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Molecular Docking study of Catechins compounds from *Camellia sinensis* against UPPS in *Staphylococcus aureus*

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ABSTRACT

Antibiotics resistant *Staphylococcus aureus* (*S. aureus*) is an emerging concern in the medical field. Due to their increasing resistance to numerous antibiotics, there is indeed essential to explore both potential targets and effective antibiotics. Therefore, we considered undecaprenyl diphosphate synthase (UPPS) as a potential target as it is an essential enzyme in cell wall biosynthesis of *S. aureus*. Earlier reports on these four major compounds from *Camellia sinensis* plant extract such as catechins (C), epicatechin (EC), epicatechin gallate (ECg) and epigallocatechin gallate (EGCg) suggested that it could be an effective antibacterial agent. Thus, we attempt to validate the antibacterial activity of these compounds against UPPS *via* molecular docking analysis. Interestingly, we found that epicatechin gallate (ECg) has the highest binding energy with UPPS protein by forming nine hydrogen bonds with the amino acid residues at the binding site of the receptor. Hence, our results infer that ECg from *Camellia sinensis* poses significant anti-bacterial activities. Thus, the aim of this study was to provide an effective antibacterial molecule and potent target which might be helpful in further modification to increase their sensitivity.

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1. INTRODUCTION

Antibiotics resistant bacteria create a serious health problem around the world. *S. aureus* is a one of those antibiotics resistant bacteria which is capable of causing a wide range of human diseases [1]. In particular, Methicillin-resistant *S. aureus* (MRSA), due to emergence and spread of this multi-drug resistance has made the treatment unsuccessful [2]. However, beta (β)-lactam antibiotics (Penicillin, Cephalosporins, Carbapenems and Monobactams) is generally used to treat this *Staphylococcus* spp. infection which inhibits the bacterial cell wall synthesis by binding with Penicillin-binding proteins (PBPs) [3]. From past decades it observed that the resistances shown by this species have been increasing to most of those antibiotics. However, there are several studies reported that the alteration of PBPs might be one of major cause for resistance due to their low binding affinity to the β-lactam antibiotics [4-6]. Thus, treating this bacterial infection has become even more complicated. Hence, this prompted us to focus on new potential target and an effective drug that might be successful in treating this Methicillin-resistant *S. aureus* (MRSA) infections.

Accordingly, based on previous study reports, we have thrown a light over isoprenoid biosynthesis pathway in bacteria. Because products of this pathway play a significant function in bacterial cell-wall biosynthesis in the very early steps of S. *aureus* and thus, it has been considered as an attractive target. This

pathway encompassed of various important steps such as to form farnesyl diphosphate (FPP), the condensation of both dimethylallyl diphosphate (DMAPP) and with two molecules of isopentenyl diphosphate (IPP) this is catalyzed by the enzyme called farnesyl diphosphate synthase (FPPS), and it is followed by the addition of eight more IPP molecules to form undecaprenyl diphosphate (UPP). Finally, the formation of UPP is catalyzed by the enzyme undecaprenyl diphosphate synthase (UPPS), then, UPP is hydrolyzed to the monophosphate, next, it converted to lipid I and lipid II, leading to formation of cell wall peptidoglycan [7, 8]. Among these proteins, UPPS is considered to be one of significant target because it has been an essential protein in peptidoglycan formation and also not found in humans [7, 9]. So, these might play an additional merit for developing or modifying a potential anti-bacterial agent.

Previous studies suggest that, plant derived compounds serve as one of the major source for the development of new antibacterial target [10]. Accordingly, we have preferred Tea plant (*Camellia sinensis*) because; it is widely consumed as beverage around the world, especially in Asia [11]. Moreover, there are several experimental reports on green tea leaves extraction contains polyphenolic components such as catechins, epicatechin (EC), epigallocatechin gallate (EGCg) and epicatechin gallate (ECg) which possess a wide range of medicinal properties in addition, it could perform antibacterial activity against both Gram positive and Gram negative bacterial species. All these catechins contain polyphenol or flavanol as a common moiety [10, 12]. Therefore, we are intended to investigate their antibacterial effects with UPPS in *S. aureus* (MRSA) *via* molecular docking studies. Our results suggest that, the ECg has inhibitory activity on UPPS which is very essential for the cell wall biosynthesis. We believe that our study might provide a base for the development of anti-bacterial molecules from *Camellia sinensis.*

2. RESEARCH METHOD

2.1 3D structure of target

The UPPS protein was essential for the formation of cell wall peptidoglycan in *Staphylococcus aureus*. From the protein data bank (PDB) [13] the three dimensional structure of undecaprenyl diphosphate synthase (UPPS) PDB ID: 4H8E [7] were retrieved. Then, all water molecules were removed and additions of hydrogen atoms to the target molecule were done on the final stage.

2.2 Active compounds identification

In this study, based on the literature report, the four major compounds from *Camellia sinensis* were selected [10, 12]. The 2D structures of these ligands were obtained from the NCBI PubChem Compound database [14]. Using the 3D converter module of the SYBYL (Tripos international, USA) [15] program, all the ligand molecules were converted into 3D structure and then energy minimization were preformed. The two dimensional structures of all four compounds were shown in Figure.1 [14]. The molecular properties of these compounds were tabulated in Table. 1 [14].

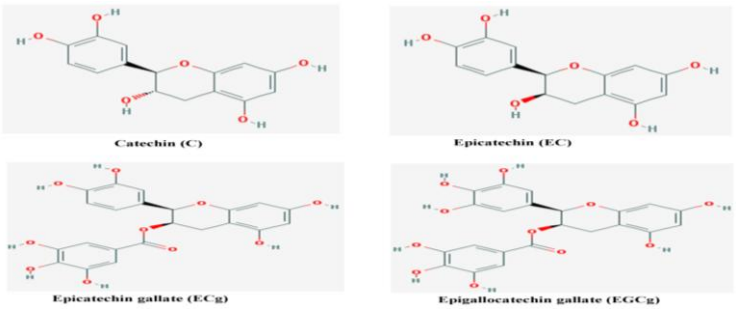

Figure 1**.** Chemical structure of compounds from *Camellia sinensis*

Tables 1. Molecular properties of active compounds from *Camellia sinensis*

S. No	Compounds	PubChem CID	Molecular Weight [g/mol]	LogP (Octanol-water partition coefficient value)	H-Bond Donor	H-Bond Acceptor
1	Catechin (C)	73160	290	0.4	5	6
2	Epicatechin (EC)	72276	290	0.4	5	6
3	Epicatechin gallate (ECg)	107905	442.3	1.5	7	10
4	Epigallocatechin gallate (EGCg)	65064	458.3	1.2	8	11

2.3 Molecular docking

In our present study, surflex-dock program (SYBYL 2.0 (Tripos international, USA)) [15] was used to perform the molecular docking simulation (SYBYL 2.0 (Tripos international, USA) [15]. This surflex-dock program was incorporated with several criteria such as Protomol generation, Gasteiger and Marsili charge calculation, Powell method, MOLCAD and Consensus Scoring (CScore) in molecular docking process. Further, the brief explanations on these criteria are described. First, by removing the unrelated substructure, the protein receptor was optimized and this was done at the beginning of the docking. Then, using the assigned default settings such as addition of water molecules, addition of hydrogen, unknown atom types the side chains of the protein structure were fixed. Moreover, to the protein atoms, the Kollman-all atom charges were assigned. Finally, using the default parameters, the whole structure was subjected to a minimization stage. In addition, Protomol was used as a computational representation of the proposed binding site which was the built from the hydrogen containing protein mol2 file where the putative ligands were aligned. This was done by the protein residues and was considered as important constitute of active site thus, the docking target was created. Furthermore, the Surflex-Dock utilizes the combination of empirical scoring function and the molecular similarity-based search (hydrophobic complementarity, polar complementarity, entropic terms and salvation terms) for scoring. There are three important steps to be carried out before docking, first, for ligands optimization, SYBYL 2.0 molecular modeling suite of Tripos were used. Then, the hydrogen atoms and charges were added using Gasteiger and Marsili charge calculation method. Finally, the ligands were minimized using Powell method. For visualization, the ligand-protein interaction was visualized through Molecular Computer Aided Design (MOLCAD) program and for creating a molecular surface, the MOLCAD were incorporated with different methods whereas, in our present study, we implicated the fast Connolly method to generate the molecular surface which were based on the marching cube algorithm.

In SYBYL, the most significant factor was the binding affinity score. The binding affinities of active compounds were represented in Consensus Scoring (CScore) which uses the multiple scoring functions to evaluate the binding affinity. This CScore includes Gold score (Gscore), Dock score (D_score), ChemScore, Potential Mean Force (PMF_score) along with polar and crash score. In particular, Gscore focus on hydrogen bonding interactions [16] and the D_score utilizes the energetic contribution from hydrophobic and electrostatic interactions and the binding energy contributions of interactions (lipophilic atoms, lipophilic interactions, metal-ligand binding and hydrogen bonding) were estimated using ChemScore and it was described by Eldridge *et al.* (1997) [17]. PMF_Score function is based on the potentials of ligand-receptor atom-pair interaction. This was found to be statistical in nature rather than empirical. On the whole the results of our study was evaluated based on Consensus score (CScore) which generate more accurate result by combining the multiple scoring functions.

2.4 ADME validation

For drug scanning, the chemo informatics tool Molinspiration [18] and admetSAR [19] was used to validate the ADME properties of the drug. This predication is based on physiochemical and pharmacological properties.

3. RESULTS AND ANALYSIS

3.1. Molecular docking

We performed Docking of four ligand molecules with UPPS using SYBYL – Surflex docking. After docking, based on the CScore, the best pose for each ligand molecules are selected. In terms of CScore, ECg is found to have best binding affinity among the four compounds. This CScore prefer multiple approaches for the evaluation of ligand-receptor interactions and it uses to rank multiple configurations of the same ligand docked with a receptor, or to rank selected configurations of different ligands docked to the same receptor. Table 2 provide better perceptive on ligands-complex interaction and their binding energies as well as the hydrogen bond interactions. The evaluation of docking depends upon the number of hydrogen bonds formed between the ligand and the protein. The best pose of our molecular docking study is shown in Figure. 2, protein structure is represented in surface model and the ligand is shown in stick models. In addition, the hydrogen bond interactions between the ECg and UPPS are shown Figure. 3. A total of nine hydrogen bonds are formed. The important binding site residues are Gly 36, Arg 46, Arg 84, Asn 81, Ser 78, Ala 76 and Asn 35.

Table 2. Surflex score of compounds from Camellia sinensis

S. No	Compounds	CScore score[a]	Crash score[b]	Polar score[c]	G score[d]	PMF score[e]	D score[f]	Chem score[g]	No. of hydrogen bonds
1	Catechin (C)	4.81	-3.98	4.80	-213.763	-50.173	-115.409	-26.530	6
2	Epicatechin (EC)	5.15	-2.80	2.41	-228.815	-33.990	-132.592	-34.016	5
3	Epicatechin Gallate (ECg)	5.59	-3.88	5.96	-222.113	-91.703	-158.457	-25.024	9
4	Epigallocatechin gallate (EGCg)	-4.77	-10.37	2.50	-266.309	-69.260	-176.367	-24.2-427	7

a CScore -consensus scoring. The multiple types of scoring functions are used to rank the affinity of ligands.
b Crash-score –It reveals the inappropriate penetration into the binding site.
C Polar region of the ligand.
d G-score –It shows the hydrogen bonding, complex (ligand-protein), and internal (ligand-ligand) energies.
e PMF-score -It indicate the Helmholtz free energies of interactions for protein-ligand atom pairs (Potential of Mean Force, PMF).
f D-score –It shows the charge and van der Waals interactions between the protein and the ligand.
g Chem-score points – It gives the hydrogen bonding, lipophilic contact, and rotational entropy, along with an intercept term.

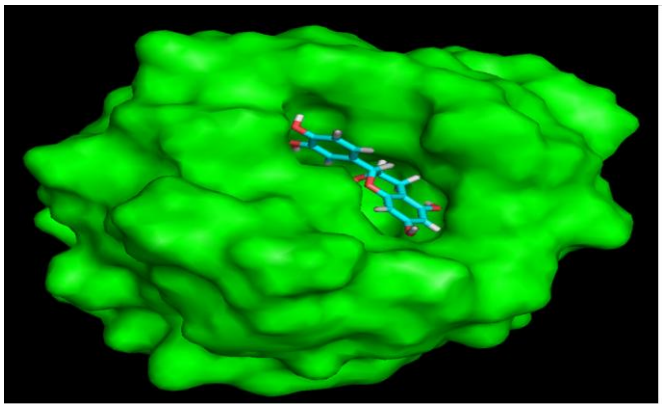

Figure 2. Docked structure of UPPS with epicatechin gallate (ECg) compound.

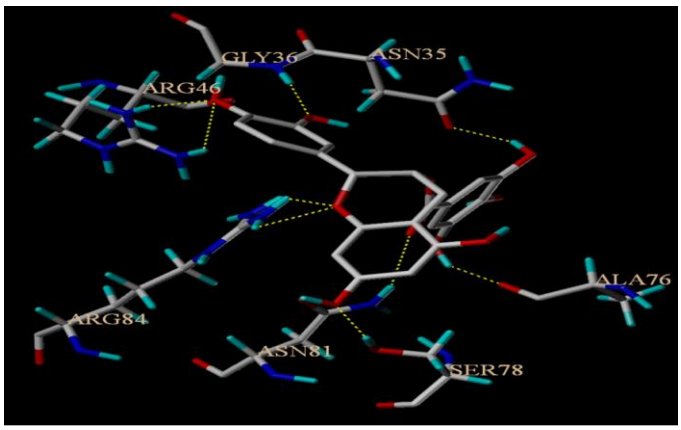

Figure 3. Docking results of epicatechin gallate (ECg) against UPPS. The interacted amino acids residues and hydrogen bond interactions in the binding pocket are shown.

3.2. ADME validation

Molecular properties and drug likeliness of the compounds are analyzed on the basis of "Lipinski's Rule of Five" [20] using the tool Molinspiration server [18]. The rule predicts important molecular properties of drug's pharmacokinetics in the human body which includes their absorption, distribution, metabolism and excretion. The drug molecule shows poor absorption and permeation when they have more than 5 hydrogen bond donors, molecular weight over 500, logP is over 5 and more than 10 hydrogen bond acceptors. For our four compounds the drug-likeliness are checked and the results are shown in Table. 1.

We performed admetSAR tool for screening ADMET profiles of the lead compounds. These compounds are passed through the filter. Among those compounds, EGCg is found to be violating against the Lipinski's rule of five. The remaining three compounds are predicted positive results by admetSAR server. These are depicted in Table. 3.

Table 3. ADMET -Toxicity prediction result of active compound

Models	C	EC	ECg	EGCg
(a) Absorption				
Blood-Brain barrier	BBB+	BBB+	BBB+	BBB+
Human Intestinal Absorption	HIA+	HIA+	HIA+	HIA+
Caco-2 Permeability	Caco2+	Caco2+	Caco2+	Caco2+
P-glycoprotein Inhibitor	Non-inhibitor	Non-inhibitor	Non-inhibitor	Non-inhibitor
Renal Organic Cation Transporter	Non-inhibitor	Non-inhibitor	Non-inhibitor	Non-inhibitor
(b) Metabolism				
CYP450 2C9 Substrate	Non-substrate	Non-substrate	Non-substrate	Non-substrate
CYP450 2D6 Substrate	Non-substrate	Non-substrate	Non-substrate	Non-substrate
CYP450 3A4 Substrate	Non-substrate	Non-substrate	Non-substrate	Non-substrate
CYP450 1A2 Inhibitor	Non-inhibitor	Non-inhibitor	Non-inhibitor	Non-inhibitor
CYP450 2C9 Inhibitor	Non-inhibitor	Non-inhibitor	Non-inhibitor	Non-inhibitor
CYP450 2D6 Inhibitor	Non-inhibitor	Non-inhibitor	Non-inhibitor	Non-inhibitor
CYP450 2C19 Inhibitor	Non-inhibitor	Non-inhibitor	Non-inhibitor	Non-inhibitor
CYP450 3A4 Inhibitor	Non-inhibitor	Non-inhibitor	Non-inhibitor	Non-inhibitor
(c) Toxicity				
AMES Toxicity	Non-toxic	Non-toxic	Non-toxic	Non-toxic
Carcinogens	Non-carcinogen	Non-carcinogen	Non-carcinogen	Non-carcinogen

C – catechin; EC- epicatechin; ECg- epicatechin gallate; EGCg- epigallocatechin gallate

MRSA has been emerging as a serious health threat with increase in the resistance to numerous antibiotics. Over a period of time, the numbers of antibiotics such as Penicillin derivatives and Cephalosporins have been continuously used for treating this bacterial infection. Recently, these bacterial pathogens confirm resistance to most of these antibiotics has been reported [2, 7]. Therefore, there is an urgent need to develop effective anti-bacterial compounds and also to identify the potent target to combat this infection. In the present study, a molecular docking is performed to explore antibacterial activity of compounds in *Camellia sinensis* leaf extracts [10, 12] against UPPS protein of S. *aureus subsp. aureus* N315 [7]. Whereas, the cell wall of *S. aureus* is composed of 30-50 peptidoglycan layers. It is a cross-link between polysaccharides and peptide complex which provides rigidity of the bacterial cell wall and protect the cell lysis from osmotic pressure [12, 21]. Since, the undecaprenyl diphosphate synthase (UPPS) is an essential enzyme in isoprenoid biosynthesis which involved in the early step of cell wall biosynthesis. In addition, UPPS plays a central role in the cell wall biosynthesis; it might acts as an attractive therapeutic target for antibacterial agent [7]. There are several studies which provide considerable evidence of UPPS enzyme as a potential drug target.

In this study, we have highlighted molecular docking studies on the inhibition of UPPS by the catechins and its analogues compounds from *Camellia sinensis*. Molecular docking is carried out using SYBYL – Surflex docking. From the results, epicatechin gallate (ECg) have a better ligand-enzyme interactions and stability which forms nine hydrogen bond with CScore of 5.59. Thus, it is considered as a one of the best inhibitors against UPPS which inturn inhibit the isoprenoid biosynthesis pathway. Our results also well correlate with the previous experimental work done by Shimamura et al. (2007) [12] which show significant antibacterial activity against *S. aureus*. ECg has also been reported to have moderate inhibitory effects on Gram negative bacteria [12]. Even though, catechin (C) shows best binding affinity in terms of hydrogen bond it shows lower values (4.81) in case of ligand-receptor atom pair interaction. Among the four analyzed compounds, the least hydrogen bond interactions are shown by EGCg, forming seven hydrogen bond interactions and lower CScore score -4.77. EC are found to have total score value of 5.59 and forms five hydrogen bond interactions than other docked compounds.

From our results, we conclude that, epicatechin gallate is known to be potent inhibitors of *S. aureus* growth by inhibiting the UPPS in cell-wall biosynthesis. The Surflex score for all four compounds are depicted in Table 2. The docked structure and hydrogen bonding interactions of ECg with UPPS represented in Figure. 2 and 3 respectively.

We analyzed the number of physiochemical and pharmaceutical properties (Molecular weight, H-bond donors, H-bond acceptors, logP (octanol/ water) and their position according to Lipinski's rule for our four compounds. They are provided in Table 3. These are developed based on high throughput and the Evaluation of Absorption, Distribution, Metabolism, and Excretion (ADME) profiling assays which facilitate the identification of active lead compounds at early drug discovery. ADME profiling in the earliest phase of the discovery process, may enhance the development of effective lead compounds in drug design process [22]. Our ADMET properties prediction on docked compounds revealed that three compounds are in acceptable range of various pharmacological parameters such as Blood-Brain barrier (BBB) penetration, P-glycoprotein substrate, renal organic cation transporter, human intestinal absorption and Caco2 permeability. We found that EGCg violating from Lipinski's rule of five. The penetration of drugs through BBB is one of the key parameters to be optimized in drug discovery [23]. This BBB is used to measure the ratio of the compound concentration in the brain to that of blood. The drug likeness of active compounds as therapeutic agents can be determined by oral bioavailability [24] which includes physiological, physiochemical and certain biopharmaceutical factors [25]. The next important parameter is cytochrome P450 (CYP), which is known as isozymes group and it is involved in the metabolism of drugs, fatty acids, steroids, bile acids and carcinogens. Considering the cytochrome P450 (CYP) analysis, all our four compounds are found to be Non-substrate and Non-inhibitor. In case of toxicity, the outcome of our result suggests that all four compounds are observed to be non-toxic in particular, ECg has potential inhibitory activity. Among four compounds (C, EC, ECg and EGCg), three compounds (C, EC, ECg) are shown positive to ADME except EGCg. Among those compounds, ECg has shown the maximum value among the analyzed properties and thus, exhibiting drug-like characteristics (Table 2 and 3).

4. CONCLUSION

Our molecular docking studies revealed that the anti-bacterial property of the four compounds - catechins (C), epicatechin (EC), epicatechin gallate (ECg) and epigallocatechin gallate (EGCg) of *Camellia sinensis* inhibit undecaprenyl diphosphate synthase (UPPS) enzymes in isoprenoid biosynthesis in *S. aureus*. It might be a potential target to combat the antibiotic resistance as the isoprenoids are involved in cell wall biosynthesis of *S. aureus*. The docking of four compounds with binding cavity of UPPS illustrates that epicatechin gallate (ECg) has more favourable interaction when compared to other three compounds (C, EC and EGCg) with better CScore score, hydrogen bonding energy and ligand-protein interaction energy. The docked compounds used in present study also satisfy ADME properties and toxicity risk assessment. They are in the acceptable range of various pharmacological parameters.

On the whole, the epicatechin gallate (ECg) in *Camellia sinensis* might serve as a good lead molecule for developing an effective antibacterial compound. Probably, this study would be useful in developing more potent molecules for countering resistant bacterial isolates of *S. aureus.*

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