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In-Silico Identification of Phytocompounds as Inhibitors to Two Key Enzymes of Shikimate Pathway of Mycobacterium Tuberculosis for Discovery of New Lead Molecule(S) for Treatment of Tuberculosis

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In-silico identification of phytocompounds as inhibitors to two key enzymes of Shikimate pathway of *Mycobacterium tuberculosis* for discovery of new lead molecule(s) for treatment of Tuberculosis.

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Abstract: **T**uberculosis(TB) is one of the most lethal respiratory infections caused by the organism Mycobacterium tuberculosis. Several drugs are available for the treatment of TB. Numerous reports have demonstrated the cause and emergence of multi drug resistance of M. tuberculosis. To improve the treatment of these strains , there is rising need to develop anti-TB effective drugs. The aim of this research was to develop an anti-tuberculosis drug.The two enymes 3-Dehydroquinate synthase(3N76) and 3-dehydroquinate dehydratase(3QBE) , of mycobacterial shikimate pathway was selected as drug targets.The structures of these two enzymes were obtained from PDB data bank.The phytocompounds from a medicinal plant,which was traditionally used in pulmonary infection, Achyranthes aspera ,were selected as ligands .Molecular docking was done against these two enzymes(receptors) by 11 phytocompounds of Achyranthes aspera by AUTODOCK vina software. The compounds which have highest binding affinity with targets was selected. Later pharmacokinetic analysis, bioactivity prediction, toxicity calculation of these compounds was done.From the docking study, the compound9(Ecdysterone 2,3-acetonide 22-O-benzoate), has highest binding affinity with enzyme 3-dehydroquinate synthase(3N76), And the compound 2(2,3,14,20,25-Pentahydroxy-6-oxocholest-7-en-22-yl benzoate) has highest binding affinity with enzyme 3-dehydroquinate dehydratase(3QBE).The druglikeness of these two compounds shows that both of them obey Lipinski's rule of 5.

Keywords: *Achyranthes aspera*, molecular docking, shikimate pathway, Pharmacokinetics

INTRODUCTION

Tuberculosis is caused by bacteria Mycobacterium tuberculosis that most often affect the lungs. It is spread from person to person through the air.TB is second leading infections killer after COVID-19. A total of 1.5 million people died from TB in 2020. This disease is curable and preventable. The first line drugs for treatment of tuberculosis are Isoniazid, rifampin, ethambutol etc. and the second line drugs are para amino salicylate, kanamycin etc. These traditionally used drugs have earned little success due to time and cost involved in development of anti TB drug.In our India most of the people are poor.They are not able to buy enough medicine.They can not take the required dose of the drug.Thus the Mycobacterium tuberculosis get a chance to develop a drug resistant strain(MDR TB). For the treatment of these multi drug resistant TB , there is an urgent need of new antituberculosis drug.To fulfil the development process of anti TB drug , first it is important to identify a suitable drug target.The intracellular metabolic pathways of M.tuberculosis are specific to this organism.So,the enzymes of this pathways are good drug targets.In this study , the two enzymes of Mycobacteial shikimate pathway are selected as drug targets.This pathway is absent in mammals. In this pathway, Phosphoenolpyruvate (PEP) and D-Erythrose-4-phosphate forms the chorismate by seven enzymatic reactions.The aromatic amino acids tryptophan, tyrosine and phenylalanine are formed from chorismate(Nunes *et al.*,2019). Thus if we can inhibit any enzyme of this pathway , the biosynthesis of tryptophan is also inhibited.Thus the bacterium can not survive within

host because of lack of tryptophan.The phytochemicals from medicinal plants related to pulmonary infections can inhibit the activity of the enzymes of this shikimate pathway. Present study includes the screening of the potent ligands of a traditionally used plant compounds, *Achyranthes aspera* , against the selected receptors (Enzymes of shikimate pathway) , 3-dehydroquinate synthase (DHQ synthase) and DHQ dehydratase through computer aided drug discovery.

The project has been aimed to screen new ligands as drug candidates using different computationally based methods. It includes following objectives:

I. To screen novel lead compounds of *Achyranthes aspera,* against the target protein 3-dehydroquinate synthase (3N76) and DHQ dehydratase (3qbe) of *Mycobacterium tuberculosis* using DOCKING software package (AutoDock Vina).

II. To study the drug like properties of the selected molecules including ADME-Tox studies to establish the selected molecules as potential lead molecules for discovery of novel anti-tuberculosis drug.

MATERIAL AND METHODS

Selection and preparation of the target protein

From the literature it has found that 3 dehydroquinate synthase and 3 dehydroquinate dehydratase are one of the good targets for the drug discovery.These two mycobacterial enzymes was searched in RCSB PDB(**rcsb.org**).The crystal structures was downloaded with their PDB iD in PDB format.The 3 dehydroquinate synthase protein consists of 1 chains and 3 dehydroquinate dehydratase consists of 1 chains. The sequences of the individual chains were aligned using **ClustalX** (http://www.clustal.org/). It wasfound that all the chains had hundred percent similarities and hence chain A is used for the study from both the target protein. With the help of Chimera the water molecules and the binded ligands along with solvents were removed and added polar hydrogen. Subsequently and the AutoDock atom types were defined using AUTODOCK Tools, graphical user interface of AUTODOCK supplied by MGL Tools. Energy minimization of the target protein was done using **VegaZZ** (http://ddl.unimi.it/) which is a file translation tool along with properties and surface calculations.

Preparation of potent inhibitors of the target proteins(ligands)

The result of GC-MS analysis was 11phytocompounds from Achyranthes aspera plant. 2D structures and Basic Chemical properties of the various *Achyranthes aspera* test compounds used in the present study are constructed in Accelrys Draw 4.2. These 11 phytocompounds were used for docking energy analysis and pharmacokinetics analysis.The default root, rotatable bonds, and torsions of the ligand were set by TORSDOF utility in AutoDock Tools. Finally, the ligand became ready into PDBQT docking format.

Determination of Drug-likeness(Pharmacokinetic properties) of ligands

Swiss ADME tox study was done to determine the pharmacokinetic properties of these phytocompounds. Swiss ADME server is an online tool which is used for determining drug like properties of these compounds by uploading smiles.After uploading smiles or simles file , the properties like solubility,GIabsorption,BBB permeant and molecular properties visible on screen.The five molecular properties determine weather ,it obeys Lipinski's Rule of 5.These properties are Molecular weight(MW<500),number of hydrogen bond donor(H bond donor<5),number of H bond acceptor<10 and calculated LogP<5. Molinspiration ,an online server was used to predict the bioactivity score.

BOILED EGG Analysis

To predict gastrointestinal absorption and brain access of small molecules, boiled egg analysis was done. Boiled egg analysis was done by Swiss ADME . By pasting smile or smiles file of small molecules(ligands) pharmacokinetics was predicted.Along with pharmacokinetics, the boiled egg analysis can be done by clicking "show boiled egg".

Determination of Oral toxicity of the Ligands

Many drugs have toxicity such as hepatoxicity.The toxicity of the phytocompounds was evaluated by ProTox-II online server.By pasting smiles of phytocompounds(ligands) , the toxicity was predicted.This method of prediction of toxicity by computational method is easy in comparison to in animal model. This type of evaluation can reduce the time.

Molecular docking

The molecular docking analysis of all 11 natural compounds of A. aspera plant accompanied by the flexible or blind docking method. The selected target proteins 3-dehyroquinate synthase (3N76) and 3-dehydroquinate dehydratase(3qbe) is docked with selected ligands from the plant compounds *Achyranthes aspera* using the AutoDock Vina software. The results exhibit different binding affinities of the target protein 3-dehydroquinate synthase and 3dehydroquinate dehydratase with the inhibitors. Finally 6 best results were selected primarily based on Lipinski's rules and observing the 3D interactions.

Visualization of the protein-ligand interaction

PyMOL is a software which can visualize the binding of receptor(protein) and ligand.PyMOL can produce high quality 3D image of small molecules and protein. The polar (hydrogen bond) and non-polar interactions between receptor and small ligand(s) were visualized by PyMOL software.

RESULT AND DISCUSSION

ADMET analysis of ligand(s)

Lipinski's rule of 5 describes the drugability of a determinate molecules.It helps to determine if a biologically active chemical is likely to have the chemical and physical properties to be orally bioavailable.The Lipinski's rule bases pharmacokinetic properties such as absorption, distribution, metabolism,and excretion on specific molecular properties such as

a)No more than 5 hydrogen bond donors

b)No more than 10 hydrogen bond acceptors

c)Molecular mass less than 500 Da

d) Partition co-efficient not greater than 5.

The violation of 2 or more of these conditions predicts a molecule as a non orally available drug.

The druglikeness of 11 phytocompounds of Achyranthes aspera is shown in table1. The compound 3,5,6,8,10 and 11 have molecular weight MW<500 Da. Next criteria of RO5< is the number of H bond donor(0-5) and number of H bond acceptor(0-10).Except the compounds 1,6 and 10 ,all the compounds belong to this range. For the forecast of oral liability of drug molecules, Lipophilicity(LogP) and Topological polar surface area(TPSA) values are crucial.Most of the compounds LogP ranges from (0.10-5),which is acceptable limits for drug to penetrate biomembrane.The ADMET analysis of phytocompounds are shown in table2.

Table 1 Pharmacokinetics properties of natural compounds according to Lipinski rule analysis for *A. aspera* plant

Sl. No.	Compound name	M.W. (g/mol)	No. of H bond acceptors	No. of H bond donor	logP	RO5
1	6-[[9-Acetyloxy-8-hydroxy-4,8a-bis(hydroxymethyl)-4,6a,6b,11,11,14b-hexamethyl-10-(2-methylbut-2-enoyloxy)-1,2,3,4a,5,6,7,8,9,10,12,12a,14,14a-tetradecahydropicen-3-yl]oxy]-4-hydroxy-3,5-bis[[3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxy]oxane-2-carboxylic acid	1131.269	24	13	0.105	NO
2	2,3,14,20,25-Pentahydroxy-6-oxocholest-7-en-22-yl benzoate	584.75	8	5	3.78	yes
3	Cocamidopropyl betaine	342.52	3	1	-2.247	yes
4	14-Hydroxy-17-[5-(3-hydroxy-3-methylbutyl)-2,2,4-trimethyl-1,3-dioxolan-4-yl]-2,6,6,18-tetramethyl-5,7-dioxapentacyclo[11.7.0.02,10.04,8.014,18]icos-12-en-11-one	560.77	7	2	5.141	yes
5	Phenylalanine betaine	117.148	3	1	-5.412	yes
6	Betaine monohydrate	117.148	7	6	-4.838	yes
7	2,3,14-trihydroxy-17-[5-(3-hydroxy-3-methylbutyl)-2,2,4-trimethyl-1,3-dioxolan-4-yl]-10,13-dimethyl-2,3,4,5,9,11,12,15,16,17-decahydro-1H-cyclopenta[a]phenanthren-6-one	520.707	7	4	3.25	yes
8	Cloral betaine	117.148	4	2	-5.412	yes
9	Ecdysterone 2,3-acetonide 22-O-benzoate	624.81	8	3	5.678	yes
10	2,3,14-trihydroxy-10,13-dimethyl-17-(2,4,7-trihydroxy-6-methylheptan-2-yl)-2,3,4,5,9,11,12,15,16,17-decahydro-1H-cyclopenta[a]phenanthren-6-one	480.642	7	6	1.296	yes
11	2,3,14,20,22,25-Hexahydroxycholest-7-en-6-one	480.642	2	0	1.359	yes

Table 2 ADMET properties of natural compounds for *A. aspera* plant

Sl. No.	Compound name	miLogP	TPSA	natoms	nrotB	nVio
1	6-[[9-Acetyloxy-8-hydroxy-4,8a-bis(hydroxymethyl)-4,6a,6b,11,11,14b-hexamethyl-10-(2-methylbut-2-enoyloxy)-1,2,3,4a,5,6,7,8,9,10,12,12a,14,14a-tetradecahydropicen-3-yl]oxy]-4-hydroxy-3,5-bis[[3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxy]oxane-2-carboxylic acid	0.105	388.049	79	16	3
2	2,3,14,20,25-Pentahydroxy-6-oxocholest-7-en-22-yl benzoate	3.78	144.51	42	8	1
3	Cocamidopropyl betaine	-2.24	69.22	24	17	0
4	14-Hydroxy-17-[5-(3-hydroxy-3-methylbutyl)-2,2,4-trimethyl-1,3-dioxolan-4-yl]-2,6,6,18-tetramethyl-5,7-dioxapentacyclo[11.7.0.02,10.04,8.014,18]icos-12-en-11-one	5.141	94.463	40	4	1
5	Phenylalanine betaine	-5.412	40.128	8	2	0
6	Betaine monohydrate	-4.83	40.128	15	5	1
7	2,3,14-trihydroxy-17-[5-(3-hydroxy-3-methylbutyl)-2,2,4-trimethyl-1,3-dioxolan-4-yl]-10,13-dimethyl-2,3,4,5,9,11,12,15,16,17-decahydro-1H-cyclopenta[a]phenanthren-6-one	3.25	116.451	37	4	1
8	Cloral betaine	-5.412	40.128	8	3	0
9	Ecdysterone 2,3-acetonide 22-O-benzoate	5.678	122.528	45	8	1
10	2,3,14-trihydroxy-10,13-dimethyl-17-(2,4,7-trihydroxy-6-methylheptan-2-yl)-2,3,4,5,9,11,12,15,16,17-decahydro-1H-cyclopenta[a]phenanthren-6-one	1.296	138.439	34	6	1
11	2,3,14,20,22,25-Hexahydroxycholest-7-en-6-one	1.359	138.439	34	4	0

*TPSA, Topological Polar Surface Area; natoms, number of atoms; nrotB, number of rotatable bonds; nVio, number of Violations

Bioactivity score prediction

The bioactivity or biological activity means the beneficial or adverse effects of a drug on living tissue.It suggests the uses of the phytocompounds in the medical applications. Molecules having bioactivity score more than 0.00 is most likely to exhibit considerable biological activity. If the values ranges from 0.50 to 0.00, are moderately active and if the score is less than 0.50, then it is inactive. Molinspiration tool was used to predict bioactivity score of phytocompounds against human receptors such as GPCRs, ION CHANNEL, KINASE, NUCLEAR RECEPTORS, PROTEASES and ENZYMES, which is shown in the table3.The results shows that except compounds 1,6,8,9 the other compounds are active against GPCR ligands.

Oral toxicity prediction

The prediction of compound toxicities is an important part of drug design development process. ProTox-II is a virtual lab for the prediction of toxicities of small molecules. Toxic doses are often given ad LD50 values in mg/kg body weight.The LD50 is the median lethal dose meaning the dose at which 50% of the test subjects die upon exposure to a compound.Toxicity classes are defined according to LD50

Class1: Fatal if swallowed(LD50<5)
Class2: Fatal if swallowed(5<LD50<50)
Class3:Toxic if swallowed(50<LD50<300)
Class4:Harmful if swallowed(300<LD50<2000)
Class5: May be harmful if swallowed(2000<LD50<5000)
Class6: Non toxic(LD50>5000)

The oral toxicity of these phytocompounds is shown in table4.

Table 3 Bioactivity Score of natural compounds for *A. aspera* plant

Sl. No.	Compound name	GPCR L	Ion CM	Ion CM	Kinase INH	Nuclear RL	Kinase INH	Protease INH	Nuclear RL	Enzyme INH	Protease
1	6-[[9-Acetyloxy-8-hydroxy-4,8a-bis(hydroxymethyl)-4,6a,6b,11,11,14b-hexamethyl-10-(2-methylbut-2-enoyloxy)-1,2,3,4a,5,6,7,8,9,10,12,12a,14,14a-tetradecahydropicen-3-yl]oxy]-4-hydroxy-3,5-bis[[3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxy]oxane-2-carboxylic acid	-3.77	-3.85	-3.89	-3.78	-3.72	-3.71		
2	2,3,14,20,25-Pentahydroxy-6-oxocholest-7-en-22-yl benzoate	0.02	-0.30	-0.47	0.49	0.15	0.36		
3	Cocamidopropyl betaine	0.34	0.32	-0.20	-0.58	0.04	0.33		
4	14-Hydroxy-17-[5-(3-hydroxy-3-methylbutyl)-2,2,4-trimethyl-1,3-dioxolan-4-yl]-2,6,6,18-tetramethyl-5,7-dioxapentacyclo[11.7.0.02,10.04,8.014,18]icos-12-en-11-one	0.02	-0.20	-0.44	0.73	0.13	0.40		
5	Phenylalanine betaine	0.01	0.30	-0.55	-1.00	-0.46	0.04		
6	Betaine monohydrate	-2.53	-1.79	-3.50	-3.75	-3.47	-2.12		
7	2,3,14-trihydroxy-17-[5-(3-hydroxy-3-methylbutyl)-2,2,4-trimethyl-1,3-dioxolan-4-yl]-10,13-dimethyl-2,3,4,5,9,11,12,15,16,17-decahydro-1H-cyclopenta[a]phenanthren-6-one	0.09	-0.04	-0.36	0.87	0.25	0.59		
8	Cloral betaine	-2.53	-1.79	-3.50	-3.75	-3.47	-2.12		
9	Ecdysterone 2,3-acetonide 22-O-benzoate	-0.24	-0.70	-0.77	0.23	-0.00	0.07		
10	2,3,14-trihydroxy-10,13-dimethyl-17-(2,4,7-trihydroxy-6-methylheptan-2-yl)-2,3,4,5,9,11,12,15,16,17-decahydro-1H-cyclopenta[a]phenanthren-6-one	0.11	0.07	-0.43	0.79	0.19	0.63		
11	2,3,14,20,22,25-Hexahydroxycholest-7-en-6-one	0.16	0.17	-0.32	0.92	0.32	0.68		

*GPCRL, G-Protein Coupled Receptor Ligand; Ion CM, Ion channel Modulator; Kinase INB, Kinase inhibitor; Nuclear RL, Nuclear receptor ligand; Protease INH, Protease inhibitor; Enzyme INH, Enzyme inhibitor

Table 4 Oral toxicity prediction of natural compounds for *A. aspera* plant

Sl.No.	Compound name	LD50 (mg/kg)	Toxic. Class (1-6)	Avg. SM	Pred. AC (%)
1	6-[[9-Acetyloxy-8-hydroxy-4,8a-bis(hydroxymethyl)-4,6a,6b,11,11,14b-hexamethyl-10-(2-methylbut-2-enoyloxy)-1,2,3,4a,5,6,7,8,9,10,12,12a,14,14a-tetradecahydropicen-3-yl]oxy]-4-hydroxy-3,5-bis[[3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxy]oxane-2-carboxylic acid	134	3	100	100
2	2,3,14,20,25-Pentahydroxy-6-oxocholest-7-en-22-yl benzoate	2450	5	59.85	67.38
3	Cocamidopropyl betaine	400	4	76.42	69.26
4	14-Hydroxy-17-[5-(3-hydroxy-3-methylbutyl)-2,2,4-trimethyl-1,3-dioxolan-4-yl]-2,6,6,18-tetramethyl-5,7-dioxapentacyclo[11.7.0.02,10.04,8.014,18]icos-12-en-11-one	4500	4	70.79	69.26
5	Phenylalanine betaine	1100	4	70.87	69.26
6	Betaine monohydrate	650	4	100	100
7	2,3,14-trihydroxy-17-[5-(3-hydroxy-3-methylbutyl)-2,2,4-trimethyl-1,3-dioxolan-4-yl]-10,13-dimethyl-2,3,4,5,9,11,12,15,16,17-decahydro-1H-cyclopenta[a]phenanthren-6-one	4500	4	71.59	69.26
8	Cloral betaine	800	4	70	68.07
9	Ecdysterone 2,3-acetonide 22-O-benzoate	1750	4	57.19	67.38
10	2,3,14-trihydroxy-10,13-dimethyl-17-(2,4,7-trihydroxy-6-methylheptan-2-yl)-2,3,4,5,9,11,12,15,16,17-decahydro-1H-cyclopenta[a]phenanthren-6-one	9000	6	97.96	72.9
11	2,3,14,20,22,25-Hexahydroxycholest-7-en-6-one	9000	6	100	100

*LD50, Lethal dose 50%; Toxic, Class- toxicity class; Avg. SM, Average similarity; Prediction accuracy

Molecular docking

The molecular docking analysis of all 11 natural compounds of A. aspera plant accompanied by the flexible or blind docking method. The selected target proteins 3-dehyroquinate synthase (3N76) and 3-dehydroquinate dehydratase(3qbe) is docked with selected ligands from the plant compounds *Achyranthes aspera* using the AutoDock Vina software. Theresults exhibit different binding affinities of the target protein 3-dehydroquinate synthase and 3dehydroquinate dehydratase with the inhibitors. Finally 6 best results were selected primarily based on Lipinski's rules and observing the 3D interactions.From the docking study, the compound9(Ecdysterone 2,3-acetonide 22-O-benzoate), has highest binding affinity with enzyme 3-dehydroquinate synthase(3N76), And the compound 2(2,3,14,20,25-Pentahydroxy-6-oxocholest-7-en-22-yl benzoate) has highest binding affinity with enzyme 3-dehydroquinate dehydratase(3QBE) is shown in table5.

Table 5 Ligand-receptor interaction of natural compounds which has highest binding affinity with M. tuberculosis 3N76 and 3QBE proteins.

Sl.No.	PDB ID	Binding affinity(Kcal/mol)					
		Lig 2	Lig4	Lig7	Lig10	Lig11	Lig9

1	3N76	-	-	-6.2	-	-	-6.7
2	3QBE	-10.1	-8.8	-9.7	-9	-8.9	-

* Lig, ligand number; PDB ID, Protein Data Bank ID

BOILED EGG ANALYSIS

The boiled egg analysis evaluates the gastrointestinal absorption (HIA) and brain penetration(BBB) in function of the position of the molecules in the WLOGP versus –TPSA referential.The white region means the high probability of passive gastrointestinal absorption ang yellow portion means the high probability of brain penetration. The points are coloured in blue if predicted as actively effluxed by P-gp(PGP^+) and in red if predicted as non substrate of P-gp(PGP^-). The boiled egg analysis of compound 2 and compound 9 was done. These two molecules are predicted as not absorbed and not brain penetrant(outside the egg), but PGP^+. The boiled egg analysis of these compounds are shown in figure 1.

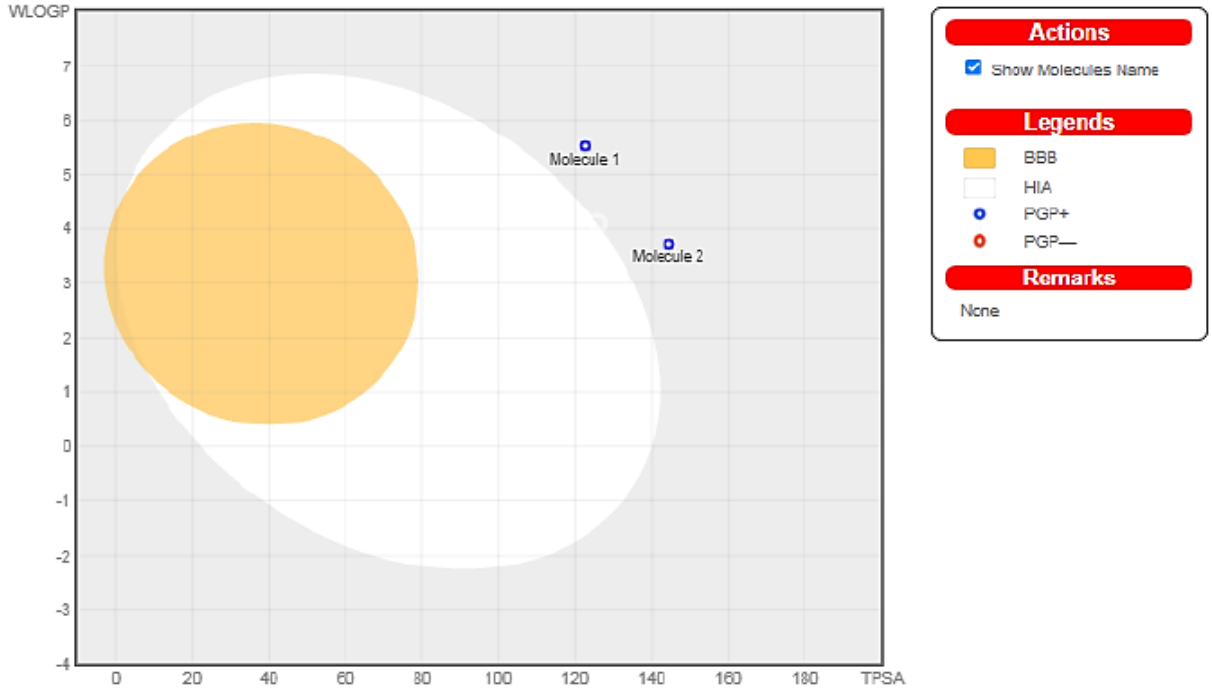

Fig1: Boiled egg analysis

Visualization of the protein-ligand interaction

From the docking study, the compound9(Ecdysterone 2,3-acetonide 22-O-benzoate), has highest binding affinity with enzyme 3-dehydroquinate synthase(3N76), And the compound 2(2,3,14,20,25-Pentahydroxy-6-oxocholest-7-en-22-yl benzoate) has highest binding affinity with enzyme 3-dehydroquinate dehydratase(3QBE).The binding interaction between receptors and ligands are visualiZed by PyMOL software.The molecular docking results suggested that seven amino acids may be important in the interaction between DHQs and compound9 which is shown in table6.The results showed that Ile 125, val 124, pro119, His 114, ser 118, His 106, val 105 are essential for function of mtDHQs. We speculated that compound 9 binds to the active center of DHQs and inhibits its catalytic activity.The interaction of compound9 with enzymeDHQ is shown in figure2.The interaction between compound2 and 3-dehydroquinate dehydratase is analyzed by molecular docking. The molecular docking results suggest that 12 amino acids may be important in the interaction between DHQase and compound2. The results showed that trp263, glu256, asn154, leu134, glu179, cys182, gly107, ala108, ala139, His265, lys228 are essential for function of mtDHQase. The interaction is shown in figure3.

Table 6 Ligand-receptor interaction with group involved in interaction of the receptor

Sl.No.	PDB ID	Ligands	Amino acids involved with interactive group
1	3N76	ligand 9	Ile 125 val 124 pro119 His 114 ser 118 His 106 val 105

2	3QBE	Ligand 2	asn154 lys228 glu75 trp263

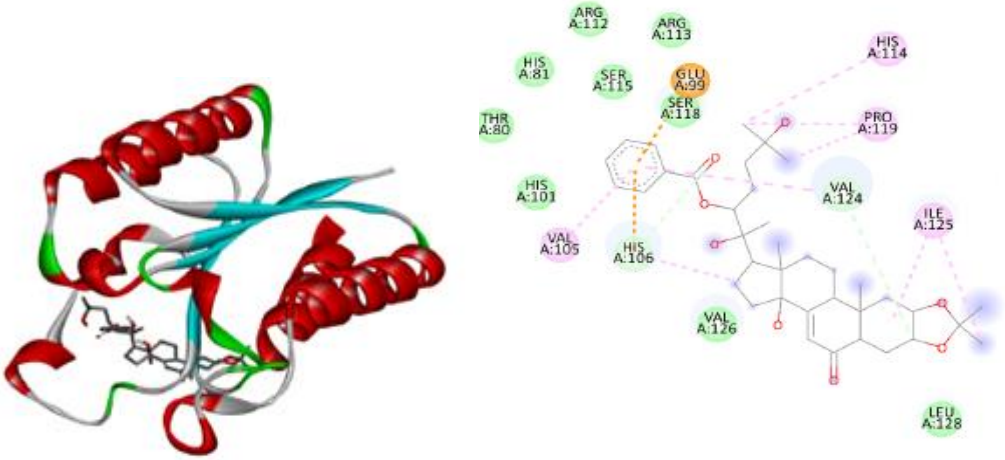

Figure 2 Binding modes of the ligand 9 as interacted with PDB ID: 3N76 which is shown in center, where green dotted line represent hydrogen bonds and purple/pink represents alkyl/pi-alkyl bonds interactions, respectively..

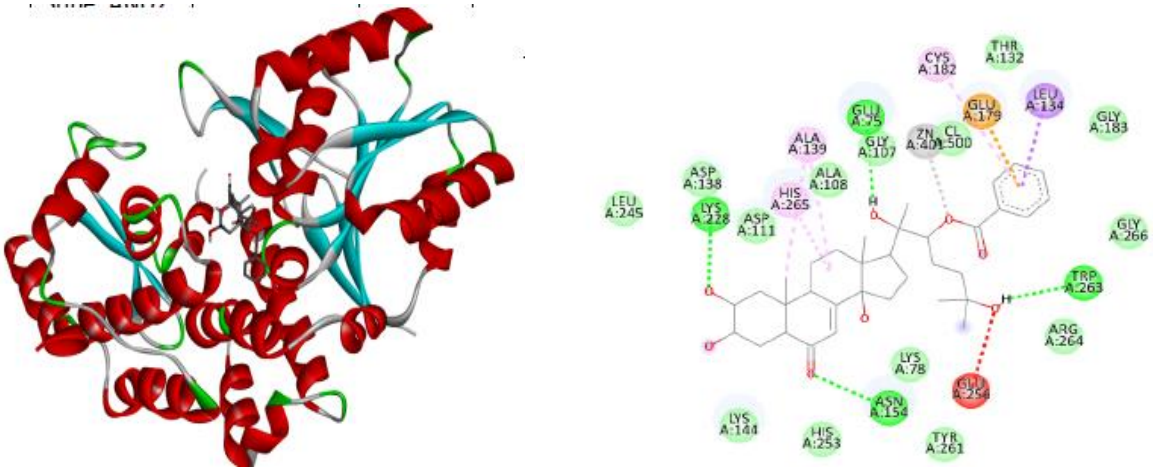

Figure 3 Binding modes of the ligand 2 as interacted with PDB ID: 3QBE

CONCLUSION

From the molecular docking study ,it is found that compound2 and compound 9 have highest binding affinities with the two enzymes of Mycobacterial shikimate pathway, 3-Dehydroquinate synthase (3N76) and 3-dehydroquinate dehydratase(3qbe). These two compounds can bind to the active sites of these enzymes,so they can inhibit the functions of these enzymes.Thus, the shikimate pathway fails and the bacterium can not produce aromatic amino acids like tryptophan.Thus the bacterium can not survive.The enzymes of shikimate pathway thus serve as novel drug target for the drug discovery process for treatment of tuberculosis.Besides, the above mentioned phytocompounds , Ecdysterone 2,3-acetonide 22-O-benzoate and 2,3,14,20,25-Pentahydroxy-6-oxocholest-7-en-22-yl benzoate have pharmacokinetic properties ,obey Lipinski's rule of 5, with one violation. These are bioactive compounds , lesser toxicity (toxic class 4 & 5). Thus, from the present analysis these molecules can be considered as potent drug molecules in tuberculosis treatment. For further validation of the compounds they are suggested for pre-clinical and clinical trials to make them successful and eventually marketed.

DATA AVAILABILITY

The datasets generated and analysed during the current study are available in the following databases:

Protein structure data are available in **Protein Data Bank(PDB) :**

http://doi.org/10.2210/pdb3N76/pdb

http://doi.org/10.2210/pdb3QBE/pdb

Chemical structures of ligands are available in database **Pubchem:**
https://pubchem.ncbi.nlm.nih.gov/compound/570764.
https://pubchem.ncbi.nlm.nih.gov/compound/44663461
The bioactivity data of phytocompounds are available in **Molinspiration**:
https://www.molinspiration.com/docu/miscreen/druglikeness.html
The pharmacokinetic data of phytocompounds are available in **Swissadme:**
https://doi.org/10.1038/srep42717

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