In-silico identification of phytocompounds as inhibitors of key enzymes in Shikimate pathway of *Mycobacterium tuberculosis* for discovery of new lead molecules

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***Abstract*:**
Tuberculosis is one of the most lethal respiratory infections caused by the microorganism Mycobacterium tuberculosis. Despite the availability of several drugs to treat TB, numerous reports have demonstrated the cause and emergence of multidrug resistance of M. tuberculosis. Hence, the need of developing effective anti-TB therapeutics against multidrug-resistant strains has always been cardinal attention for the past two decades. In this study, to identify potent anti-tuberculosis drugs, two enzymes namely, 3-dehydroquinate synthase and 3-dehydroquinate dehydratase of mycobacterial Shikimate pathway were selected as drug targets for inhibiting their regulatory mechanisms. The medicinal plant Achyranthes aspera has been traditionally used in pulmonary infection. Therefore, the phytocompounds from this plant were selected for carrying out the computational evaluation of their binding affinities and drug-like properties against the selected enzymes. Molecular docking was done for 11 phytocompounds against these two enzymes (receptors) using AutoDock Vina software. The compounds which exhibited the highest binding affinities with targets were selected for pharmacokinetic analysis, bioactivity prediction and toxicity calculation. From the docking study, it was concluded that the compound-9 (ecdysterone 2,3-acetonide 22-O-benzoate) and compound-2 (2,3,14,20,25-pentahydroxy-6-oxocholest-7-en-22-yl benzoate) showed the highest binding affinities with the enzymes3-dehydroquinate synthase and 3-dehydroquinate dehydratase, respectively. Eventually, both the compound exhibited similar druglikeness by obeying Lipinski's rule of 5.

***Keywords*:** *Achyranthes aspera*,Molecular docking, Shikimate pathway, Pharmacokinetics.

I. INTRODUCTION

Tuberculosis (TB) is caused by the bacterium *Mycobacterium tuberculosis*which commonly affects the lungs but can also affect the brain, kidneys, or spine. It is spread from infected persons to healthy persons through the air via coughing, sneezing and spitting.TB is the second leading infectious disease after COVID-19. Although the disease is curable and preventable, a total of 1.6 million people died worldwide from TB in 2021 (https://www.who.int/news-room/fact-sheets/detail/tuberculosis, 27 October 2022). The first-line drugs for the treatment of TB are isoniazid, rifampin, ethambutol, etc. and the second-line drugs are para-amino salicylate, kanamycin, etc. All of these drugs have been used traditionally and earned little success due to the time-consuming and cost-intensive strategies for development of anti-TB drugs.Management and control of such an infectious disease are cumbersome for a massively populated developing country like India. Due to the lack of proper awareness, the drugs recommended to treat TB are commonly misused or mismanaged, which causes the emergence of multidrug-resistant (MDR) strains. Multidrug-resistance is developed by the microbe when it becomes resistant to at least isoniazid and rifampicin, the two most promising first-line anti-TB drugs. Moreover, MDR-TB strains are resistant to any of the fluoroquinolones and to at least one of the three injectable second-line drugs. Even more alarming reality is that MDR-TB patients are most likely to be infected with extensively drug-resistant strains of*M. Tuberculosis*(XDR-TB).The contemporary treatment approved by the WHO for the cases of drug-sensitive TB is a 6-month administration of four first-line drugs namely, isoniazid, rifampicin, ethambutol and pyrazinamide [1]. Despite the prolonged and expensive treatment regimens adopted for curing MDR-TB with toxic drugs, the success rate of such treatment procedure is only about 56%[1]. Hence, there is an intense necessity of effective treatment for MDR-TB with new and more powerful anti-TB agents. In order to accomplish the new drug development

process, the first and foremost important step is to identify the suitable drug targets, especially to inhibit the functions of certain proteins (enzymes/receptors) present within the microbe or host.The intracellular metabolic pathways of *M.tuberculosis* are unique and specific to cause its infection. Therefore,the enzymes of these pathways are essentially the promising targets for developing new drugs.The*M. tuberculosis* Shikimate pathway is a unique pathway involved in the biosynthesis of aromatic rings from carbohydrate precursors via a range of extraordinary chemical transformations and is absent in mammals. The pathway starts with the substrates phosphoenolpyruvate (PEP) and D-erythrose-4-phosphate and terminates with the production of chorismate, the precursor of aromatic amino acids, by seven enzymatic reactions.The aromatic amino acids tryptophan, tyrosine and phenylalanine are formed from chorismate[1].

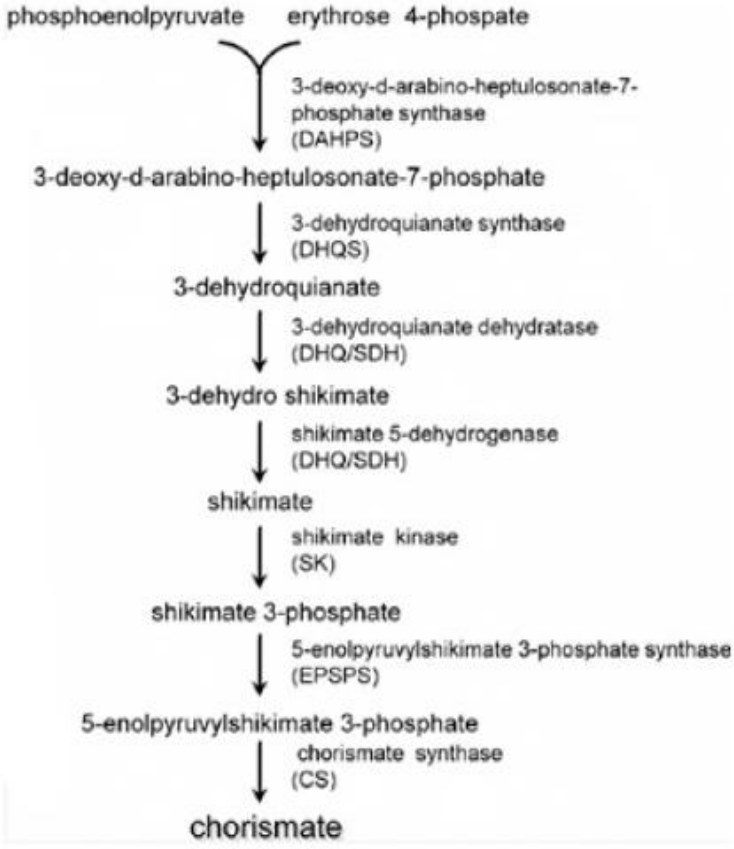

Fig. 1. The Shikimate Pathway

An efficient mechanism of killing the microbe afforded by the human host is the inhibition of any enzyme of this pathway which prevents tryptophan biosynthesis and increases the likelihood of malnourishment of the microbe.The phytochemicals from medicinal plants traditionally used to treat pulmonary infections might interact with the Shikimate pathway of the microorganism and established to inhibit the activity of the enzymes involved in the pathway[2].The present study includes the screening of potent phytochemical compounds from a traditionally used medicinal plant, *Achyranthes aspera*, against the selected enzymes of the Shikimate pathway, 3-dehydroquinate synthase (DHQ synthase) and 3-DHQ dehydratase using computational techniques.This study was aimed to screen new phytochemicals from *A. aspera*as drug candidates by evaluating their binding affinities with 3-DHQ synthase and 3-DHQ dehydratase of *M. tuberculosis* Shikimate pathway using molecular docking protocol followed by evaluation of drug-like properties of the selected molecules including ADME-Tox studiesto establish the selected phytochemical compounds as potential leadmolecules for the discovery of novelanti-TB drugs.

II. MATERIALSAND METHODS

A. Selection and Preparation of the Target Enzymes

The X-ray crystallographic structures of the two mycobacterial enzymes, 3-DHQ synthase and 3-DHQ dehydratase were searched and obtained from RCSB Protein Data Bank (PDB) with PDB IDs 3N76 and 3QBE, respectively. Both enzymes consisted of single polypeptide chains.Co-crystallized ligands and solvents associated with the PDB files were removed and polar hydrogen atoms were added using UCSF Chimera [3]. Subsequently, theAutoDock atom types were defined using AutoDock Tools provided by the graphical user interface from MGL Tools[4].Energy minimization of the target proteins was performed using **VegaZZ**(http://ddl.unimi.it/), a file translation tool equipped with properties and surface calculations of biomolecules.

B. *Preparation of Inhibitors (Ligands)*

The Gas Chromatography-Mass Spectrometry(GC-MS) analysis wasdone to identify the phytocompounds from the medicinal plant *A. aspera*. The result of GC-MS analysis was 11phytocompounds, 2D structures and basic chemical properties ofwhich were evaluated usingAccelrys Draw 4.2 (https://accelrys-draw.software.informer.com/4.0/). These 11 phytocompounds were used for docking andpharmacokinetics analysis.The default root, rotatable bonds andtorsional degrees of freedom of the compounds were set by TORSDOF utility in AutoDock Tools to prepare the compounds compatible to docking format.

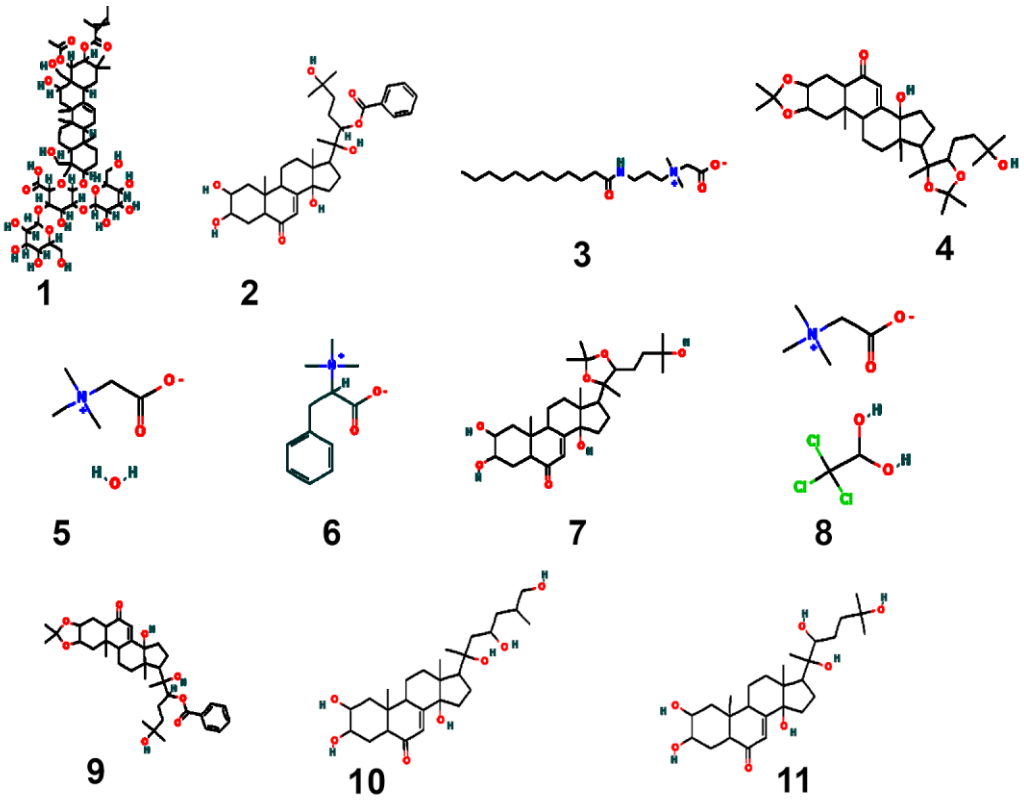

Fig. 2. 2D structures of11 phytocompounds from *Achyranthes aspera* plant

C. *Molecular Docking*

The molecular interaction analysis of all 11 natural compounds of *A. aspera* was performed by the flexible or blind docking method. The selected ligands obtained from the plant were docked with the selected targets 3-DHQ synthase (3N76) and 3-DHQ dehydratase(3QBE) using the AutoDock Vina software (https://vina.scripps.edu/). The results depicted different binding affinities of the phyocompounds with the target proteins. Finally 6 best compounds were selected based on the Lipinski's rules and observed 3D interactions.

D. *Visualization of the Protein-Ligand Interaction*

The PyMOL Molecular Graphics System, Version 2.0 Schrödinger, LLCwas used for visualizing the binding modes of the ligands with the receptors (selected enzymes protein). The software was also used to produce high quality 3D images of the protein–ligand complexes with polar (hydrogen bond) and non-polar interactions.

E. *Pharmacokinetic Analysis of Ligands*

Swiss ADME-Tox studies were carried out in order to determine the pharmacokinetic properties of the screened phytochemical compounds[5]. The drug-likeness analyses such as solubility, GI-absorption, Blood–Brain Barrier permeability along with the Lipinski's Rule of 5 were evaluated by providing the SMILES strings of the compounds to the server. Another online tool Molinspiration, was used to predict the bioactivity score. Similarly, toxicity of the phytocompounds was evaluated by ProTox-II server (https://tox-new.charite.de/protox_II/index.php?site=compound_input).

III. RESULTAND DISCUSSION

A. *Molecular Docking*

The molecular interaction analysis of all 11 natural compounds of *A. aspera* was accomplished by the flexible or blind docking method. The selected compounds from the plant were docked with the target enzymes3-DHQsynthase (3N76) and 3-DHQ dehydratase(3QBE) using the AutoDock Vina software [6]. The results exhibited different binding affinities of the phytocompounds with the target proteins. Eventually,6 best compounds were selected primarily based on Lipinski's rules and observed 3D interactions.From the docking study, the compound-9 (ecdysterone 2,3-acetonide 22-O-benzoate), showed the highest binding affinity with enzyme 3-DHQsynthase(3N76), whereas, the compound-2(2,3,14,20,25-pentahydroxy-6-oxocholest-7-en-22-yl benzoate) expressed the highest binding affinity with enzyme 3-DHQ dehydratase (3QBE) as listed in Table I.

TABLE I. LIGAND–RECEPTOR INTERACTION OF NATURAL COMPOUNDS WHICH EXHIBITED THE HIGHEST BINDING AFFINITY WITH*M. tuberculosis* 3N76 AND3QBE PROTEINS.

Sl.No.	**PDB ID**	**Target Enyme**	**Binding affinity(kcal/mol)**					
			Lig2	Lig4	Lig7	Lig9	Lig10	Lig11
1	3N76	3-DHQ synthase	-	-	-6.2	-6.7	-	-
2	3QBE	3-DHQ dehydratase	-10.1	-8.8	-9.7	-	-9	-8.9

B. *Visualization of the Protein-Ligand Interaction*

From the docking study, it was found that the compound-9(ecdysterone 2,3-acetonide 22-O-benzoate), exhibited the highest binding affinity to3-DHQ synthase(3N76) and the compound-2(2,3,14,20,25-pentahydroxy-6-oxocholest-7-en-22-yl benzoate) showed the highest binding affinity to 3-DHQdehydratase(3QBE).The binding interactions between the receptors andligands were visualized using PyMol.The molecular docking results suggested that seven amino acids might be important for the interaction between DHQs and compound-9 which as listed inTable VI.The results showed that Ile125, Val124, Pro119, His114, Ser118, His106 andVal105 were found essential for function of 3-DHQ synthase.Thus, it could be speculatedthat the compound-9 bound to the active center of 3-DHQsynthase and inhibited its catalytic activity. The interaction of compound-9 with 3-DHQ synthase is shown in Fig. 2. Similarly, the molecular docking results of interactions between compound-2 and 3-DHQdehydratase suggested that 12 amino acids might be crucial for conferring potential inhibition of3-DHQ dehydratase by compound-2. The results showed that Trp263, Glu256, Asn154,Leu134, Glu179, Cys182, Gly107, Ala108, Ala139, His265 and Lys228 were essentialfor proper function of the enzyme (Fig. 3).

TABLE II. PROTEIN–LIGANDINTERACTIONS WITH THE AMINO ACIDS GROUP INVOLVEDIN THE INTERACTIONS.

Sl.No.	**Target Enyme**	**Compound**	**Amino acids involved with interactive group**
1	3-DHQ synthase	Compound-9	Ile125 Val124 Pro119 His114 Ser118 His106 Val105
2	3-DHQ dehydratase	Compound-2	Asn154 Lys228 Glu75 Trp263

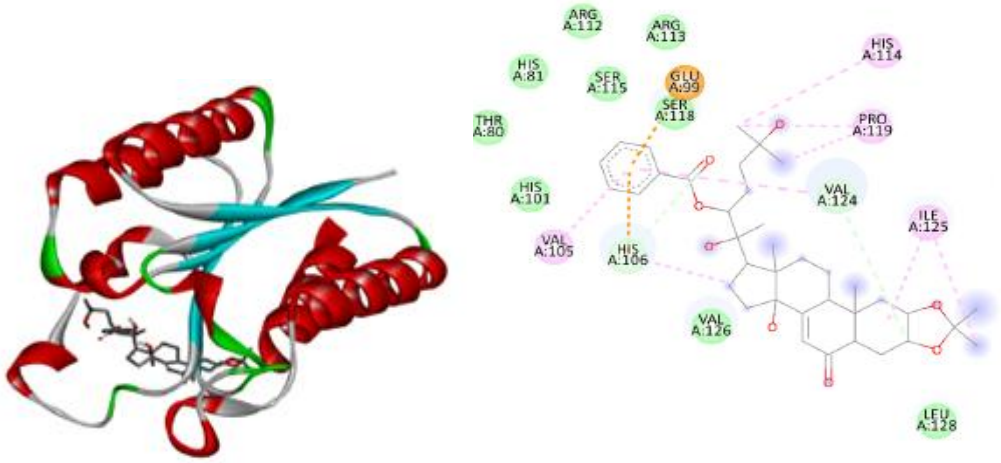

Fig. 2. The best binding mode of the Compound-9 interacting with 3-DHQ synthase in the centre of the enzyme. Green dotted line represents the hydrogen bonds and purple/pink represents alkyl/pi-alkyl bonds interactions, respectively.

C. ADMET Analysis of Ligand(s)

The druglikeness of 11 phytocompounds of was listed in Table I. The compounds 3,5,6,8,10 and 11 possessed molecular weight (MW) <500 Da. However, except the compounds 1,6 and 10,all the compounds obeyed the hydrogen bonding criteria of Lipinski's rule. Moreover, the lipophilicity(logP) and topological polar surface area(TPSA) values are crucial for forecasting oral liability of drug molecules. In this study, logP values of the most of the compounds ranged from 0.10 to 5which were in good agreement for an ideal drug to penetrate the biomembrane.The ADMET analyses of phytocompounds were listed shown in Table III.

TABLEIII. PHARMACOKINETICS PROPERTIES OF THE NATURAL COMPOUNDS ACCORDING TO THE LIPINSKI'S RULE ANALYSIS.

Sl. No.	Compoundname	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 IV. ADMETPROPERTIESOFTHENATURALCOMPOUNDS.

Sl.No.	Compound name	miLogP	TPSA[a]	natoms[b]	nrotB[c]	nVio[d]
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

a. TPSA, Topological Polar Surface Area
b. natoms, number of atoms
c. nrotB number of rotatable bonds
d. nVio, number of Violations

D. Boiled Egg Analysis

The boiled egg analysis evaluates the gastrointestinal absorption (HIA) and blood–brain barrier (BBB)penetration in function of the position of the molecules in the WLOGP versus TPSA referential.The white region represented the highest probability of passive GIA and yellow portion depicted the highest probability of BBB penetration. The points were 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 suggested that these two molecules could neither be absorbed by the gastrointestinal tract nor they could cross the BBB (outside the egg), but the compounds were actively effluxed by P-gp as represented by PGP^+(Fig. 4).

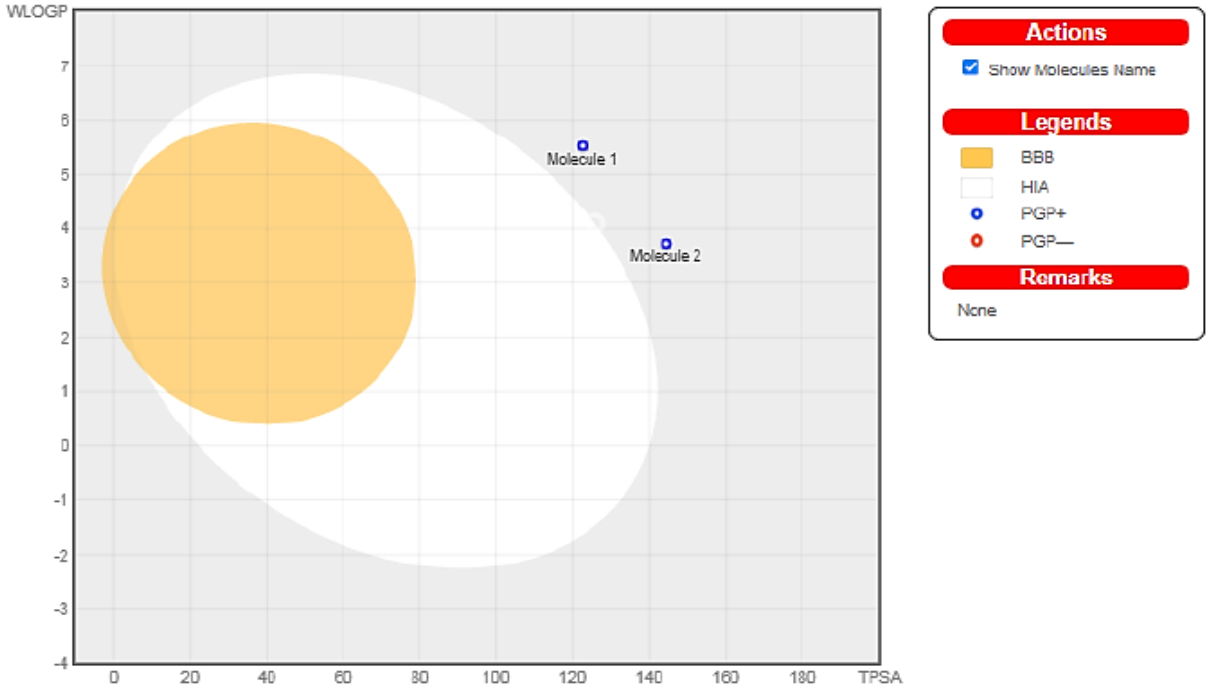

Fig. 4. Boiled egg analysisofthe two selectedligands 2,3,14,20,25-pentahydroxy-6-oxocholest-7-en-22-yl benzoate(compound-2) andecdysterone 2,3-acetonide 22-O-benzoate(compound-9)

*E. **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 by indicating abioactivity score. For instance, the molecules having the scores more than 0.00 are most likely to exhibit considerable biological activity. If the values range from 0 to 0.50, the compounds are likely to be moderately active and if the score is less than 0.50, then the compounds are inactive. Molinspirationtool was used to predict bioactivity score of the phytocompounds of this study against the human receptors such as G-protein coupled receptors (GPCRs), ion channels, kinases, nuclearreceptors, proteases and enzymes. Results were tabulated in Table IV and it was observed that except the compounds 1,6,8, and 9, the other compounds were found to be active against GPCRligands.

*F. **Oral Toxicity Prediction***

The prediction of compound toxicities is an important part of rational drug design. For predicting the toxicities of the small molecules under this study, ProTox-II– a virtual lab was used. According to the online tool, the toxic doses are often represented by LD50 values in mg/kg body weight. Moreover, LD50 is the median lethal dose which means that the dose required to kill 50% of the test subjects upon exposure to a chemical compound. The toxicity classes are defined as Class1 and Class 2 to be “Fatal”with LD50<5 and 5<LD50<50, respectively, if swallowed. The Class3 represents as “Toxic”with 50<LD50<300, whereas the Class4 implies “Harmful”with 300<LD50<2000, if swallowed. Likewise, Class5 denotes “May be harmful” with 2000<LD50<5000if swallowed and Class6 means Non toxic with LD50>5000.The oral toxicity of the phytocompounds of this study was listed in Table VI. LD50 of compound 1 is 134, which belongs to toxic class 3, that is, toxic. LD50 of compounds 3, compound5, compound6, compound 8, compound 9, ranges between 300–2000, belong to class 4 and are harmful. LD50 of compound 2, compound 4 and compound 7 ranges between 2000–5000, toxic class 5 and they may be harmful. LD50 of compound 10 and compound 11 is 9000, belong to the class 6 and they are non-toxic.

TABLE V.BIOACTIVITY SCORE OF THE NATURAL COMPOUNDS.

Sl. No.	Compound name	GPCR L[e]	Ion CM[f]	Kinase INH[g]	Nuclear RL[h]	Protease INH[i]	EnzymeI NH[j]
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

e. GPCR L, G-Protein Coupled Receptor Ligand

f. Ion CM, Ion channel Modulator

g. Nuclear RL, Nuclear receptor ligand

h. Kinase INB, Kinase inhibitor

i. Protease INH, Protease inhibitor

j. Enzyme INH, Enzyme inhibitor

TABLE VI. ORALTOXICITYPREDICTIONOFTHENATURALCOMPOUNDS.

Sl.No.	Compoundname	LD50(mg/kg)[k]	Toxic. Class (1-6)[l]	Avg. SM[m]	Pred. AC (%)[n]
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-oxocholes t-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-trimethy l-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

k. LigandLD50, Lethal dose 50%

l. Toxic, Class- toxicity class

m. ligandAvg. SM, Average similarity

n. Pred. AC,Prediction accuracy

Evaluation of binding affinities of the selected phytocompounds was carried out against the target enzymes3-DHQ synthase and 3-DHQ dehydratase of *M. tuberculosis* using AutoDock Vina. Further, analyses of the protein–ligand complexes were carried out using PyMOL to visualize the proper binding of the ligands with the receptors. The molecules which were bound to the surface area and having five hydrogen bonds with the receptor were discarded. Furthermore, 11 most favourable docked poses between the ligands and receptors were analyzed by SwissADME Tox to evaluate pharmacokinetics, druglikeness properties and medicinal chemistry features of these small molecules. The molecules were then screened through theLipinski's filter for the assessment of bioavailability of the compounds. Boiled Egg Analysis was performed topredict the passive gastrointestinal absorption and BBB permeability of small molecules useful fordrug discovery and development. Thus, from the present analysis, the molecules 2,3,14,20,25-pentahydroxy-6-oxocholest-7-en-22-yl benzoate (compound-2) and ecdysterone 2,3-acetonide 22-O-benzoate (compound-9) can be considered as potent druglike molecules for the treatment of TB. Further validation of the compounds can be performed on animal models as pre-clinical trial which can project these molecules for human clinical trials to make them successful and eventually marketed.

IV. CONCLUSION

This study deciphered thatcompound-2 and compound-9 exhibited the highest binding affinities with the two enzymes, 3-DHQsynthase and 3-DHQdehydratase of Mycobacterial Shikimate pathway, respectively. It was also evaluated that these two compounds could effectively bind to the active sites of these enzymes there by inhibiting the functions of these enzymes.Thus, the Shikimate pathway would be interrupted and the bacterium would not produce aromatic amino acids for its survival.The enzymes of Shikimate pathway thus serve as novel drug target for the drug discovery process for treatment of TB.Besides, the abovementioned phytocompounds,ecdysterone 2,3-acetonide 22-O-benzoate and2,3,14,20,25-pentahydroxy-6-oxocholest-7-en-22-yl benzoate exhibited promising pharmacokinetic properties,obeyed Lipinski's rule of 5, with only one violation and also conferred less toxicity (toxic class 4 and 5). Therefore, these molecules can be considered as potent drugmoleculesin TB treatment.

V. DATA AVAILABILITY

The datasets generatedandanalysedduring the current study areavailable in the followingdatabases:
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 ofphytocompoundsare availablein **Molinspiration**:
https://www.molinspiration.com/docu/miscreen/druglikeness.html
The pharmacokineticdata ofphytocompounds areavailable in**Swissadme:**
https://doi.org/10.1038/srep42717

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