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Research Article

MOLECULAR DOCKING STUDIES OF *RICINUS COMMUNIS* PHYTOCHEMICALS AGAINST BETA-LACTAMASE FROM *ENTEROCOCCUS FAECALIS* AND *STAPHYLOCOCCUS AUREUS*

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ABSTRACT

Objective: The objective of this study is to investigate the antibacterial activity of *Ricinus communis* phytochemicals against beta-lactamase from *Enterococcus faecalis and Staphylococcus aureus* through molecular docking studies.

Methods: The three-dimensional (3D) structure of beta-lactamase from *E. faecalis* was modeled using modeler 9v9 and validated. The 3D structure of beta-lactamase from *S. aureus* (PDB ID: 1 GHP) was retrieved from PDB database. The 2D structures of 29 phytochemical compounds from the methanol leaf extracts of *R. communis* were drawn in ACD-Chemsketch and converted into 3D structures. The 3D structure of *R. communis* leaf compounds and cefotaxime (control) was virtually screened in the binding pockets of β-lactamase proteins from *E. faecalis* and *S. aureus* using FlexX docking program.

Results: The docking studies revealed that ferulic acid and hyperoside exhibited promising minimum binding and docking energy that is closely related to the docking score of standard antibiotic cefotaxime.

Conclusion: The result of the present study indicates that ferulic acid and hyperoside are potential compounds that could be effectively used in the treatment of infections caused by *E. faecalis* and *S. aureus.* However, further clinical studies are required to ascertain the antibacterial activity and potential toxic effects of ferulic acid and hyperoside *in vivo*.

Keywords: Homology modeling, Molecular docking, *Ricinus communis*, *Enterococcus faecalis*, *Staphylococcus aureus*, β-lactamase.

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INTRODUCTION

Staphylococcus aureus is a versatile pathogen capable of causing both community-acquired as well as hospital-acquired infections ranging from skin infections, surgical site infections, sepsis, pneumonia, to bloodstream infections. *Enterococcus faecalis* is the causative agent of urinary tract infection, surgical site infections, coleocystis, or septicemia. Furthermore, the prevention and treatment of post-operative surgical site infections have been complicated by the drug-resistant pathogens, especially, beta-lactamase producing *E. faecalis* and *S. aureus*. Multidrug-resistant *S. aureus* strains that exhibit coresistance to methicillin, vancomycin, linezolid, and tigecycline are on the rise. Serious enterococcal infections can be optimally treated with the synergistic combination that includes a cell wall active agent (ampicillin/vancomycin) and an aminoglycoside. However, the increasing incidence of high-level aminoglycoside-resistant strains and Bla$^+$ enterococci has nullified this possibility [1].

The continuous use and misuse of antibiotics have led to the emergence of multidrug-resistant pathogens. In the past few decades, the emergence and spread of beta-lactamase producing Gram-positive bacteria, especially *E. faecalis* and *S. aureus,* have become a serious public health concern. The CTX-M enzymes have become the most prevalent extended spectrum β-lactamases that mainly targets cefotaxime. The change in activities of CTX-Ms leading to the evolution of more variants may be due to point mutations present either inside or outside of its active site omega loop [2-4]. Thus, the identification of the potential compound from a plant source that overcomes these strategies might serve as a novel agent that can challenge these situations. Although various efforts are underway in the development of novel antimicrobial chemotherapeutic agents, i.e., synthetic drugs, the majority of these drugs in the pipeline suffer the higher production cost and adverse effects compared to the plant-derived drugs [5,6]. Hence, there is a growing need for the search of potential compounds from plant sources that possess antibacterial activity against these beta-lactamase producing strains [7]. The increase in the search for phytotherapeutic agents is largely based on the fact that plants continue to survive environments with high bacterial density and might possess protective means against bacterial infections [8].

Ricinus communis L. (Castor bean [in English] and amanakku/kottaimuthu [in Tamil]), a native of India, is an important non-edible oilseed crop, that belongs to the *Euphorbiaceae* (spurge) family. Castor bean is an annual or perennial shrub that is widely cultivated for its economic value in many tropical, subtropical, and warm temperate regions around the world. The leaves of *R.* communis L. have pronounced activity on the female generative organs and lactation. These leaves are used as anti-inflammatory, anticonvulsant, and analgesic activity [9]. Several animal studies have documented the hepatoprotective effect of the alcoholic extracts of *R.* communis L. leaves [10].

In recent decades, advances in computational techniques have enabled virtual screening of drug discovery. Virtual screening exploits molecular docking which implies the prediction and orientation of ligand molecules within the catalytic sites of receptors to form a stable complex. However, the choice of "best-fit" orientation of a ligand persists as an optimization problem, which would describe the ligand's inhibition activity against a particular protein of interest [11]. Docking conventionally reports two important pieces of information such as correct conformation of a ligand-receptor complex and its binding affinity which represents an approximation of the binding free energy relevant to the formation of the complex [12]. Many studies on the screening of phytochemical compounds from medicinal plants have made an impact on the identification of compounds as inhibitors [13,14]. Thus, in the present study, the β-lactamase protein from *E. faecalis* was modeled through homology modeling, and the available X-ray three-dimensional (3D) structure of β-lactamase from *S. aureus* was used for docking studies with the phytochemical compounds from the methanolic extract of *R. communis* (leaf) that is revealed through gas chromatography-mass spectrometry (GC-MS) analysis, as it might lead to the design of a novel antibacterial compound against the β-lactamase-producing *S. aureus* and *E. faecalis.*

METHODOLOGY

Sequence analysis for potential templates

The β-lactamase protein sequence of *E. faecalis* was retrieved from the UniProtKB database (UniProt ID: Q6LDJ1). Using basic local alignment search tool (BLASTP) [15], similarity search tool against PDB database, the most homologous sequence was obtained and considered as a potential template for homology modeling and its respective atomic coordinate file from PDB was obtained for homology modeling [16]. The sequence alignment and alignment errors were refined using ClustalW [17] program as the sequence alignment reflects the quality of the homology models. The X-ray 3D structure of β-lactamase from *S. aureus* was retrieved from PDB database (PDB ID: 1 GHP) [18].

Homology modeling

Using the homology modeling tool, Modeler9v9, the homology models of β-lactamase protein sequences from *E. faecalis* were built by employing the target-template sequence alignment files. A total of five 3D models of the target sequences were built from the starting structure of the templates by satisfying the spatial restraints through random generation. Among the generated models, the least RMSD value in comparison with template structure was considered for selecting the best model and its energy was minimized through 20 steps of steepest descent and conjugate gradient using GROMOS [19] of Swiss-PdbViewer, and the final energy-minimized model was used for further analysis.

Model validation

The stereochemical parameters of the energy-minimized models were considered to evaluate the quality of the generated models. The phi and psi angles representing the stereochemical parameters of the model through PROCHECK [20], the compatibility of a generated 3D structure with its own amino acid sequence through verify 3D [21], and the regions of the modelled structure that can be rejected at the 95 % and 99 % confidence intervals through ERRAT were determined at Structural Analysis and Verification Server (SAVES) [22].

Binding pocket prediction

The binding efficiency of the phytochemical compounds from the *R. communis* leaf was determined through predicting the binding pocket of modeled β-lactamase protein structures of *E. faecalis* using DoGSiteScorer [23]. The binding site of β-lactamase complexed with Penicillin in *S. aureus* is considered for the docking studies.

Lead compounds

The 2D structure of the phytochemical compounds from leaf extracts of *R. communis* was drawn in ACD-Chemsketch [24], and their SMILES notation was obtained. They were converted into SDF files using “online SMILES convertor and structure file generator” [25] for further docking studies.

Virtual screening

The obtained 3D structure of *R. communis* leaf compounds in SDF format was virtually screened to reveal their binding efficiencies through docking in the predicted binding pockets of modeled β-lactamase proteins from *E. faecalis* and X-ray crystal structure of β-lactamase from *S. aureus* using FlexX [26] with docking parameters such as triangle matching base placements, zero full score and no score contributions, and threshold for full score and no score contributions of 30 and 70, respectively; Clash handling values of 2.9 A03 and 0.6 for protein-ligand clashes with maximum allowed overlap volume and intra-ligand clash factors while considering the hydrogen in internal clash tests and 200 as the default docking values for maximum number of solutions per iteration and also per fragmentation [27].

Docking interactions

The docking interactions that envisage the binding affinities of the phytochemical compounds from GC-MS analysis of methanol extracts of *R. communis* leaf with the predicted binding pocket amino acids in the modeled β-lactamase proteins from *E. faecalis* and X-ray crystal structure of β-lactamase from *S. aureus* were analyzed using poseview module of LeadIT [28] which clearly picturized the Hbond and non-bond interactions.

RESULTS AND DISCUSSION

The emergence and widespread dissemination of multidrug-resistant strains of enterococci and staphylococci have become a serious public health concern. In this scenario, the development of novel antibacterial agents with minimal side effects has become the need of the hour. In western countries, there is a greater demand for phytopharmaceutical p2oducts of medicinal plant origin [29]. Hence, the present study was designed to assess the antibacterial activities of the methanolic extract of *R. communis* (leaf), against beta-lactamase producing *E. faecalis* and *S. aureus*.

Target-template alignment for homology modeling

The BLASTP analysis of target sequence of the β-lactamase protein from *E. faecalis* against PDB shows the X-ray crystal structure of β-lactamase from *S. aureus* (PDBID: 1KGF A_chain) as homologous sequences with sequence similarity of 92.6 % at an E-value of 1.10e–32. The template-target sequence alignment is shown in Fig. 1. It is worth mentioning that the obtained template sequence is of β-lactamase and is from the same genera, which has resulted in the better template-target alignment and has left the choice of considering these as homologous sequences and as template structure for generating homology models.

Homology modeling

The template-target sequence alignment files were used to generate a bundle of 5 initial models of β-lactamase proteins from *E. faecalis* in the automated homology modeling tool Modeler9v9 by applying spatial restraints from the initial structure. The discrete optimized protein energy (DOPE) score that significantly reveals the structural compatibility of the models was considered to rank the models. Model-3 of β-lactamase proteins from *E. faecalis* with the lowest DOPE assessment score was considered as the best model with the most stable minimized energy and considered for further analysis. The best models of β-lactamase from *E. faecalis* and the X-ray crystal structure of β-lactamase (PDBID: 1GHP) from *S. aureus* are shown in Fig. 2.

Model assessment

The quality of the modeled structure was assessed through SAVES of UCLA-DOE Lab. The phi and psi angles that explore the stereochemical parameters of the energy-minimized model of β-lactamase protein from *E. faecalis* was determined using PROCHECK; the 1D–3D structure compatibility of the best models and the regions of the modeled structure that can be rejected at the 95% and 99% confidence intervals were predicted through verify3d and ERRAT programs. The Ramachandran plot of the energy-minimized model of a β-lactamase protein from *E. faecalis* showed most of the residues in the most favorable region and 0.0% in the disallowed region. The Ramachandran plot of all the generated models of β-lactamase protein from *E. faecalis* was analyzed and model-3 was considered best as it exhibited a number of residues in the most favorable regions and also the low number of residues in the disallowed region (Fig. 3). Furthermore, the other quality factor

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   Seqres MKELNDLEKKYNAHIGVYALDTKSGKEVKFNSDKRFAYASTSKAINSAIL  50
1kgf.1.A -KELNDLEKKYNAHIGVYALDTKSGKEVKFNSDKRFAYASTSKAINSAIL  50

   Seqres LEQVPYNKLNKKVHINKDDIVAYSPILEKYVGKDITLKALIEASMTYSDN 100
1kgf.1.A LEQVPYNKLNKKVHINKDDIVAYSPILEKYVGKDITLKALIEASMTYSDN 100

   Seqres TANNKIIKEIGGIKKVKQRLKELGDKVTNPVRYEIELQYYSPKSKKDTST 150
1kgf.1.A TANNKIIKEIGGIKKVKQRLKELGDKVTNPVRYEIELQYYSPKSKKDTST 150

   Seqres PAAFGKTLNKLIANGKLSKENKKFLLDLMLNNKSGDTLIKDGVPKDYKVA 200
1kgf.1.A PAAFGKTLNKLIANGKLSKENKKFLLDLMLNNKSGDTLIKDGVPKDYKVA 200

   Seqres DKSGQAITYASRNDVAFVYPKGQSEPIVLVIFTNKDNKSDKPNDKLISET 250
1kgf.1.A DKSGQAITYASRNDVAFVYPKGQSEPIVLVIFTNKDNKSDKPNDKLISET 250

   Seqres AKSVMKEF                                           258
1kgf.1.A AKSVMKEF                                           258
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Fig. 1: Template-target sequence alignment considered for homology modeling of β-lactamase protein from *Enterococcus faecalis*

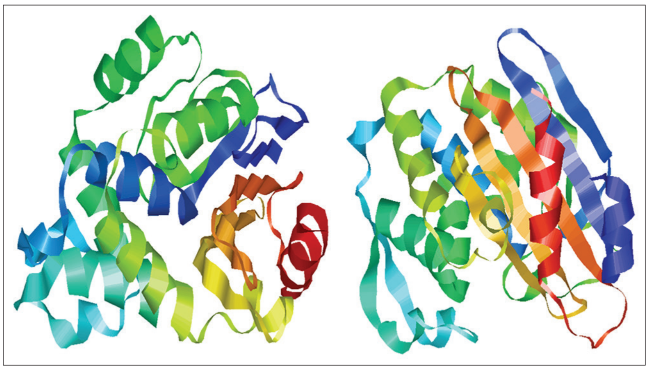

Fig. 2: 3D structures of beta-lactamase protein shown in cartoon model. (a) Modeled beta-lactamase from *Enterococcus faecalis*, (b) the x-ray crystal structure of β-lactamase from *Staphylococcus aureus*

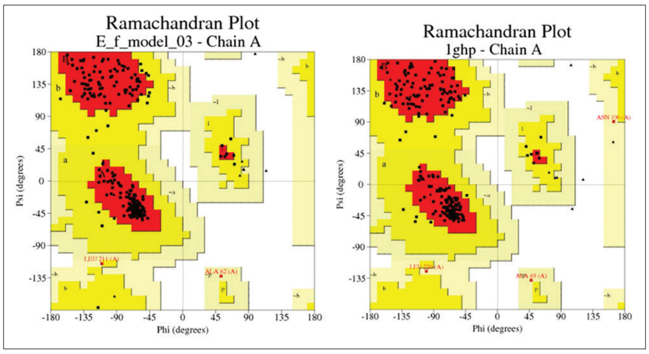

Fig. 3: Ramachandran plot for the modeled β-lactamase protein from *Enterococcus faecalis* and X-ray crystal structure of β-lactamase from *Staphylococcus aureus*

values that determine the generated β-lactamase protein models from *E. faecalis* as reliable and of good quality through verify3d and ERRAT are given in Table 1.

Binding site prediction

The modeled β-lactamase protein from *E. faecalis* was subjected to DoGSiteScorer to predict the possible binding sites. The server revealed seven binding sites with their predicted volume [Å^3], surface [Å^2], lipo surface [Å^2], depth [Å], and drug score (Table 2). Among these, based on the predicted drug score, it is considered the P0 site from modeled beta-lactamase with the drug score of 0.80. Thus, the P0 site is considered as the most potential binding sites for further docking studies. The binding site of β-lactamase complexed with pencillin from *S. aureus* was considered for the docking studies.

Docking studies

The 15 prominent phytochemicals compounds (Fig. 4) from the leaf extracts of *R. communis* were used to determine their antibacterial activity against the β-lactamases from *E. faecalis* and *S. aureus* by revealing its binding efficiency through docking studies.

Docking interactions against β-lactamases from *E. faecalis*

Among the 15 compounds, the best docking interaction score of −17.4484 kJ/mol was observed for the ferulic acid. This interaction is favored by the formation of Hbond with Gln204, Lys201, Asn181, and Ser202 and hydrophobic interactions with Lys201, Ser97, Ser202, Gln204, Ser39, and Gly203. It is observed that the standard drug cefotaxime exhibited the dock score of −13.1677 kJ/mol. This interaction is favored by Hbonds with Gln204, Asn99, and Ser30 and non-bonded interactions with Ser97, Tyr72, Asn137, Ser39, and Gln204. Interestingly, it is observed that the other compounds such as a p-coumaric acid (−16.5195 kJ/mol), N-demethylricinine (−16.3299 kJ/mol), shikimic acid (−15.3777 kJ/mol), and gallic acid (−13.9769 kJ/mol) exhibited better docking scores than that of the standard antibiotics. The binding of remaining compounds exhibited the docking score ranging from −12.2281 kJ/mol to −1.4897 kJ/mol. The docking interactions of those compounds that exhibited better docking score than the standard cefotaxime were ferulic acid, p-coumaric acid, N-demethylricinine, shikimic acid, gallic acid, and standard cefotaxime as shown in Fig. 5a-f. The weak binding interaction was observed for the corilagin, while the compound beta-amyrin does not exhibit any docking interactions.

The results indicate that the compounds such as ferulic acid, p-coumaric acid, N-demethylricinine, shikimic acid, and gallic acid observed in *R. communis leaves* exhibited promising inhibitory activity when compared to the standard drug cefotaxime. Thus, these compounds from *R. communis* might serve as a potential antimicrobial compounds and may be considered as good inhibitors of β-lactamase of *E. faecalis*.

Docking interactions against β-lactamases from *S. aureus*

Among the 15 compounds, all the 14 compounds showed encouraging binding and docking energies, and the compound beta-amyrin does not exhibit any docking interactions. The best docking interaction score of −16.5031 kJ/mol was observed for the hyperoside. This interaction is favored by the formation of Hbond with Ser202, Ser97, Asn99, Ser39, and Gln204 and hydrophobic interactions

Table 1: Validation parameters of the generated beta-lactamase protein model from *S. sciuri* and *E. faecalis* by SAVES

Protein	PROCHECK					ERRAT	Verify 3D (%)
	G-factor	Ramachandran plot (residues %)					
		MFR	AAR	GAR	DAR		
Modeled β-lactamase (*E. faecalis*)	−0.29	91.9	7.3	0.9	0.0	96.787	95.2
X-ray crystal structure of T-lactamase (*S. aureus*)	−0.13	91.5	7.3	1.3	0.0	97.590	93.39

MFR: Most favored region, AAR: Additionally allowed region, GAR: Generously allowed region, DAR: Disallowed region, structural analysis and verification server, *S. aureus: Staphylococcus aureus, S. sciuri: Staphylococcus sciuri, E. faecalis: Enterococcus faecalis*

Table 2: Pockets and descriptors calculated for modeled beta-lactamase protein from *E. faecalis*

Binding site	Volume [Å≥]	Surface [Å^2]	Lipo surface [Å^2]	Depth [Å]	Drug score
P0	366.59	496.26	282.03	21.05	0.80
P1	233.02	170.48	127.50	12.66	0.51
P2	206.59	420.85	254.59	15.10	0.56
P3	204.10	331.92	205.06	13.63	0.50
P4	170.11	330.02	182.97	8.56	0.30
P5	153.41	285.99	130.16	7.85	0.25
P6	152.00	377.50	313.90	9.58	0.28
P7	148.86	203.17	116.51	10.59	0.34
P8	125.70	315.91	215.29	10.86	0.34

E. faecalis: Enterococcus faecalis

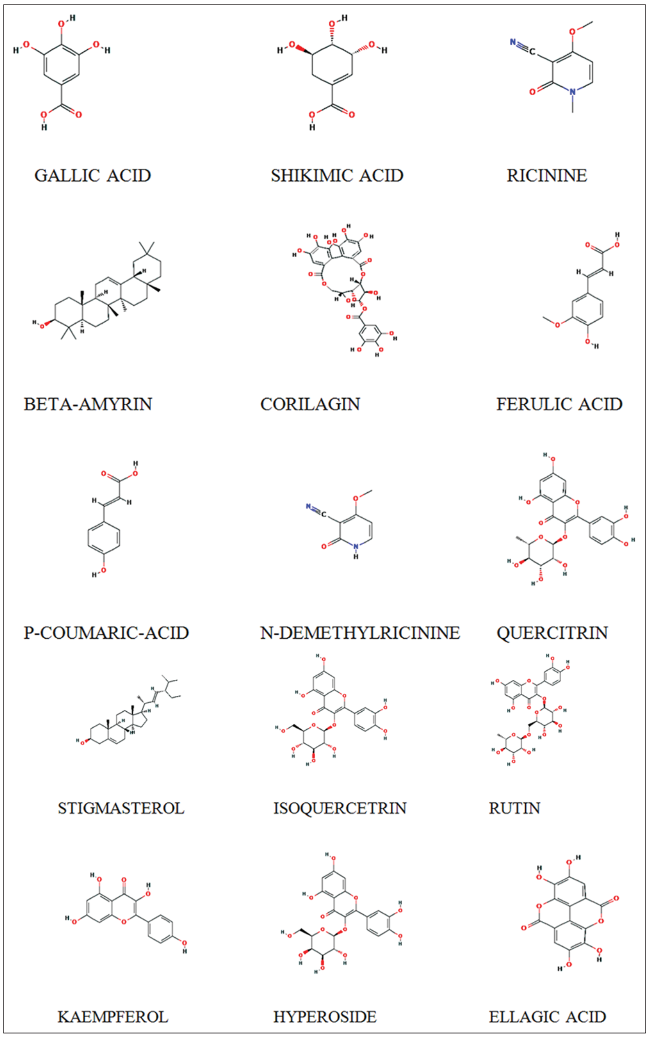

Fig. 4: The two-dimensional structures of the phytochemical compounds from *Ricinus communis* used in the study

with Ser39, Ser97, Gln204, Asn99, and Tyr72. It is observed that the standard drug cefotaxime exhibited the dock score of −13.3997 kJ/mol. This interaction is favored by Hbonds with Gln204, Ser39, and Asn99 and non-bonded interactions through Gln204, Ser39, Gln137, Tyr72, Ser97, and Asn99. Interestingly it is observed that the other compounds such as a shikimic acid (−15.2636) and gallic acid (−14.5674) exhibited better docking scores than that of the standard antibiotics. The binding of remaining compounds exhibited the docking score ranging from −13.0194 kJ/mol to −0.8872 kJ/mol. The docking interactions of the two compounds such as shikimic acid and gallic acid that exhibited better docking score than the standard cefotaxime are shown in Fig. 6a-c. The weak binding interaction was observed for the corilagin.

The results indicate that the compounds such as shikimic acid and gallic acid observed in the methanolic extracts of *R. communis* exhibited promising inhibitory activity while compared to the standard drug cefotaxime. Thus, these compounds from a methanolic extract of *R. communis* might be the potential source of antimicrobial activity and may be considered as good inhibitors of β-lactamase from *E. faecalis* and *S. aureus*.

The docking studies also imply that the conserved amino acids glutamine (Gln), serine (Ser), and asparagine (Asn) in the active site of β-lactamase receptors are crucial in binding compounds with this receptor. These docking interactions imply that the =O (keto group) present in the compounds and NH (amino group) on the amino acids favors the H-bond interactions. Hence, these findings throw light for the design of novel compounds with antimicrobial activity envisages that these amino acids should be considered during its design for implying its action as novel antibiotics that target β-lactamase from *E. faecalis* and *S. aureus*.

CONCLUSION

The development of drug-resistant strains of *E. faecalis* and *S. aureus* are the major concerns in the health-care systems. In line with this, the 3D structure of β-lactamase from *E. faecalis* was modeled using modeler and validated through SAVES server. The phytochemical constituents from *R. communis* with better inhibition activity were explored through molecular docking studies. The docking studies revealed that the compounds ferulic acid and hyperoside exhibited the promising inhibitory activity against the β-lactamases from *E. faecalis* and *S. aureus,* respectively, when compared to the standard antibiotic cefotaxime. Interestingly, the *in vitro* studies of the methanolic extracts of *R. communis* exhibited the significant activity in terms of zone of inhibition against the both *S. aureus* and *E. faecalis*. The docking studies imply that the conserved amino acids such as glutamine (Gln), serine (Ser), and asparagine (Asn) in the binding pockets of β-lactamase are key in favoring the binding interactions with the ligands. Thus, this study significantly suggests that the ferulic acid and hyperoside can be considered as good inhibitors against the most troublesome drug-resistant strains of *E. faecalis* and *S. aureus*.

CONFLICTS OF INTEREST

We declare that we have no conflicts of interests.

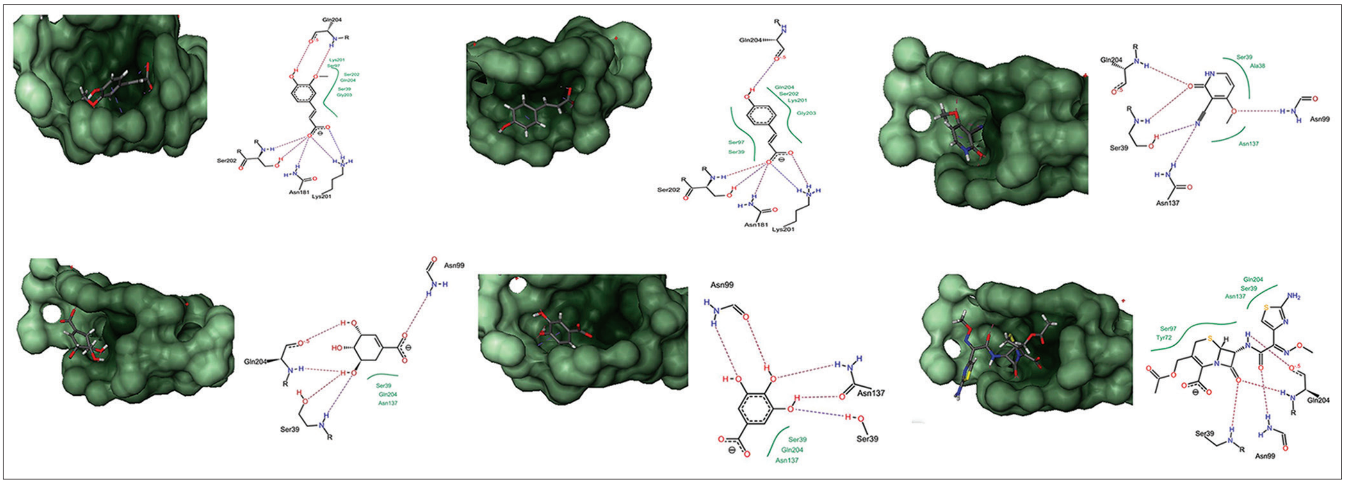

Fig. 5: Docking complex and interactions of *Ricinus communis* phytochemical compounds against modeled β-lactamase of *Enterococcus faecalis*, (a) docking complex and interaction of ferulic acid (dock score: −17.4484 kJ/mol), (b) docking complex and interaction of P-coumaric acid (dock score: −16.5195 kJ/mol), (c) docking complex and interaction of N-demethylricinine (dock score: −16.3299 kJ/mol), (d) docking complex and interaction of shikimic acid (dock score: −15.3777 kJ/mol), (e) docking complex and interaction of gallic acid (dock score: −13.9769 kJ/mol), (f) docking complex and interaction of cefotaxime (dock score: −13.1677 kJ/mol)

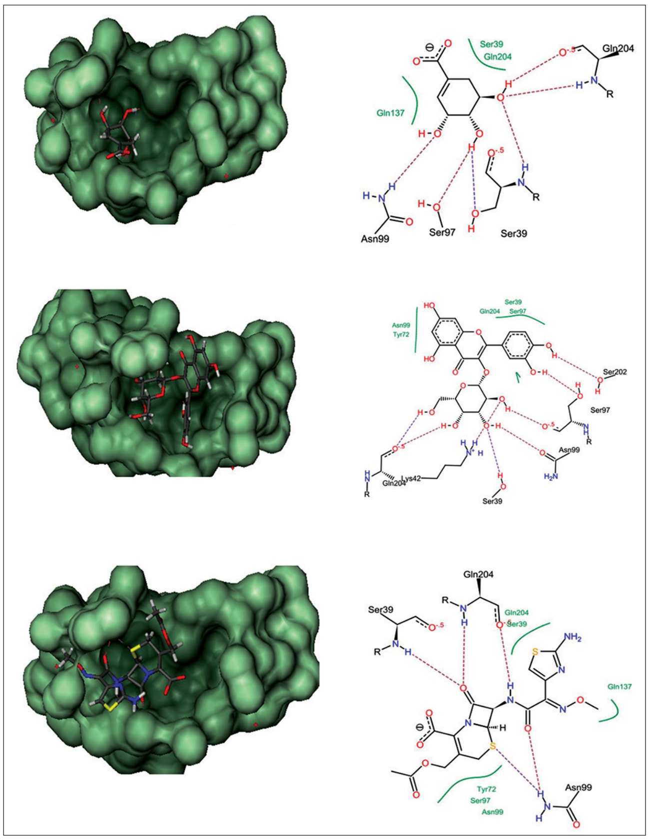

Fig. 6: Docking complex and interactions of *Ricinus communis* phytochemical compounds against modeled β-lactamase of *S. aureus*, (a) docking complex and interaction of hyperoside (dock score: −16.5031 kJ/mol), (b) docking complex and interaction of shikimic acid (dock score: −15.2636kJ/mol), (c) docking complex and interaction of cefotaxime (dock score: −13.3997 kJ/mol)

AUTHORS' CONTRIBUTION

TU and DT performed the experiments; KP and GS supervised the experiments; GS, RSV, and KP analyzed the data; TU wrote the article with contributions of all the authors. All authors discussed the results.

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