

Ethyl Iso-allochololate from a Medicinal Rice *Karungkavuni* Inhibits Dihydropteroate Synthase in *Escherichia coli*: A Molecular Docking and Dynamics Study

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Malathi, *et al.*: *E. coli* DHPS Inhibition by Ethyl Isoallochololate

Medicinal rice varieties have defensive and therapeutic properties against many human disorders. Drug resistance has become a major problem in recent years. *Escherichia coli* have developed resistance to most of the antibiotics including sulfonamides that target dihydropteroate synthase. In the present study, we attempted to identify a novel inhibitor for the dihydropteroate synthase from a medicinal rice variety *Karungkavuni*. The phytochemical composition of the rice *Karungkavuni* was analysed through gas chromatography-mass spectrometry. The isolated compounds with reported antimicrobial activity were subjected to molecular docking procedures to understand the binding behaviour of the ligands with the target. These analyses revealed that ethyl iso-allochololate and 9,12,15-octadecatrienoic acid-2,3-dihydroxypropyl ester were the best binding compounds. Molecular dynamics studies revealed dihydropteroate synthase-ethyl iso-allochololate complex was more stable than other ligands throughout the simulation. Our study demonstrated that ethyl iso-allochololate isolated from *Karungkavuni* could serve as a potent inhibitor of dihydropteroate synthase.

Key words: Dihydropteroate synthase, drug resistance, sulfonamide, binding affinity, folate biosynthesis, molecular docking, molecular simulation

Rice is a monocotyledonous plant, which belongs to the family of poaceae^[1]. Several studies suggest that *Oryza sativa* is domesticated from *O. rufipogon*, which is an Asian common wild rice^[2]. Rice is the main cereal crop of the world and it is the main staple food in most Asian countries. Approximately, 95% of rice is produced in Asia. Rice is consumed mostly in a cooked form for obtaining energy and nutrition. Rice contains nutrients such as carbohydrates, proteins, dietary fiber, vitamins and minerals^[3]. Medicinal rice varieties have many defensive and therapeutic properties against human disorders like colon cancer, epilepsy, chronic headache, paralysis, skin diseases, indigestion, rheumatism, arthritis, blood pressure, diabetes, internal rejuvenation of tissues and postnatal weaknesses^[4]. Whole grain of rice has some health promoting components. It is suggested that regular intake of whole grains and whole grain products reduces the risk of chronic diseases such as type 2 diabetes and cardiovascular diseases^[5]. Antibiotic resistance

has become a major concern in recent decades. Pathogenic bacteria have the potential to transmit and acquire resistance to all available therapeutic agents. Development of drug resistance is a major cause that limits the usage of existing antimicrobials that includes sulphonamides, earlier which were quite effective against infections caused by both Gram-positive and Gram-negative bacteria. Hence, sulfonamide resistance is a serious problem in health care settings. Bacterial folate biosynthetic pathway is an ideal target for the antimicrobial therapy^[6]. Dihydropteroate synthase (DHPS, EC 2.5.1.15) is a triose-phosphate isomerase enzyme with eight β -strands and eight α -helices. DHPS is the key enzyme in folic acid metabolism

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as it catalyses the formation of tetrahydrofolate, an important precursor for the synthesis of pyrimidines, purines and amino acids. DHPS plays a vital role in the proliferation of microorganisms and it is encoded by the gene folP^[7]. DHPS is involved in the conversion of 6-hydroxymethyl-7,8-dihydropteridine-pyrophosphate and para-amino benzoic acid (PABA) to pyrophosphate and 7,8-dihydropteroate^[8]. PABA is synthesized through the upregulation process by the chorismate pathway and supplemented into the folate biosynthesis pathway. DHPS has been identified as the best target for the sulfonamide group of compounds. Generally, sulfonamides inhibit the target by mimicking as a competitive inhibitor and substrate analogs of PABA and leads to “dead end” in the pathway^[9]. Resistance to sulfonamides are associated with chromosomal mutations within the folP gene. Drug resistance is the major factor that restricts the current use of sulfa-based inhibitors against pathogenic bacteria including *E. coli*. Over the past few years, there is an increasing interest in the study of chemical composition of rice varieties. Many researchers have reported the phytochemical composition of the rice and rice by-products^[3,10,11]. There are a few reports on the antimicrobial activity of the rice extracts, rice by-products and components isolated from rice^[12,13]. However, there are not many reports that describe the mechanism and bioactivity of compounds isolated from medicinal rice varieties.

Thus, in our present study we aim to explore the phytochemical composition in the medicinal rice variety, *Karungkavuni* through Gas chromatography-mass spectrometry (GC-MS) analysis. The compounds with reported antimicrobial activity are selected for the molecular docking study to understand the binding behaviour. Similar to sulpha group of compounds, the selected compounds competitively inhibit the DHPS and might disrupt the folic acid pathway. The stability of the docked complexes is then analysed through molecular dynamics (MD) simulation.

MATERIALS AND METHODS

Karungkavuni paddy was procured from the organic rice farmers in Tanjavur and Tiruvarur Districts of Tamil Nadu, India.

Preparation of the extract:

Fifty grams of *Karungkavuni* rice was pulverized by using pestle and mortar. Two grams of powdered homogenized rice sample was added in a centrifuge tube containing 20 ml of ethanol. The contents were

mixed properly using cyclomixer for 10 min. The resultant mixture was then centrifuged at 10 000 rpm for 10 min. After centrifugation, the separated supernatant was filtered through 0.2 µ filter. Then, the filtrate was stored in a glass vial.

GC-MS analysis:

GC-MS analysis of the ethanol extract of *Karungkavuni* rice was carried out on the Thermo Fisher TSQ Quantum XLS GC-MS/MS using DB5-MS capillary column measuring 30 m×0.25 mm×0.25 µm and composed of 5% phenyl, 95% dimethyl polysiloxane cross bonded liquid phase. Helium of 99.9995% was used as a carrier gas with a flow rate of 1.0 ml/min. Sample vial with 1 ml sample was kept in the auto-injector and 1 µl of sample was injected into the GC column using syringe in split mode. At the time of injection, the system temperature was kept at 290°. The sample was injected with split ratio of 10:1. Initially, the column temperature was programmed at 80° for 2 min, then it was increased to 150° with 40°/min then to 240° with 4°/min then slowly increased to 255° with 2°/min and finally it was increased to 285° with 4°/min keeping for 3 min. Total GC cycle run time was set to 30 min. The MS scan mode of m/z 50 to 650 was operated and data were evaluated using total ion count. The spectrum of compounds was compared with reference in the National Institute of Standards and Technology (NIST) database.

Statistical analysis:

Tests were performed in triplicates (n=3) and the area of percentage observed in GC-MS analysis was expressed as mean±standard deviation.

Dataset:

After GC-MS analysis, the compounds reported with antimicrobial activities were retrieved from the National Center for Biotechnology Information (NCBI) PubChem database^[14] and the structures were energy minimized. The 3-Dimensional (3-D) structure of the target protein *E. coli* DHPS (1AJ0)^[15] was retrieved from the protein data bank^[16]. The water molecules were removed and the hydrogen atoms were added to the protein at the end.

Molecular docking:

Molecular docking is a standard procedure in drug discovery process. Surflex-Dock program interfaced with SYBYL X 2.0 (Tripos international, USA) was used for the docking study. The compounds isolated

from rice variety with reported antimicrobial activity were docked into the active site of the 3-D structure of DHPS. At first, the optimization of the protein structure was done by detaching the unrelated substructures. Similarly, the other standard parameters such as, protein side chain fixation, addition of hydrogen, removal of water molecules and allocation of unknown atom types were also utilized. The relaxation of bumps was carried out to define the correct configuration and tautomeric states of the protein structure. The protein atoms were assigned with the Kollman-all charges^[17]. The whole protein structure was energy minimized by using the AMBER7 F99 force field. Finally, the ligands were energy minimized by using the Powell's energy minimization algorithm^[18]. SYBYL X 2.0 program docks a ligand automatically into the active site of a target protein. It is a protomol-based method and an empirical score is provided. The scoring functions employ properties that include solvation, crash, hydrophobic, polar entropic and repulsive properties. These functional scores explain the interaction between the protein and ligand. The final bioactive conformation was selected based on the highest consensus scoring (C-score) and number of H-bond formation. The C-score also utilizes the other surflex dock scores, crash score, polar score, gold score (G-score), potential mean force (PMF) score, dock score (D-score) and chem-score.

Absorption, distribution, metabolism and excretion (ADME) screening:

The compounds retrieved from the NCBI PubChem database were subjected to ADME screening for the prediction of both physiochemical and pharmacological properties. The molinspiration server was used for the prediction of ADME and drug-likeness of the compounds isolated from rice variety. For this, simplified molecule input line entry specification of the compounds was submitted to the molinspiration server, which predicts the number of hydrogen (H)-bond donors (HBD) and number of H-bond acceptors (HBA), log P, molecular volume and rotatable bonds. This online server calculates molecular properties and drug likeness of a given compound rapidly. Based on the predicted properties, the acceptability of the drugs was evaluated by Lipinski's rule of 5^[19], which was important for the structure-based drug designing.

MD simulation:

The MD simulation of free DHPS, DHPS-ethyl iso-allocholate and DHPS-9,12,15-octadecatrienoic acid-

2,3 dihydroxypropyl ester complexes were performed using Gromacs 4.5.5 package^[20]. In the first step, enzyme topology was generated by the default parameters of the Gromacs, whereas the ligand topology was created by the online tool PRODRG server^[21]. Gromos96 53a6 force field was used for the generation of topology and interaction parameters. The structures were solvated in the cubic box containing simple-point-charge water molecules. The distance of 1.2 nm was set between the box and protein-ligand complex. Since, proteins were positively charged, the system must be neutralized by the addition of counter ions (Cl⁻ or Na⁺). The concentration of NaCl was set as 0.145 M. For energy minimization, steepest descent algorithm was applied for first 1000 steps. In the next step, system was equilibrated under restraints position to relax the solvent under constant volume and temperature dynamics (NVT) for 100 ps at 300 K, followed by the production simulations that implemented under constant pressure and temperature dynamics (NPT) for 100 ps. Linear constraint solver algorithm was used for restrain the lengths of all bonds. A coulomb type particle mesh Ewald method is applied for calculating electrostatic interactions. The coulomb and Van der Waals Cut off radii interactions were set to 10.0 Å and 14.0 Å, respectively. Each system was subjected to 50 ns MD simulation. Xm grace tool was used for the generation of graphs^[22].

RESULTS AND DISCUSSION

Seventeen different compounds are observed in the GC-MS chromatogram of ethanolic extract of the medicinal rice variety *Karungkavuni*. The chromatogram of the rice is depicted in fig. 1 and the compounds are tabulated in Table 1. These phytochemical compounds are identified using NIST library. Some of the prominent phytochemicals noted in i rice variety are sitosterol, tetradecanoic acid, hexadecanoic acid, cis-vaccenic acid, eicosanoic acid and tocopherol. The identified phytochemical compounds signify the medicinal activity of this rice variety. The biological activities of these compounds are listed in Table 2^[23-34]. Reports suggest that compounds 9,12,15-octadecatrienoic acid-2,3-dihydroxy propyl ester^[26], sitosterol^[28,29], squalene^[30,31] and ethyl iso-allocholate^[33] have antimicrobial activity. We selected these four compounds for docking studies to reveal the binding orientation of ligands with the target. The compound structures are retrieved from the NCBI PubChem database and their PubChem IDs are listed in Table 3. These retrieved compounds are docked into the active site of the *E. coli* DHPS. After

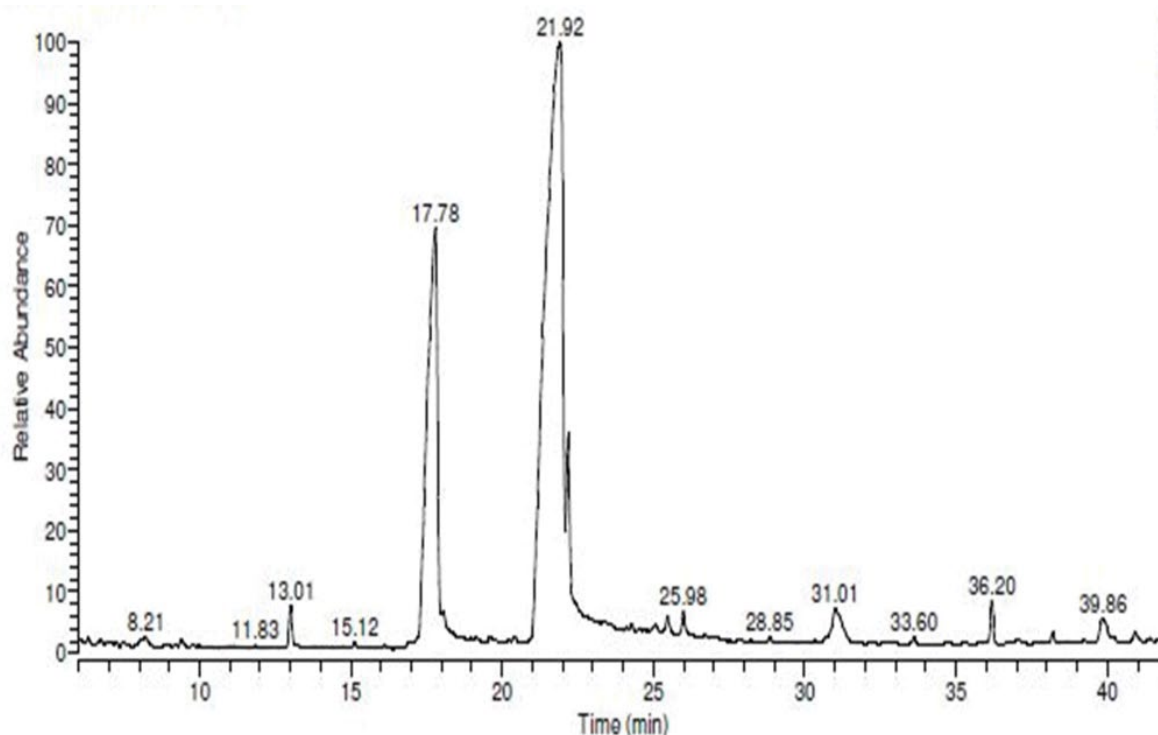


Fig. 1: GC-MS chromatogram of the ethanolic extract of medicinal rice variety *Karungkavuni*.

TABLE 1: PHYTOCHEMICALS IDENTIFIED IN ETHANOLIC EXTRACT OF MEDICINAL RICE VARIETY KARUNGKAVUNI USING GC-MS PEAK

RT ^a	Name of the compound	Similarity index (%)	MW ^b	Area (%)
5.69	D-ribo-hexose, 2,6-dideoxy-3-o-methyl-	85	176	0.88±0.03
6.31	Ascaridole epoxide	82	184	0.13±0.02
8.2	D-glucopyranoside	79	504	0.86±0.07
9.39	3-tret-butyl-4-hydroxyanisole	84	180	0.28±0.03
13.01	Tetradecanoic acid	86	228	0.86±0.08
17.76	Hexadecanoic acid	89	256	26.76±0.92
21.89	Cis-vaccenic acid	92	282	64.40±0.77
25.06	9,12,15-octadecatrienoic Acid-2,3-dihydroxy propyl ester	86	352	0.32±0.06
25.47	Cis-13-eicosenoic acid	81	310	0.48±0.05
25.98	Eicosanoic acid	88	312	0.49±0.05
31.03	Sitosterol	86	414	2.13±0.10
36.2	Squalene	85	410	0.86±0.07
38.21	Heptacosane	82	380	0.21±0.25
39.86	9,19-cyclolanostan-3-ol,24-methylene-(3a)	89	440	0.97±0.02
40.94	Tocopherol	91	416	0.47±0.03
42.13	Ethyl iso-allocholate	93	436	0.25±0.03
43.25	Olean-13-(18)-ene	86	410	0.29±0.04

^aRT-retention time; ^bMW-molecular weight. Analysis was done in triplicates; data presented as mean±standard deviation

docking, the observed results are evaluated based on the C-score and number of H-bonds formed between the target protein and ligand. Out of four compounds screened, ethyl iso-allocholate has the best binding affinity with DHPS, which has C-score value of 4. DHPS-ethyl iso-allocholate interaction displayed

totally 5 H-bonds and the residues Arg63, Ser222 and Thr62 of DHPS are involved in the H-bond formation. Ethyl iso-allocholate forms two H-bonds with Arg63 and ser222. The residue Thr62 forms only one H-bond with ethyl iso-allocholate (fig. 2). Similarly, 9,12,15-octadecatrienoic acid-2,3-dihydroxypropyl

TABLE 2: BIOLOGICAL ACTIVITIES OF PHYTOCHEMICALS PRESENT IN THE ETHANOLIC EXTRACTS OF MEDICINAL RICE VARIETY KARUNGKAVUNI

Name of the compound	Compound nature	Bioactivity
D-ribo-hexose, 2,6-dideoxy-3-o-methyl-	Heterocyclic	Sugar moiety of cardiac glycosides
Ascaridole epoxide	Heterocyclic	Anthelmintic
D-glucopyranoside	Heterocyclic	Preservative
3-tret-butyl-4-hydroxyanisole	Phenolic	Antioxidant, antimutagenic activity
Tetradecanoic acid	Acid	Antioxidant, anticancer
Hexadecanoic acid	Acid	Antioxidant, hypocholesterolemic
Cis-vaccenic acid	Acid	Anticancer, antiinflammatory
9,12,15-octadecatrienoic acid-2,3-dihydroxy propyl ester	Alpha linolenic acid	Antimicrobial activity, antiinflammatory, anticancer
Eicosanoic Acid	Acid	Anticancer, antioxidant, antiinflammatory, cardioprotective
Sitosterol	Phytosterol	Antimicrobial activity, reduces blood levels of cholesterol
Squalene	Tritrepene	Antibacterial, antioxidant, antitumor, cancer preventive
Heptacosane	Heterocyclic	Insect pheromone constituent
9,19-cyclolanostan-3-ol, 24-methylene-(3a)	Tritrepene alcohol	Used as an active ingredient of a drug
Tocopherol	Alcohol	Antioxidant, antiageing
Ethyl iso-allocholate	Steroid derivative	Antimicrobial activity
Olean-13-(18)-ene	Triterpenoids	Antitumor activity

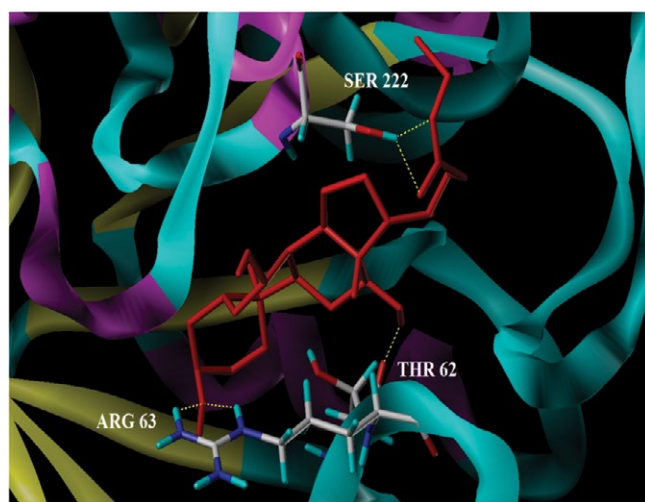


Fig. 2: Docking results of ethyl iso-allocholate against DHPS. H-bonds formed between ethyl iso-allocholate (red sticks) and DHPS (ribbon model) are indicated in yellow dots.

ester also have binding affinity with DHPS, the C-score value is 3. DHPS-9,12,15-octadecatrienoic acid-2,3-dihydroxypropyl ester interaction has 4 H-bonds formation. The 9,12,15-octadecatrienoic acid-2,3-dihydroxypropyl ester has formed two H-bonds with the residues Asp185 and Lys221 of DHPS as in fig. 3. To understand the interaction of ligands with the protein, different binding energy scores and number of H-bonds formed are tabulated in Table 4.

Rice contains a consortium of many bioactive components. The compounds isolated from rice

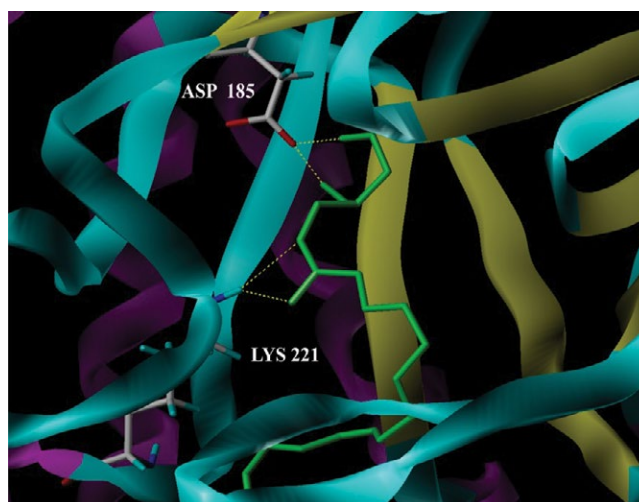
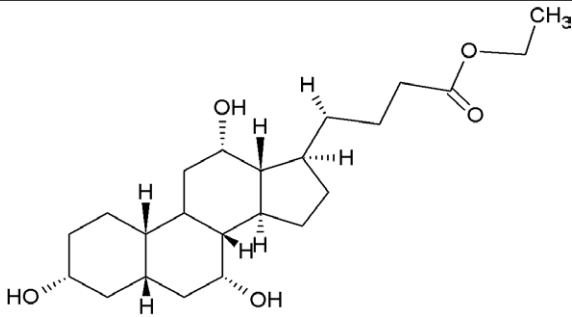
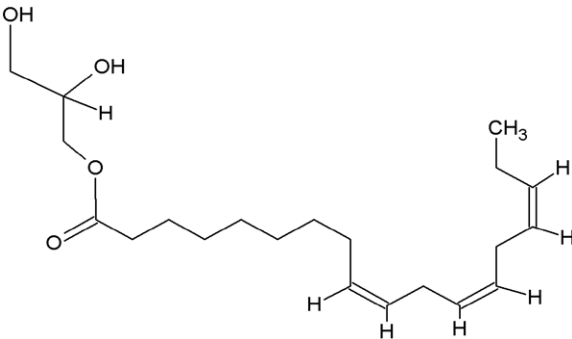
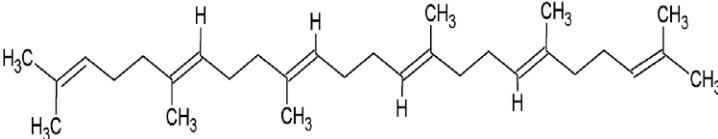
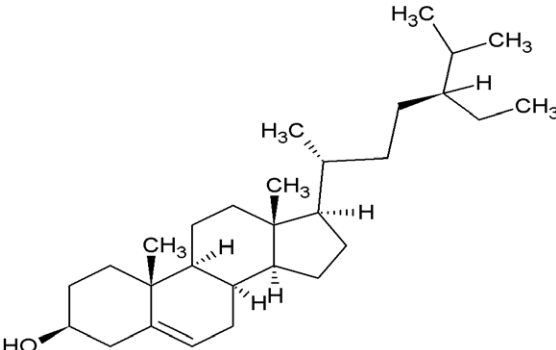


Fig. 3: Docking results of 9,12,15-octadecatrienoic acid-2,3-dihydroxypropyl ester against DHPS. H-bonds formed between 9,12,15-octadecatrienoic acid-2,3-dihydroxypropyl ester (green sticks) and DHPS (ribbon model) are indicated in yellow dots.

varieties are subjected to physicochemical and pharmaceutical property prediction by the Lipinski's rule of 5. It accepts compounds with one violation. This rule explains about the importance of physiochemical property prediction in the pharmacokinetics of a drug in the human body and also with ADME prediction. This rule restricts compounds with less than 5 HBD, molecular weight below 500 Da, less than 5 log P and 10 HBA. The Molinspiration prediction reveals that the 4 compounds have shown possible drug

TABLE 3: COMPOUNDS REPORTED WITH ANTIMICROBIAL ACTIVITY AND THEIR PUBCHEM IDs

Pubchem ID	Compound name	Structure
6452096	Ethyl iso-allocholate	
5367328	9,12,15-octadecatrienoic acid-2,3-dihydroxypropyl ester	
1105	Squalene	
222284	Sitosterol	

likeness characteristics as listed in Table 5. The compounds squalene and sitosterol has log P value greater than 5. Also, the compounds squalene and 9,12,15-octadecatrienoic acid-2,3-dihydroxypropyl ester has more than 10 rotatable bond counts. All the 4 compounds are having the drug likeness properties. The rotatable bonds are used as a filter to distinguish the drug molecule. Increase in the rotatable bond count may leads to the decreased oral bioavailability^[35].

MD simulations of free DHPS, DHPS-ethyl iso-allocholate and DHPS-9,12,15-octadecatrienoic acid-2,3 dihydroxypropyl ester complexes are conducted to understand the overall stability of the complexes

on a nanosecond time scale. The complex structures are subjected to 50 ns MD simulations. To investigate the stability of conformations in MD simulations, we analyse root-mean-square deviation (RMSD), root mean-square fluctuations (RMSF), R_g and number of intermolecular H-bonds formed between the protein and ligand. RMSD is used to calculate the average displacement of atoms for a particular frame with respect to the reference frame. RMSD backbone values of free DHPS, DHPS-ethyl iso-allocholate and DHPS-9,12,15-octadecatrienoic acid-2,3-dihydroxypropyl ester complexes are calculated against the simulation time scale between 0 and 50 000 ps (fig. 4). This

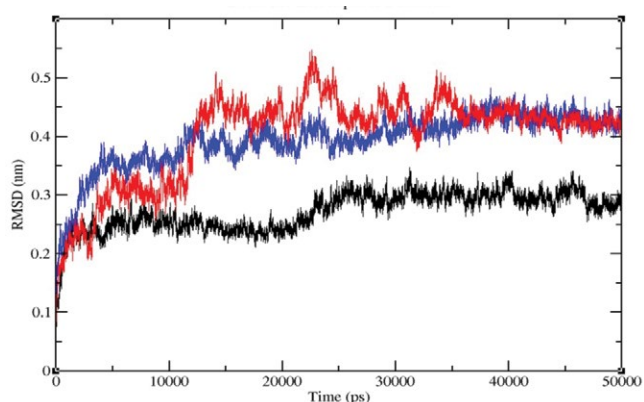
TABLE 4: SURFLEX DOCK SCORES OF COMPOUNDS ISOLATED FROM MEDICINAL RICE VARIETY KARUNGKAVUNI

Pubchem ID	Compound name	Crash score ^a	Polar score ^b	D score ^c	PMF score ^d	G score ^e	Chem score ^f	C score ^g	H bond
6452096	Ethyl iso-allocholate	-10.21	1.18	-143.39	-25.77	-328.52	-25.78	4	4
5367328	9,12,15-octadecatrienoic acid- 2,3-dihydroxy propyl ester	-1.73	3.29	-86.65	-35.92	-168.63	-16.74	3	4
1105	Squalene	-3.67	0.00	-169.21	-18.13	-346.84	-36.97	3	-
222284	Sitosterol	-5.85	0.29	-114.79	-29.34	-275.419	-25.235	2	1

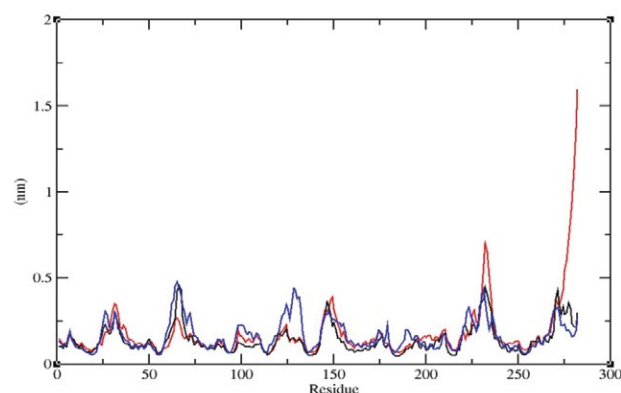
TABLE 5: MOLINSPIRATION PREDICTED MOLECULAR PROPERTIES OF THE COMPOUNDS ISOLATED FROM MEDICINAL RICE VARIETY KARUNGKAVUNI

Pubchem ID	Compound name	LogP (<5)	Molecular weight (<500 Da)	HBA count (<10)	HBD count (<5)	Rotatable bond count (<10)
222284	Sitosterol	8.62	414.72	1	1	6
5367328	9,12,15-octadecatrienoic acid- 2,3-dihydroxypropyl ester	4.87	352.51	4	2	17
1105	Squalene	9.62	410.73	0	0	15
6452096	Ethyl iso-allocholate	4.01	436.63	5	3	6

plot reveals that three trajectories have the RMSD values between 0.1 nm and 0.54 nm throughout the simulation. For free DHPS system, the RMSD value is increased to 0.45 nm at 38 000 ps and there after the value gets decreased to 0.42 nm. For DHPS-9,12,15-octadecatrienoic acid-2,3-dihydroxypropyl ester complex, initially the RMSD value increase to 0.45 nm till 15 000 ps and then, there is a slight fluctuations in the value. After 35 000 ps, the values get decreased and reach 0.42 nm. In case of DHPS-ethyl iso-allocholate complex, the RMSD value increased up to 0.3 nm at 25 000 ps. Then, there is no further increase in the RMSD value and the DHPS-ethyl iso-allocholate system attains equilibrium. Comparing the values of the DHPS-ethyl iso-allocholate and DHPS-9,12,15-octadecatrienoic acid-2,3-dihydroxypropyl ester systems with the free DHPS system, it reveals that DHPS-ethyl iso-allocholate system shows a lower RMSD value. The decrease in the RMSD value of the DHPS-ethyl iso-allocholate system indicates increased stability and rigidity of DHPS upon binding with the ethyl iso-allocholate. RMSF is calculated to analyse the flexibility of residues on protein structure and the ligand-binding regions. The fluctuation scores reveal the behaviour of the residues throughout the simulation. The flexibility observed in the residues of free DHPS, DHPS-ethyl iso-allocholate and DHPS-9,12,15-octadecatrienoic acid-2,3-dihydroxypropyl ester complexes are shown in fig. 5. The free DHPS residues GLY31, GLY65, SER98 and ALA128 have highest fluctuations of 0.29, 0.46, 0.21 and 0.43 nm, respectively. In case of DHPS-ethyl iso-allocholate

**Fig. 4: Root mean square deviation plot.**

Free DHPS is shown in blue colour, DHPS-ethyl iso-allocholate complex in black colour and DHPS-9,12,15-octadecatrienoic acid-2,3-dihydroxypropyl ester complex in red colour.

**Fig. 5: Root mean square fluctuation plot.**

Free DHPS is shown in blue colour, DHPS-ethyl iso-allocholate complex in black colour and DHPS-9,12,15-octadecatrienoic acid 2,3-dihydroxypropyl ester complex in red colour.

complex, it shows more fluctuations at 0.26, 0.42, 0.12 and 0.13 nm, respectively. For DHPS-9,12,15-

octadecatrienoic acid-2,3-dihydroxypropyl ester complex, the fluctuations are seen at 0.34, 0.25, 0.18 and 0.15 nm, respectively. When comparing the fluctuation scores of free DHPS system with other two complexes, it reveals that the DHPS-ethyl iso-allocholate complex has the lesser fluctuation. These results interpret that the DHPS system shows more fluctuation without ethyl iso-allocholate. Hence, the DHPS becomes more rigid as a result of binding of ethyl iso-allocholate, when compared to the binding of 9,12,15-octadecatrienoic acid-2,3-dihydroxypropyl ester. R_g is plotted to identify the level of compaction in the structure of free DHPS, DHPS-ethyl iso-allocholate complex and DHPS-9,12,15-octadecatrienoic acid-2,3-dihydroxypropyl ester complex. The R_g of C α atoms of free DHPS, DHPS-ethyl iso-allocholate and DHPS-9,12,15-octadecatrienoic acid-2,3-dihydroxypropyl ester complexes versus time at 300 K are plotted in fig. 6. The R_g value of DHPS structure has shown to increase up to 1.86 nm, later the value get decreased to 1.78 nm and after 35 000 ps, the value again increases and attains equilibrium at 1.82 nm. For DHPS-ethyl iso-allocholate complex, the R_g plot shows fluctuation at the beginning and after 35 000 ps, it attains constant value of 1.78 nm. Further, not much variation is observed in R_g plot. In case of DHPS-9,12,15-octadecatrienoic acid-2,3-dihydroxypropyl ester, the R_g value get increased and reach 1.86 nm at 25 000 ps and started decreasing and reach 1.75 nm after 40 000 ps. It shows decreased value for DHPS-9,12,15-octadecatrienoic acid-2,3-dihydroxypropyl ester complex. Finally the

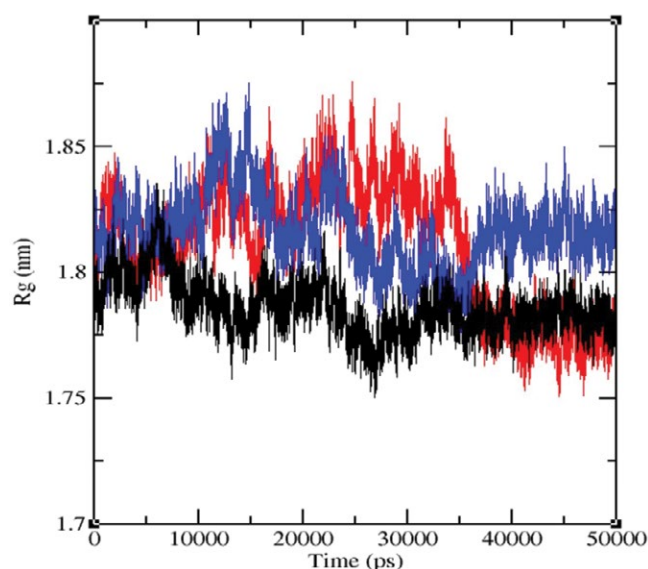


Fig. 6: Radius of gyration plot.

Free DHPS is shown in blue colour, DHPS-ethyl iso-allocholate complex in black colour and DHPS-9,12,15-octadecatrienoic acid-2,3-dihydroxypropyl ester complex in red colour.

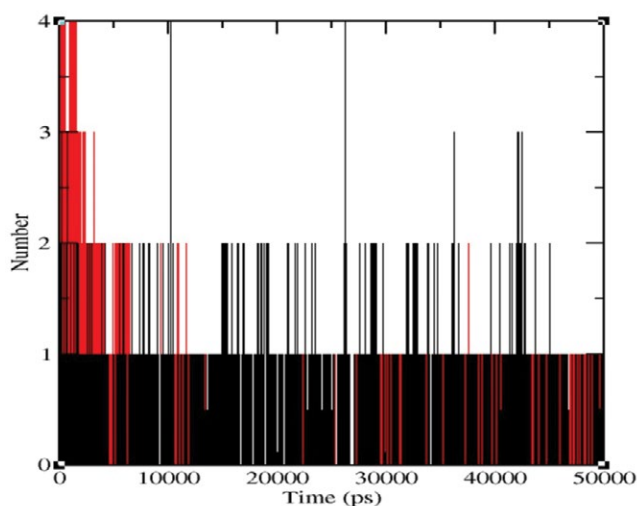


Fig. 7: Inter-molecular H-bond analysis plot.

DHPS-ethyl iso-allocholate complex is shown in black colour and DHPS-9,12,15-octadecatrienoic acid-2,3-dihydroxypropyl ester complex in red colour.

intermolecular H-bond analysis reveals that a total of 4 H-bonds are formed between DHPS-ethyl iso-allocholate complex and on average of 2 H-bonds are found throughout the simulation (fig. 7). For DHPS-9,12,15-octadecatrienoic acid-2,3-dihydroxypropyl ester, 4 H-bonds are formed and on average of 1 one H-bond is found throughout the simulation. The number of H-bonds formed between the complexes is involved in the stabilization of the complex. Overall, our results indicate that ethyl iso-allocholate isolated from *Karungkavuni* rice variety could serve as a potent inhibitor for *E. coli* DHPS. We believe our results will be a good starting point for further experimental validation on ethyl iso-allocholate to be developed as a potent antibacterial agent.

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Conflict of interest:

The authors declare that there is no conflict of interest.

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