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COMPARATIVE MOLECULAR DOCKING ANALYSIS OF VARIOUS PHYTOCHEMICALS AS β-LACTAMASE INHIBITORS

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ABSTRACT

The management of the multi-drug resistant strains of both Gram-negative and Gram-positive pathogens has become increasingly difficult because of the β-lactamase production which confers resistance to the β-lactam antibiotics by catalyzing the cleavage of the β-lactam ring to yield inactive products. The present study was formulated to analyze the drug-likeliness and β-lactamase inhibitory potential of fifteen phytochemicals by ADME (Absorption, Distribution, Metabolism and Excretion) and docking studies. *In silico* docking studies were carried out using GLIDE. The results showed that all the phytochemicals showed GLIDE score ranging between -2.36 to -8.20 when compared with that of the standard Clauvenic acid (-2.16). *In silico* studies also showed that flavonoids and sterols exhibited significant inhibitory potential as compared to other classes of phytochemicals. Docking and ADME studies revealed that out of 15 phytochemicals, β-Sitosterol (GLIDE Score -8.20) possessed the appreciable inhibitory profile against β–lactamase and scientifically validates its use as a β-lactamase inhibitor.

KEYWORDS: Antibiotic-resistance, β-Lactamase, β-lactam antibiotics, docking, β-Sitosterol.

INTRODUCTION

Infectious disease – the fourth horseman of the apocalypse – is the largest source of premature deaths and suffering; accounting for deaths of 15 million people annually [(1)]. The World Health Organization estimates that nearly 50,000 people die each day throughout the world from infectious diseases. Clearly, understanding and combating the spread of disease is among the most serious challenges faced by us and problem is more aggravated when the disease causing microbes becomes resistant to the antimicrobial agents. The discovery of antibiotics was an essential part in combating these infectious diseases. But the irrational use of antibiotics led to the emergence and dissemination of antibiotic resistant strains. Antibiotic resistance genes database lists the existence of more than 20,000 potential resistance genes (r genes) of nearly 400 different types, predicted from available bacterial genome sequences [(2)]. Several resistance mechanisms have been developed in bacteria which include metabolic pathway alteration, target site alteration, efflux pumps and enzymatic cleavage of antibiotics (β-lactams, aminoglycosides and chloramphenicol). Among these mechanisms, resistance in bacteria acquired by enzymatic cleavage of β-lactam antibiotics remains critical.β-lactams are a large group of antibiotics having the β-lactam ring. Penicillins, cephalosporins, carbapenems and monobactems are four major groups, which differ from one another in the nature of the additional ring attached to the β-lactam ring. β-lactams account for approximately 50% of global antibiotic consumption and this heavy usage leads to bacterial resistance [(3)]. The resistance to β-lactams is mediated by the production of β-lactamases [(4)]that catalyze the hydrolysis of the β-lactam ring to yield inactive products. The first β-lactamase was identified in *Escherichia coli* prior to the release of penicillin for use in medical practice [(5)]. Now, five hundreds of different β-lactamases have been identified [(6)]. Today, the management of the MDR strains of both Gram-negative and Gram-positive pathogens has become increasingly difficult because of the β-lactamase production in *Staphylococcus, Bacillus, Pseudomonas, Proteus, Klebsiella, Neisseria, Salmonella, Haemophilus* etc [(7)] β-lactamase activity of S. *aureus* has caused resistance to most penicillin

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derivatives including the oxacillin and now is identified as MRSA, which has become major cause of nosocomial infections worldwide. Bacterial enzymes that degrade or modify antibiotics are themselves important targets for drug action since their inhibition protect the antibiotic from degradation [8]. To prevent the breakdown of β-lactam antibiotics due to β-lactamases, clavulanic acid (β-lactamase inhibitor) is used in combination with β-lactam compounds such as amoxicillin - clavulanate. Thus, the co-administration of β-lactamase inhibitors is an important strategy for restoring the activity of β-lactam antibiotics [9]. It is therefore of great interest to find co-therapeutic agents that inhibit antibiotic degrading β-lactamases. Various phytochemicals may have the potential to either inhibit the modified targets or exhibit a synergy by blocking one or more of the other targets in the metabolic pathway, thus causing cell death [10]

Literature is rich with the reports of the analysis of β –lactamase inhibitory potential of various plants by *in vitro* approach. However, limited information is published regarding β –lactamase inhibitory potential of phytochemicals by *in silico* approach. Therefore, in consideration of paucity of data, the present *in silico* study was formulated to evaluate the β –lactamase inhibitory potential of fifteen phytochemicals which are the main constituents of the plants already reported to have anti β-lactamase activity. The affinity of phytochemicals to β–lactamase may be determined by computational analysis of inhibitor/substrate docking in the enzyme active site. Thus, the present study was focused on targeted inhibition of β –lactamase by a set of natural compounds using Schrodinger suite. The study also provides a comparative account of the efficacy of phytochemicals to that of commercially available inhibitor Clauvenic acid.

MATERIALS AND METHODS

Selection of Target Proteins

The X- ray crystal structure of the proteins β–lactamase (PDB ID: 1KE4) with refinement of 1.72Å was downloaded from the RCSB protein databank (www.rcsb.org/pdb).

Protein preparation

Protein preparation and refinement studies were performed on 1KE4 using protein preparation module (Schrodinger suite, LLC) in which the water molecules were removed, hydrogen atoms were added, bond orders were assigned and orientation of hydroxyl groups were optimized. Finally, energy minimization was carried out using default constraint of 0.3 Å RMSD and OPLS 2005 force field.

TABLE: 1 DOCKING RESULTS OF SYNTHETIC PHYTOCHEMICAL

Synthetic Standard Active Compound	Compound ID	Molecular Weight	XP Glide Score with β –Lactamase	Number of hydrogen bond	Interacting Residues
Clauvenic acid	CID:5280980	199.16	-2.16	2	Arg148, Glu272, Ser64, Thr68, Tyr112, Trp271 and Val65.

Ligand preparation

The structure of the ligands (15 phytochemicals + standard Clauvenic acid) was downloaded from PubChem (http://www.ncbi.nlm.nih.gov/pubchemcompound). Ligand structures were geometrically minimized using OPLS_2005 force field by Ligprep module of Maestro 9.1 (Schrödinger suite, LLC). Ligprep produces a single, low energy, 3D structure for each input structure with various ring conformations, ionization states and tautomers using various criteria including molecular weight or specified numbers and types of functional groups present. The prepared ligands can be used for docking.

Molecular docking using GLIDE

GLIDE uses a hierarchical series of filters to search for possible locations of the ligand in the active site region of the receptor. The receptor grid was generated at the receptor site bound by a ligand. The ligands were then docked to the target proteins using Glide module of Schrodinger. The docking was done in Extra Precision mode (XP). Glide XP scoring protocols were used for the docking. The

TABLE: 2 DOCKING RESULTS OF VARIOUS NATURAL PHYTOCHEMICALS

Herbal Active Compound	Compound ID	Molecular Weight	XP Glide Score with β – Lactamase	Number of hydrogen bond	Interacting Residues
Afzelone A	CID:641712	540.525	-2.90	2	Asp275, Gly270, Glu272, Leu274, Trp271, Trp276.
Baicalin	CID:64982	446.36	-5.22	4	Ala231, Glu61, Ile227, Leu62, Met230 and Phe60.
Crinamean	CID:541205	343.37	-4.25	0	Glu272, Lys67, Ser64, Thr68, Tyr150 and Val65.
acetate					Lys67, Ser64 and Tyr150.
Ferulic acid	CID:445858	194.18	-2.57	1	Asn237, Leu238, Leu274 and Val234.
Galangin	CID:5281616	270.24	-4.23	1	Arg148, Gly270, Glu272, , Ile283, Leu274, Met265, Thr68 and Trp271
Globularin	CID:44148071	478.45	-3.21	5	Gly270, Lys67, Ser64, Thr68, Trp271, Tyr112 and Tyr150
Methyl Phenol	CID:248913	138.16	-2.69	1	Asn237, Glu272, Gln256, Trp271 and Val234
					Glu272, Gln256, Ile283, Leu274, Met273, Thr68 and Trp271
Pinenone	CID:29025	150.22	-2.68	0	Glu272, Ile274, Ile283, Lys67, Met265, Ser64, Trp271 and Tyr112.
Quercetin	CID:5280343	302.24	-4.04	2	Ala29, Ala31, Ala231, Glu61, Ile33, Ile227, Leu62, Leu238, Phe60, Met28, Met230, Val32, Val65 and Val234
Schaftoside	CID:442658	564.49	-6.97	2	Glu272, Leu274, Thr68, Trp271, Trp276, Met 273, Val65 and Val278
β-Sitosterol	CID:222284	414.71	-8.20	1	Arg148, Glu272, Leu62, Ser64 and Tyr112.
Ursolic acid	CID:64945	456.70	-2.36	1	Asn237, Ile283, Leu274, Glu272, Trp271and Val 278.
Vicenin	CID:442664	594.52	-7.33	7	Asn237, Gln256, Glu272, Ile238, Ile283, Leu274,
Violanthin	CID:44257675	578.50	-6.20	2	Lys67, Met 273, Ser64, Thr68, Trp271, Val65, Val278 and Val234
Xylosyl vitexin	CID:631110	690.74	-3.92	0	Arg158, Glu282, Leu82 and Tyr132.
Ursolic acid					
Vicenin					
Violanthin					
Xylosyl vitexin					

docked protein and the ligands were viewed with Glide Pose Viewer. Non-bonded interactions like hydrophobic was observed using LigPlot program and these interactions can increase the binding

affinity between target drug interfaces. The images of the best docked poses of the ligand and the protein were saved as .jpg files.

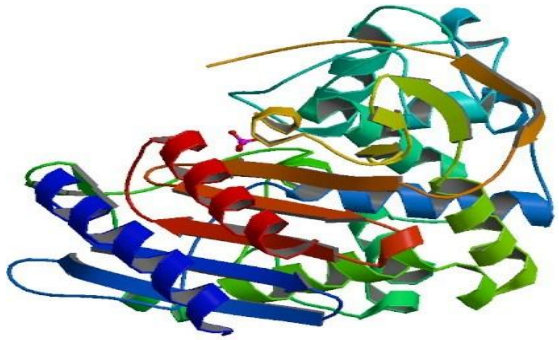

FIGURE 1 : X- ray crystal structure of β–lactamase of *e. coli* retrieved from the RCSB PDB

ADME studies

The drug likeliness of various herbal compounds was screened by ADME studies. The QikProp 3 module predicts physically significant descriptors and pharmaceutically relevant properties of organic molecules. In addition to predicting molecular properties, Qikprop provides ranges for comparing a particular molecule's properties with those of 95% of known drugs. The Absorption, Distribution, Metabolism and Excretion (ADME) studies of the prepared ligands were done using QikProp module of Schrodinger.

evaluation should be comprehensive enough to ensure the efficacy of the compounds. Screening of the compounds by Pre-ADMET tools facilitates the elimination of weak candidates and selection of the potential lead candidates. Absorption, distribution, metabolism, excretion (ADME) studies are the most important part of pharmacological studies. Hence, all 15 ligands and standard were tried for their drug-likeliness, ADME profile, blood brain barrier penetration and permeability analysis by Pre-ADMET. Drug-likeliness was evaluated by Lipinski rule of 5 and lead like rule. Absorption was predicted by human intestinal/oral absorption model. Distribution was predicted by blood brain barrier penetration. Caco2-cell (heterogeneous human epithelial colorectal adenocarcinoma cell lines) and MDCK (Madin-Darby Canine Kidney) cell models were used for predicting oral drug absorption and skin permeability.

The structure of the target protein β–lactamase (*Escherichia coli*) was obtained from the Protein Data Bank and refined using the protein preparation wizard (Figure 1). Various phytochemicals; Afzelone A, Baicalin, Crinamean acetate, Ferulic acid, Galangin, Globularin, Methyl Phenol, Pinenone, Quercetin, Schaftoside, β-Sitosterol, Ursolic acid, Vicenin, Violanthin and Xylosyl vitexin were screened for their β-lactamase inhibitory activities by molecular docking studies. These phytochemicals were selected for the study as these are the main constituent of the plants, which are reported to have

TABLE: 3 ADME PROFILE OF SYNTHETIC PHYTOCHEMICAL

Standard/ Herbal Active Compound	Lipinski's Rule	Lead like Rule	Human Oral Absorption	Caco2 Cell Permeability	MDCK Cell Perme-ability	Skin Perme-ability	Blood Brain Barrier Penetration
Clauvenic acid	Suitable	Suitable	Moderate	Low	Low	High	High

RESULTS AND DISCUSSION

Computer-aided drug design (CADD) and discovery methods put emphasis on the identification of novel active compounds that are selective against target families or individual targets and can be used as molecular probes for specific functions [(11)]. Compounds with favorable pharmacokinetics are vital for structure based drug discovery. Therefore, the preclinical pharmacokinetic

anti β-lactamase activity by *in vitro* study [(12,13,14,15,16,17,18,19)].

The docking results revealed that all ligand–protein complexes were stabilized by hydrogen bonds, hydrophobic bonds and electrostatic interactions except Crinamean acetate, Pinenone and Xylosyl vitexin. This indicates that Crinamean acetate, Pinenone and Xylosyl vitexin binds to the target molecule β-Lactamase via weak bonds (hydrophobic bonds and electrostatic interactions)

other than H-bonds. Although maximum seven hydrogen bonds were formed by the phytochemical Vicenin with good docking score but the candidature of compound as a drug was not supported by the ADME profile. However ADME profile supports the drug candidature of Methyl Phenol and Pinenone but GLIDE score reveals that these compounds don't exhibit strong affinity for target. Docking results and ADME profile of the phytochemicals are summarized in Table 1, 2, 3 & 4.

The binding mode of the phytochemicals with in the active site of β–lactamase was also analyzed. The amino acid residues responsible for the binding interactions of the phytochemicals with the β–lactamase were Ala29, Ala31, Ala231, Arg148, Asn237, Asp275, Gly270, Glu272, Glu61, Gln256, Ile33, Ile227, Ile238, Ile274, Ile283, Leu62, Leu238, Leu274, Lys67, Met28, Met230, Met265, Met273, Phe60, Ser64, Thr68, Tyr150, Tyr112, Trp271, Trp276, Val32, Val65, Val234 and Val278. The binding site of the standard Clauvenic acid was found to have Arg148, Glu272, Ser64, Thr68, Tyr112, Trp271 and Val65. These results proved that the effective binding orientations were present in the tested phytochemicals when compared with the standard Clauvenic acid.

The GLIDE score of different phytochemicals revealed that the β–lactamase had a strong affinity for Baicalin, Schaftoside, β-Sitosterol, Vicenin and Violanthin; β-Sitosterol is a phyosterol while others are the flavonoids. Thus, the present investigation revealed that the flavonoids and the sterols have more inhibitory potential as compared to other classes of phytochemicals. The *in silico* inhibitory potential of flavonoid is also reported by Ganugapati *et al*., 2011 [20]. The tested phytochemicals showed GLIDE score ranging between -2.36 to -8.20 when compared with that of the standard (-2.16). All the tested phytochemicals showed more affinity to the target in comparison to the standard. This proves that tested phytochemicals consist of potential β–lactamase inhibitory binding sites similar to that of the standard. On the basis of GLIDE score and ADME profile, it was inferred that out of 15 phytochemicals, β-Sitosterol possessed maximum affinity for β–lactamase (GLIDE Score -8.20) and the reasonable stability & bioavailability, thus having the appreciable inhibitory profile against β–lactamase and could be considered as the lead compound for

TABLE: 4 ADME PROFILE OF NATURAL PHYTOCHEMICALS

Standard/ Herbal Active Compound	Lipinski's Rule	Lead like Rule	Human Oral Absorption	Caco2 Cell Perme-ability	MDCK Cell Permeability	Skin Perme-ability	Blood Brain Barrier Penetration
Afzelone A	Suitable	Suitable	Moderate	middle	Low	High	High
Baicalin	Suitable	Suitable	Poor	Low	Low	High	High
Crinamean acetate	Suitable	Suitable	Well absorbed	High	Middle	High	High
Ferulic acid	Suitable	Suitable	Moderate	Middle	Middle	High	High
Galangin	Suitable	Suitable	Moderate	Middle	Middle	High	High
Globularin	Suitable	Suitable	Moderate	Middle	Low	High	High
Methyl Phenol	Suitable	Suitable	Well absorbed	High	High	High	High
Pinenone	Suitable	Suitable	Well absorbed	High	High	High	High
Quercetin	Suitable	Suitable	Moderate	Low	Low	High	High
Schaftoside	Suitable	Suitable	Poor	Low	Low	High	High
β-Sitosterol	Suitable	Suitable	Well absorbed	High	High	High	High
Ursolic acid	Suitable	Suitable	Well absorbed	Middle	Middle	High	High
Vicenin	Suitable	Suitable	Poor	Low	Low	Low	Low
Violanthin	Suitable	Suitable	Poor	Low	Low	High	Low
Xylosyl vitexin	Suitable	Suitable	Moderate	High	High	Low	High

further analysis. All the bonded and non-bonded interactions of β-Sitosterol with β–lactamase are shown in Figure 2.

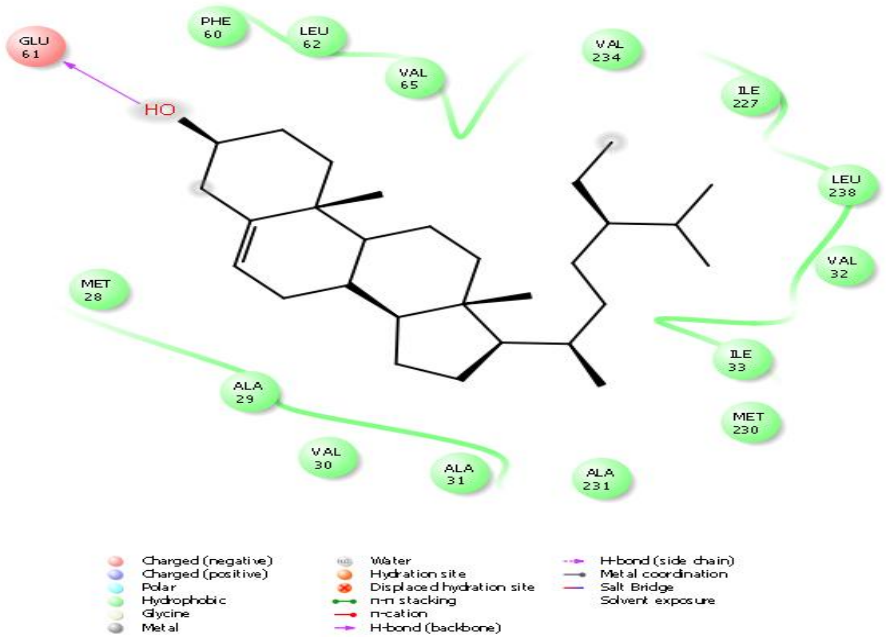

FIGURE 2 Ligplot image of interaction between β–lactamase with β-sitosterol

Thus, the study revealed that the phytochemicals have great potential to inhibit β–lactamase. Role of some other phytochemicals as β–lactamase inhibitor was also reported by Lakshmi *et al.,* 2011[21] but this is the first report of these 15 phytochemicals regarding their β–lactamase inhibitory potential.

CONCLUSION

In order to contain the problem of antibiotic resistance to the β-lactam antibiotics, development of new β-lactamase antagonists should be advanced. An effort was made in this direction and the results of the present investigation clearly demonstrate the β-lactamase inhibitory potential of β-Sitosterol. The ADME profile also supports its bioavailability and it was found to be more efficient than already commercially available inhibitor Clauvenic acid, satisfying all the criteria and the necessary parameters to act as drug. Thus, β-Sitosterol has great potential to reverse β–lactam resistance, so can be co-administered as β-lactamase inhibitors to restore the activity of β-lactam antibiotics. Further investigations are necessary to develop potential chemical entity for the prevention of resistance to the β-lactam antibiotics.

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