J Young Pharm, 2020; 12(1) : 37-41.
A multifaceted peer reviewed journal in the field of Pharmacy
www.jyoungpharm.org | www.phcog.net

Original Article

Enzymatic Inhibition of Phytochemical from *Garcinia imberti* on Homology Modelled Beta-lactamase Protein in *Staphylococcus sciuri*

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ABSTRACT

Objectives: To analyze the interactions of modeled Beta-lactamase from *Staphylococcus sciuri* with phytochemical compounds from medicinal plant, *Garcinia imberti* in the docked complex. **Methods:** The protein-protein blast (BLASTP) analysis of target sequence of Beta-lactamase protein from *Staphylococcus sciuri*, against protein data bank (PDB) resulted that the X-ray crystal structure of beta-lactamase from *Staphylococcus aureus* was carried out. The sequence alignment was performed to build the initial model of Beta-lactamase protein from *Staphylococcus sciuri* using Modeler 9v9 by applying spatial restraints from the initial structure, a bundle of 3 models were developed using random generation for further analysis. The Ramachandran plot of the energy minimized model of Beta-lactamase protein from *Staphylococcus sciuri* was also carried out. The modeled Beta-lactamase protein from *S. sciurii* was subjected to difference of Gaussian (DoG) site scorer to predict the possible binding sites. **Results:** The results indicate that 4-Butylanisole and trans-9-Octadecene exhibited promising inhibitory activity. The docking studies also implies that the conserved amino acids Glutamine and Asparagine, Lysine and Phenyl alanine in the active site of beta-lactamase are critical in binding compounds with these receptor. These docking interactions implies that the =O (keto group) present in the compounds and NH (amino group) on the amino acids favors the H bond interactions. **Conclusion:** The present findings throws light for the design of novel beta lactamase inhibitor compounds with antimicrobial activity envisages that the amino acids Glutamine (Q) and Asparagine (N), Lysine (L) and Phenyl alanine (F) should be considered during its design for implying its action as a best antimicrobial compound to target *S. sciurii*.

Key words: Beta-lactamase, Docking, Phytochemicals, *Staphylococcus sciuri, Garcinia imberti*.

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DOI: 10.5530/jyp.2020.12.8

INTRODUCTION

Root canal infection seems to be a major oral health problem throughout the world. It develops mainly due to the survival of microbes that are arising from endogenous source. The infected root canal system is capable of harbouring the pathogenic microbes. Also this system receives virulent product of microbes. These products are responsible for the development of apical periodontitis.[1] Apical periodontitis have a polymicrobial etiology.[2] In this infection, *Prevotella* sp., *Porphyromonas* spp. *Fusobacterium* sp., *Enterococus* spp. *and Candida* spp are mostly involved.[3] To eliminate these organism from the root canal, several irrigants like NaOCl, Chlorohexidine and Hydrogen peroxide,[4] nevertheless the efforts to be taken to treat this infection is failed frequently, due to procedural errors as well as capable of surviving multi drug resistant microbes within the root canal. Moreover the irrigant causes tissue damage and tissue necrosis. In order to prevent this, the present study helps to formulate a new suitable herbal irrigant for the successful endodontic treatment.

To fill the huge gap between the annotated sequence and 3D structures of the proteins, Theoretical or homology modeling serves as less expensive and faster computational resources to successfully determine the 3D structures of protein.[5] Comparative modeling plays an important role in the absence of experimentally derives structures and remains as a viable cost-effective alternate for structure based drug designing.[6] As the homology modeling builds the 3D structures of proteins based on template structures, it relies upon the percentage of sequence similarity and its accuracy in alignments. The accuracy of the built model always depends upon the choice of template, alignment accuracy and refinement of the model. Generally, the models built with the templates exhibiting over 70% identities are enough accurate for drug discovery applications.[7]

In line with this, various applications associated with the homology modeling, less expensive technique to generate reasonable accurate model, provides to identify the conserved regions that significantly helps to predict the functional sites and aid to trace the evolutionary relationship between sequences by multiple template alignments and also helps to identify the accurate binding modes to study the protein – ligand interactions and aid the mutagenesis studies.[8] In general, the interaction between two molecules - the ligand and the protein that interacts with each other to form a complex is derived by means of certain forces at their point of intercept (binding sites of receptor).[9-11] The fitness function describes the interactions between the ligand and the receptor. Docking conventionally reports two important 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 complex.[12]

Thus in this present study the homology modeling of Beta-lactamase protein from *Staphylococcus sciuri* and docking studies of phytochemical compounds of methanol extract revealed through gas chromatography –

mass spectrophotometry (GC-MS) analysis were carried out as it might lead to the design of novel antibiotic as a therapeutic agents in the form of irrigant against the troublesome multidrug resistant *Staphylococcus sciuri* for the successful endodontic treatment.

MATERIALS AND METHODS

Target sequence and potential template search

The Beta-lactamase protein sequence of *Staphylococcus sciuri* was retrieved from the UniprotKB database (uniprot ID: U6EG14).[13] NCBI-BlastP (basic local alignment search tool) was used to search the homologous sequences against PDB and obtained homologous sequence was considered as the potential template structure for homology modeling.[14] The atomic co-ordinate file of the template structure was obtained from the PDB.[15] The sequence alignment and alignment errors were refined by using ClustalW program as homology modelling relies on the sequence alignment.[16]

Homology modeling

The automated homology modeling software Modeler9v9 was used to built the model based on the final sequence alignment file of target and the template sequence.[17] The atomic coordinate file of the template structure was used to build the 3D model by generating the satisfaction of spatial restraints.[18] A bundle of 3 models were calculated from the starting structure by random generation. The best model was selected based on the least root – mean – square deviation (RMSD) value. This best model was energy minimized by applying 20 steps of each steepest descent and conjugates gradient using GROMOS of SwissPDBviewer and was used for further analysis.[19]

Model assessment

The quality of the generated model was assessed by checking the stereo chemical parameters using a program to check the stereochemical quality of protein structures (PROCHECK),[20] Verfiy3D[21] and Errat[22] at Save server.[23]

Prediction of binding site

To determine the binding affinities between modeled Beta-lactamase protein sequence of *Staphylococcus sciuri* and the phytochemical compounds identified through GC-MS were predicted through difference of Gaussian site (DoG) Site Scorer.[24]

Ligand generation and flexible docking

The 3D structure of identified phytochemical compounds from GC-MS analysis of Methanol extracts of *Garcinia imberti* was retrieved as SDFile from PubChem Database. The obtained SDF structures was docked with the amino acids in the predicted binding site of modeled Beta-lactamase protein from *Staphylococcus sciuri* using FlexX[25] with following parameters i) default general docking information ii) base placement using triangle matching, iii) scoring of full score contribution and threshold of 0,30 and No score contribution and threshold of 0,70. iv) Chemical parameters of clash handling values for protein ligand clashes with maximum allowed overlap volume of 2.9 A^{03} and intra-ligand clashes with clash factor of 0.6 and considering the hydrogen in internal clash tests. v) Default docking details values of 200 for both the maximum number of solutions per iteration and maximum number of solutions per fragmentation.

Prediction of legend- receptor interactions

The interactions of phytochemical compounds with modeled Beta-lactamase protein from *Staphylococcus sciuri* in the docked complex were analyzed by the pose-view of LeadIT.[26]

RESULTS

The BLASTP analysis of target sequence of Beta-lactamase protein from *Staphylococcus sciuri*, against PDB resulted that the X-ray crystal structure of beta-lactamase from *Staphylococcus aureus* as the homologous sequences with sequence similarity of 71.6% at an E-value of 1.10e-20**.** The template- target sequence alignment is shown in Figure 1. As both the sequences are of beta-lactamase and from same genera the resultant homologous sequence was selected as template structure for homology modeling. The sequence alignment file was used as input to build the initial model of Beta-lactamase protein from *Staphylococcus sciuri* using Modeler 9v9 by applying spatial restraints from the initial structure, a bundle of 3 models were developed using random generation and the best model was selected for further analysis based on its structural compatibility (structure with lowest Discrete Optimized Protein Energy (DOPE) score). The modeled structure was shown in Figure 2.

The overall stereo chemical quality of the model was assessed by PROCHECK, Verfiy3D and Errat of Saves server. The Ramachandran plot of the energy minimized model of Beta-lactamase protein from *Staphylococcus sciuri* showed 92.1% of the residues in the most favorable region, 7.0% in the additionally allowed region, 0.9% in the generously allowed region and 0.0% in the disallowed region (Figure 3). The Ramachandran plot of the all generated models of beta-lactamase Protein were analyzed and considered the best model as it exhibited more number of residues in the most favourable regions and also the low number of residues in disallowed region. The total quality G-factor was -0.13. Further the overall quality factor and compatibility of an atomic model (3D) with amino acid sequence (1D) for the modelled Beta-lactamase protein from *Staphylococcus sciuri* was observed as 83.328 and 97% from ERRAT and Verify3D respectively and were given in Table 1. The results of ERRAT and Verify-3D also confirm the model was reliable and of good quality.

The modeled Beta-lactamase protein from *S. sciurii was* subjected to DoG site scorer 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 dock score it is considered that the P0 site with the highest dock score as the most potential binding site for the further docking studies.

The GC-MS analysis of methanol extracts of *Garcinia imberti* revealed the presence of nearly 250 compounds, among these compounds, the 3D conformations for only 83 compounds were able to generate. Thus, these 83 compounds were used to determine their inhibition activity against *S. sciurii* by revealing its binding efficiency through docking studies. The determined binding site P0 was considered as potential binding site five important compounds with generated 3D conformations (Figure 4). The best docking interactions score of-22.1230 kJ/mol was observed for the 1-butyl-4-methoxybenzene with the modeled Beta-lactamase protein from *Staphylococcus sciurii.* This interaction is favored by the formation of Hbond with Gln41 and hydrophobic interactions with Asp39, Phe126, Asn40, Trp65, Phe111, Thr109 and Lys67. It is observed that the standard

Figure 1: Template-target sequence alignment considered for homology modeling.

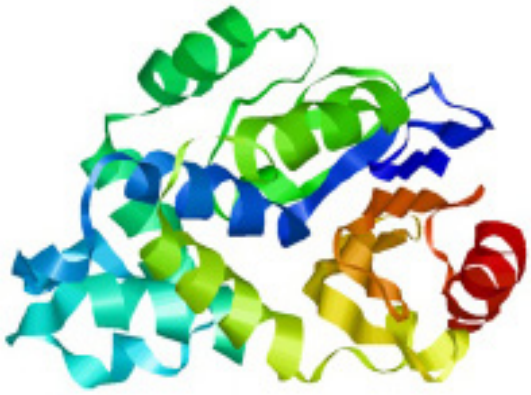

Figure 2: Modeled Beta-lactamase protein from *S.sciuri* shown in cartoons model.

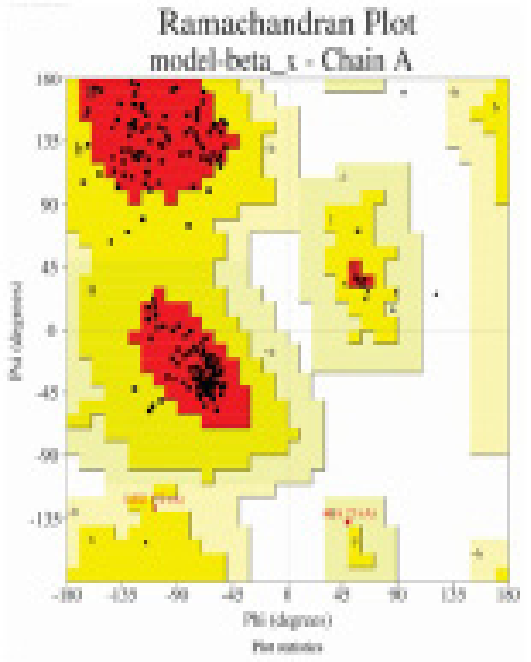

Figure 3: Ramachandran plot for the modeled Beta-lactamase protein from *S.sciuri.*

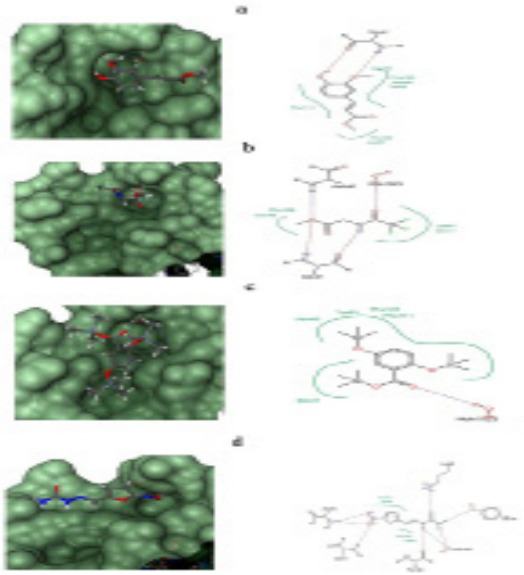

Figure 4: Docking complex and interactions of best three compounds with modeled Beta-lactamase protein from *S. sciurii*. (a) Docking complex and interactions of 1-butyl-4-methoxybenzene within the binding site of modeled Beta-lactamase protein from *Staphylococcus sciurii*, (b) Docking complex and interactions of Trans-octadec-9-ene within the binding site of modeled Beta-lactamase protein from *Staphylococcus sciurii*, (c) Docking complex and interactions of 2-methyloctadec-7-yne within the binding site of modeled Beta-lactamase protein from *Staphylococcus sciurii* and (d) Docking complex and interactions of standard antibiotic Gentamycin within the binding site of modeled Beta-lactamase protein from *Staphylococcus sciurii*.

Table 1: Validation of the model Beta-lactamase protein from *S.sciurii* by SAVES Server.

Protein	PROCHECK					ERRAT	VERIFY-3D
	G-Factor	Ramachandran Plot					
		MFR	AAR	GAR	DAR		
Beta-lactamase	-0.13	92.1%	7.0%	0.9%	0.0%	83.328	97%

MFR-Most Favoured Region; AAR- Additionally allowed Regions; GAR-Generously allowed Regions; DAR-Disallowed Regions.

antibiotic Gentamycin exhibited the dock score of -20.0425 kJ/mol. The binding of remaining compounds exhibited the docking score ranging from -22.2263 kJ/mol to -0.4577 kJ/mol. The docking interactions of the best three compounds were shown in Figure 4. The weak binding interaction was observed for the compound-6. This interaction is favored by Hbond with Asn40 and non-bonded interactions by the means of Gln41, Trp65, Asn40, Phe126, Thr109 and Phe111 with the docking score of -0.4577 kJ/mol.

Theoretically, all the compounds showed encouraging binding and docking energy (Table 3). Among them, the 1-butyl-4-methoxybenzene and Decyl trifluoroacetate exhibited the minimum binding revealed in terms of highest dock scores of -22.1230 kJ/mol and -21.2263 kJ/mol with modeled Beta-lactamase protein from *S. sciurii*, when compared to that of standard antibiotic Gentamycin (dock score -20.0425 kJ/mol). The interactions of the 1-butyl-4-methoxybenzene is favored by the formation of Hbond with Gln41and hydrophobic interactions with Asp39, Phe126, Asn40, Trp65, Phe111, Thr109 and Lys67. The interactions of the Decyl trifluoroacetate are favored by hbonds with Asn40 and Gln41 and non bonded interactions with Lys42, Gln41, Phe126 and Asn40. The standard antibiotic gentamycin with effective antimicrobial activity exhibited the dock score of -20.0425 kJ/mol. This interaction is favored by Hbond formation with Lys42, Tyr130, Asn40, Gln41, Phe126 and Ser127 and also by hydrophobic interactions with Asn40, Gln41, Phe126 and Lys42. I is also observed that the 2-methyloctadec-7-yne exhibited the dock score of -19.6326 kJ/mol, which is very close to that of the standard antibiotic Gentamycin. This interaction is favored by Hbond formation with HOH1023 and non bonded interactions with Gln41, Asn40, Trp65, Phe126 and Phe111.

DISCUSSION

GC-MS analysis of two extracts showed potent antimicrobial activity, which was described earlier[27] was considered for docking. The results indicate that compound-4-Butylanisole and trans-9-Octadecene exhibited promising inhibitory activity in comparison to that of the standard antibiotic Gentamycin and 2-methyloctadec-7-yne (2-methyloctadec-7-yne) with the close margin dock score to standard drug. From the *in-*

Table 2: Pockets and descriptors calculated for modeled Beta-lactamase protein from *S. Sciuri*.

Name	Volume [Å^3]	Surface [Å^2]	Lipo surface [Å^2]	Depth [Å]	Drug Score
P0	485.25	733.51	544.94	32.49	0.90
P1	322.11	354.47	174.67	19.71	0.76
P2	291.33	459.95	303.92	13.62	0.60
P3	216.00	350.64	209.16	11.49	0.45
P4	168.83	285.99	230.16	9.38	0.35
P5	142.14	342.98	253.82	10.25	0.32
P6	113.73	253.69	136.94	7.74	0.24

Table 3: Docking scores of five most important compounds within the predicted P0 active site of modeled Beta-lactamase protein from *Staphylococcus sciuri*.

Sl.No.	Compound Name	CAS-Number	Docking score (kJ/mol)
1	4,4-dimethyl-8-methylidene-1-oxaspiro[2.5]oct-6-ene	54345-60-7	-15.1452
2	1-butyl-4-methoxybenzene	18272-84-9	-22.1230
3	Decyl trifluoroacetate	333-88-0	-17.5766
4	Trans-octadec-9-ene	7206-25-9	-21.2263
5	2-methyloctadec-7-yne	35354-38-2	-19.6326

vitro studies of the methanolic extracts of *Garcinia imberti*, the methanol extract had significant activity in terms of zone of inhibition. Interestingly, it was observed that among the docked compounds, the 1-butyl-4-methoxybenzene and Decyl trifluoroacetate that exhibited the promising minimum binding and docking energy and the close related dock score for 2-methyloctadec-7-yne in comparison to that of the standard antibiotic Gentamycin where the active constituents of methanol extract as revealed by GC-MS analysis.[27] Thus these compounds can be considered as good inhibitors against *S. sciurii*. Recently, molecular docking and inhibition studies on the interactions of *Bacopa monnieri*'s potent phytochemicals against pathogenic *Staphylococcus aureus. B.* extract and its compound luteolin have potent antimicrobial activity against *S. aureus*. Molecular binding interaction revealed that luteolin has more specificity towards the DNA gyrase enzyme binding site and could be a novel antimicrobial compound.[28] Recently molecular docking studies were carried out to investigate the antibacterial activity of *Ricinus communis* phytochemicals against beta-lactamase from *Enterococcus faecalis* and *Staphylococcus aureus* through molecular docking. The docking studies revealed that ferulic acid and hyperoside exhibited promising minimum docking and binding energy that is highly related to the docking score of standard antibiotic cefotaxime.[29]

The docking studies also implies that the conserved amino acids Glutamine (Q) and Asparagine (N), Lysine (L) and Phenyl alanine (F) in the active site of beta-lactamase are crucial in binding compounds with these receptor. These docking interactions implies that the =O (keto group) present in the compounds and NH (amino group) on the amino acids favors the Hbond interactions. Hence these findings throws light for the design of novel compounds with antimicrobial activity envisages that the amino acids Glutamine (Q) and Asparagine (N), Lysine (L) and Phenyl alanine (F) should be considered during its design for implying its action as a best antimicrobial compound that targets *S. sciurii*.

CONCLUSION

In root canal infection the chemical irrigants such as chlorohexidine and sodiumhypochlorite have been applied to treat various multi drug resistance bacterial strains. However, these chemical irrigants caused serious side effects such as tissue necrosis, gastritis and local inflammation. The phytochemicals of *Garcinia imberti* especially, 4 Butylanisole and trans-9-Octadecene have immense potential against various beta lactamase producing multiple drug resistance bacteria. In our study *Staphylococcus sciuri* was used as the model organism to study the inhibitory effect of active molecules from *Garcinia imberti* by molecular docking. The plant phytochemicals have inhibitory effect on beta lactamase and may have potent application as natural irrigant. This natural irrigant is safe and an alternate to chemical irrigant.

ACKNOWLEDGEMENT

The authors extend their sincere appreciation to Dr. Jaya Balaji Mano Christaine Angelo, Ganapathy Murugan and Muthu Kumar for their help in manuscript writing.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

ABBREVIATIONS

BLASTP: Basic Local Alignment Search Tool; **DoG:** Difference of Gaussian; **DOPE:** Discrete Optimized Protein Energy; **GC-MS:** Gas Chromatography and Mass Spectrophotometry; **RMSD:** *Root-mean-square deviation;* **GROMOS:** GROningen Molecular Simulation; **PDB:** Protein data bank, **PROCHECK:** A program to check the stereochemical quality of protein structures.

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Article History: Submission Date : 24-07-2019 ; Revised Date : 02-10-2019 ; Acceptance Date : 10-12-2019
Cite this article: Ramkumar SRS, Sivakumar N, Vijayaraghavan P. Enzymatic Inhibition of Phytochemical from Garcinia imberti on Homology Modelled Beta-lactamase Protein in *Staphylococcus sciuri*. J Young Pharm. 2020;12(1):37-41..