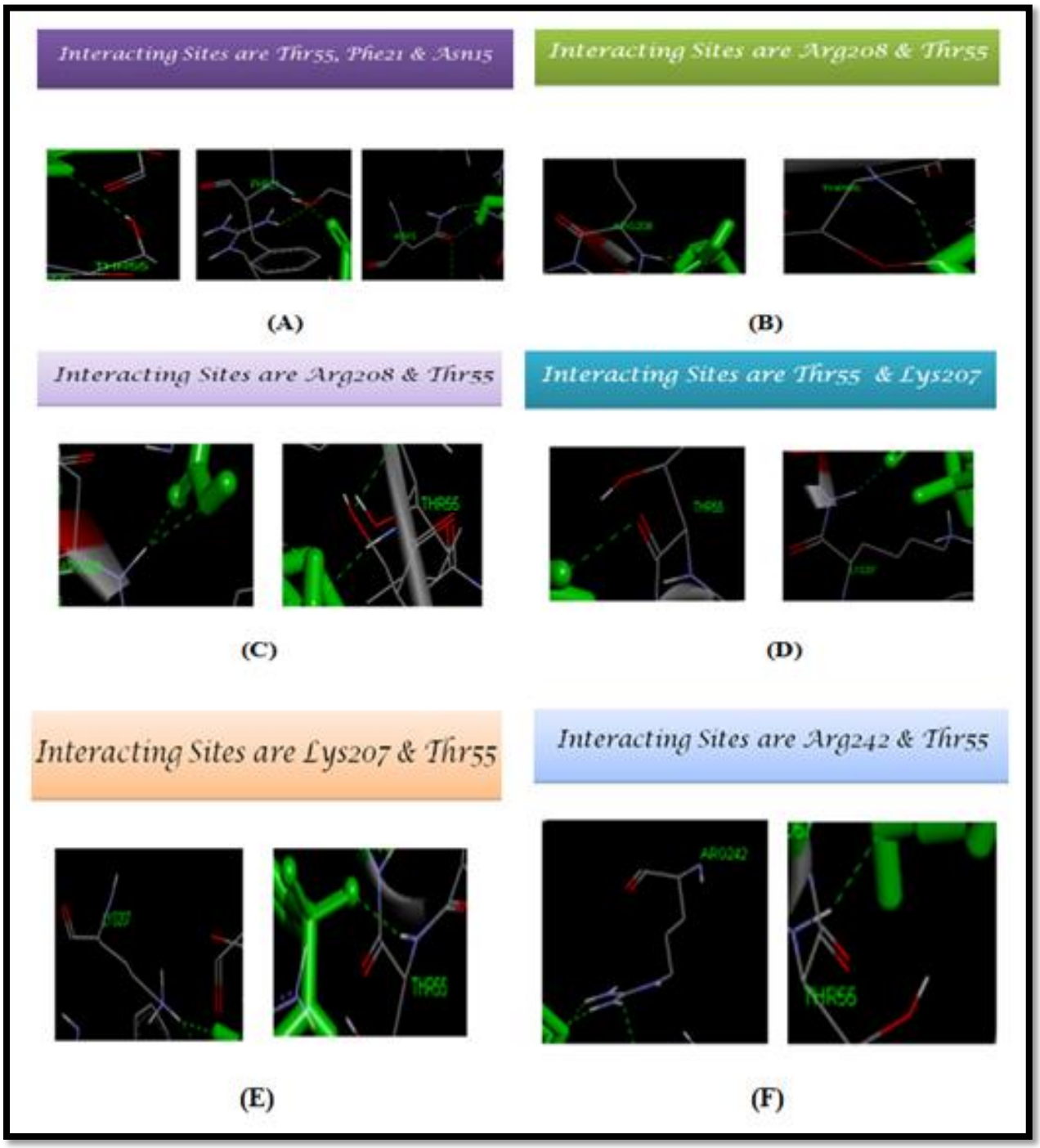
Fig (8): Interacting Sites of DHFR Protein of *E.faecalis* with Inhibitors i.e. (A) Chlorogenic acid,(B) Ellagic acid, (C) Gallic acid, (D) Hippuric acid and(E) Quercetin.



Fig(9): Interacting Sites of DHFR Protein of *E.faecalis* with Antibiotics i.e. (A)Clavulanic acid, (B) Cephalosporin,(C) CephalosporinC, (D) Penicillin, (E) Sulfamethoxazole and (F) Trimethoprim.



Fig(10): Interacting Sites of DHPS Protein of *E.faecalis* with Inhibitors i.e. (A) Chlorogenic acid,(B) Ellagic acid, (C) Gallic acid, (D) Hippuric acid and (E) Quercetin.



Fig(11): Interacting Sites of DHFR Protein of *E.faecalis* with Antibiotics i.e. (A)Clavulanic acid, (B) Cephalosporin,(C) CephalosporinC, (D) Penicillin, (E) Sulfamethoxazole and (F) Trimethoprim.

III. DISCUSSION

(UTIs) are the most common infections caused by *Enterococcus faecalis*. Little is known about the bacterial factors necessary for *E. faecalis* to cause infections in general, and even less has been reported related to the urinary tract. Many researchers have exposed the emergence of multidrug resistance in *Enterococci faecalis* to all clinically useful antibiotics (15, 17).

Insilico studies with DHFR & DHPS showed a high binding affinity towards the ligands viz., Chlorogenic acid, Ellagic acid, Gallic Acid, Hippuric acid and Quercetin and as well as the prescribed standard antibiotics viz., Clavulanic acid, Cephalosporin, Cephalosporin C, Penicillin, Sulfamethoxazole and Trimethoprim.

Molecular docking has been carried out to check the efficiency of these ligands and Standard antibiotics to bind to the active site of the DHFR protein of the folate pathway. On comparing the various inhibitors and standard antibiotics docked

upon DHFR and DHPS of *E.faecalis*, Quercetin, Ellagic acid, Penicillin and Cephalosporin show higher binding affinity. Hence, it can be concluded that the compounds Quercetin and Ellagic acid have significant potential to bind to the active site of DHFR.

Our study also shows that, inhibitors like Clavalunic acid and Gallic acid efficiently bind to the active site of the DHPS of *E.faecalis*. Quercetin and Chlorogenic acid have the highest binding affinity to the ligand binding pocket of DHPS of *E.faecalis*. When compared to inhibitors i.e Quercetin and Chlorogenic acid, antibiotics like Cephalosporin & Penicillin show higher interactions towards DHPS of *E.faecalis*.

Even though, Standard antibiotics viz., Cephalosporin and Penicillin do show strong interactions with both the proteins of the folate synthesis pathway, the clinical isolates of *E.faecalis* have developed resistance to these. Hence, a better alternative, which binds to DHFR is Quercetin and Ellagic acid while inhibitors that binds to DHPS are Quercetin and Chlorogenic acid.

Protein binding with various ligands, indicate that various inhibitors viz., Chlorogenic acid, Ellagic acid, Gallic Acid, Hippuric acid and Quercetin of DHFR and DHPS can be utilized for the treatment of MDR- UTI after due *invivo*, *invitro* and ADMET testing, since they have been proved to posses potential antibacterial activities.

IV. CONCLUSION

Antibiotics have been a high success till date for curbing bacterial infections. But, the widespread and uncontrolled use of antibiotics has led to the emergence of multidrug-resistant (MDR) bacteria (15). Along with limited treatment options and increased mortality the MDR seems grave. Hence, there is an urgent need to search for a new antibacterial agent. The molecular docking programs aid to establish new ligands/inhibitors for the selected target receptor proteins from the different available databases, based on their efficiency to bind the active sites on the receptor (15). Our study shows that inhibitors viz., Quercetin and Ellagic acid show best interactions and binding energy with DHFR while Quercetin and Chlorogenic acid show best interactions and binding energy with DHPS of the folate synthesis pathway of *E.faecalis*. These *in silico* studies supported with *invivo*, *invitro* and ADMET testing will certainly help towards developing candidates for treatment of MDR-UTI in the future. More studies on mutations are needed for corroborating the role of *quercetin*, *chlorogenic acid* and *ellagic acid* as antibacterial agents to treat MDR *E. faecalis* mediated uropathological infections.

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