Research Paper

Anti-Tuberculosis Activity in *Punica granatum: In silico* Validation and Identification of Lead Molecules

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Discovery of novel drug against tuberculosis is inevitable since resistant bacterial strains are evolved against existing drugs and rapidly active single drug in short period is necessary for effective treatment. When compared to synthetic drugs, natural drugs particularly phytochemicals induce less side effects and long term stability. In Indian traditional medicine, several plant species have been used for treating tuberculosis and each plant species contains a plethora of phytochemicals. The efficacy of such treatment system and identification of the phytochemical with drug activity from plants has been seldom investigated scientifically. In this backdrop, the authors have validated the efficacy of anti-tuberculosis activity and identified lead molecule from a widely used plant species against several disease including tuberculosis, viz *Punica granatum* L. The four promising target proteins viz mycolyltransferase antigen protein 85C involved in cord factor synthesis, filamentous temperature sensitive protein Z involved in bacterial cell division, pantothenate kinase involved in co-enzyme A pathway and decaprenylphosphoryl β-D-ribofuranose-2 epimerase involved in the synthesis of virulent factor arabinan were docked with 243 phytochemicals derived from *Punica granatum*. The docked molecules having binding energy ≤-6 kcal/mol were considered as active/hit molecules. The docked results showed that out of 243 phytochemicals, 126 have inhibitory activity on all selected target proteins. Further docking study using Glide and absorption, distribution, metabolism, excretion and toxicity studies revealed that the compound derived from the flowers, pomegranate can be recommended as the best lead compound against tuberculosis.

Key words: *Mycobacterium tuberculosis*, multi drug resistance, extensively drug resistance, totally drug resistance, *Punica granatum*, molecular docking

Tuberculosis (TB) remains as one of the top killer diseases worldwide from a single infectious agent, *Mycobacterium tuberculosis*. Globally 10 million people infected with TB and 1.5 million died in 2018[1]. Although several drugs are available to treat against TB, the emergence of resistance strains of *M. tuberculosis* such as MDR (Multi Drug Resistance) and TDR (Totally Drug Resistance Strains) is a major threat. Besides, TB patients with HIV are more vulnerable. The forgoing problems necessitate novel drug against TB. Generally, the drug molecules derived from natural sources especially from plants have long-term stability, effectiveness and find more potential therapeutic uses with no or less side effects to the human body since those molecules are derived within the living system through repeated interactions with other bio molecules and modifications through a long term evolutionary process. Moreover, the traditional herbal treatment systems serve as real indicator to make the drug discovery process easy and reduce the initial investment. In these backdrops, search on novel drugs from plants are considered as the best source.

The Greek physician Hippocrates said that "Let food is thy medicine and medicine is thy food". In natural food materials, nutrients and medicines are enriched in a balanced form and consumption of such food materials can control or prevent ailments in the human body. *Punica granatum* (common name pomegranate), a widely used fruit, has been used as a polychrest against many ailments in traditional

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medicine in all over the world since time immemorial. It's nutritional and medicinal values have been well reviewed[2-4]. The research reports demonstrated its anti-cancer, anti-inflammatory, anti-atherogenic, anti-diabetes, hepatoprotective and antioxidant activity[5-7]. It's antimicrobial activity has also been reported[8,9]. The present investigation was aimed to validate the efficacy of anti-tuberculosis activity of *P. granatum* and identification of lead molecules through *in silico* method.

MATERIALS AND METHODS

Selection and preparation of target protein

The crystal structures of the target proteins viz. mycolyl transferase antigen 85C protein (Ag85C/FbpC, PDB ID: 1DQY), filamentous temperature sensitive protein Z (FtsZ, PDB ID: 2Q1X), pantothenate kinase (PanK, PDB ID: 4BFS) and decaprenyl phosphoryl β-D-ribose 2'-epimerase (DprE1, PDB ID: 4FDO) were retrieved from Protein Data Bank (PDB). The foregoing target proteins were analyzed using a web servers, VADAR (Volume, Area, Dihedral Angle Reporter) [10] and ProtParam[11]. Active sites of the proteins were determined using Computed Atlas of Surface Topography of proteins (CASTp) server and PDBSum server[12].

Selection and preparation of ligands

A total of 243 phytochemicals reported from *Punica granatum* were selected as ligand molecules. Of these, information on 219 phytochemicals was collected from literature and databases like PubChem, Foodb, ChEBI and ZINC. The remaining 24 phytochemicals were identified from fruit extract through Gas chromatography-mass spectrometry (GC-MS) analysis (fig. 1). The structure-data file (SDF) or MOL formats of the phytochemical structures were retrieved from open access databases and converted to PDB format using Open Babel version 2.4.1[13]. The Open Babel represents chemical toolbox which is designed to search, convert, analyze or store data from molecular modeling, chemistry or related areas.

Preparation of fruit extract and GC-MS analysis

Fresh pomegranate fruits (2 kg) collected from farmers' field in Wayanad district of Kerala, India were cleaned well first in running tap water followed by distilled water thrice. Then water content was removed from the surface of the fruit using blotting paper. The fruits were cut open and removed the peels. The arils along with

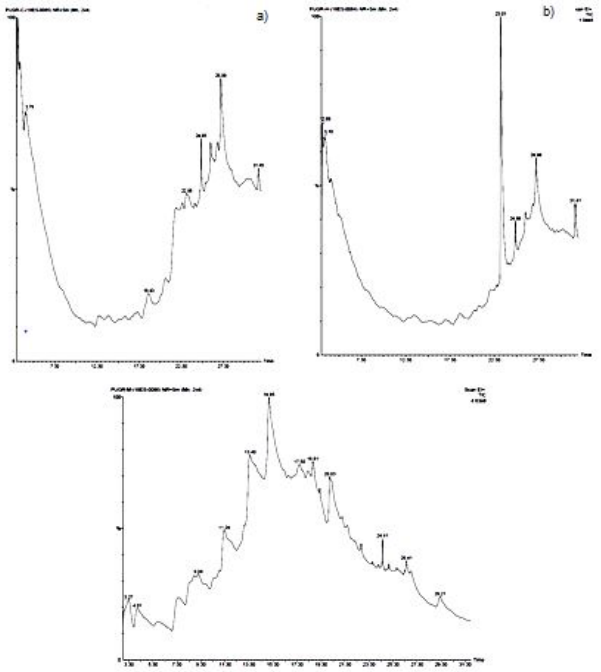

Fig. 1: GC-MS chromatograms of a) Chloroform extract; b) Hexane extract and c) Methanol extract

the seeds were weighed (1.300 kg) and oven dried at a temperature 55° for 3 d till the weight becomes constant. The dried sample was powdered and stored in an air tight glass container. 25 g samples were taken and extracted with 250 ml each of the solvents such as hexane, chloroform and methanol using soxhlet apparatus for 6 h according to their boiling point. Further, the extracts were concentrated in a rotary evaporator and the dried samples were stored in air tight bottles.

The GC-mass analysis was performed using Perkin Elmer-Clarus 680 which employs a fused silica column, packed with Elite-5MS (5 % biphenyl 95 % dimethylpolysiloxane, 30 m×0.25 mm ID×250 μm df) and the components were separated using helium as carrier gas at a constant flow of 1 ml/min. 1 μL of extract sample was injected into the instrument. The injector temperature was set at 260° during the chromatographic run. Initial temperature of the oven was set at 600° for 2 min followed by ramp to 300° at the rate of 10° min^{-1}; and at 300°, where it was held for 6 min. The mass detector conditions include a transfer line temperature of 240°, ion source temperature 240° and ionization mode electron impact at 70 eV, a scan time 0.2 s and a scan interval of 0.1 s. The fragments from 40 to 600 Da were detected using this technique. The spectrums of the components were compared with the database, TurboMass ver 5.4.2. spectrum of known components stored in the GC-MS National Institute of Standards (NIST) (2008) library.

Molecular Docking

Molecular docking procedure was carried out using an open access molecular docking program AutoDock Vina[14] and is compatible with Molecular Graphics Laboratory (MGL) Tools. Vina uses a hybrid scoring function and an iterated local optimization algorithm-Broyden-Fletcher-Goldfarb-Shanno algorithm. Prior to virtual screening, the PDB format of the macromolecules and phytochemicals were converted to PDBQT using MGL Tools wherein the Gasteiger partial charges and polar hydrogens were added. Like Autodock, Vina also require the specification of 3D search space centered on the protein's active site in the form of grid points and grid box in different dimensions along with spacing. Vina allows the user to make the side chains of the target protein flexible as in Autodock. Hence both rigid and flexible part of the target proteins were saved separately. In Vina, spacing is always set to 1 Å. In order to execute the screening process, a configuration file in .txt format has to be setup wherein we should specify all the above mentioned parameters. Virtual screening of 243 phytochemicals was performed with the help of bash scripting.

Post virtual screening analysis

The docked complexes of the protein together with the top hit phytochemicals were analyzed using Hbind tool[15]. Hbind rigorously calculate and define intermolecular H-bonds by donor/acceptor chemistry and geometric constraint. The criteria for finding H-bonds is well described[16,17].

Analysis of molecular properties and ADMET properties

The hit molecules were subjected for molecular property calculation using OSIRIS Data Warrior. OSIRIS Data Warrior is a free cheminformatics program for data visualization and analysis[18]. Pharmacokinetics refers to the movements of drug into, through and out of the body, during the process of absorption, bioavailability, distribution, metabolism and excretion. The absorption, distribution, metabolism, excretion and toxicity (ADMET) property analysis was performed using the tool pkCSM, a platform for the analysis and optimization of pharmacokinetic and toxicity properties. Further, Extra Precision Glide[19] docking and QikProp analysis of the top prioritized hit molecules were also performed to finalize the best lead compound[20]. Qikprop analysis provides accurate results in predicting properties for molecules with novel scaffolds as for analogs of well-known drugs.

RESULTS AND DISCUSSION

Most of the screening studies for anti-TB agents considered only small homogenous molecules against only one target[21], while in the present study a total of 243 phytochemicals derived from *Punica granatum* were screened against four target proteins namely, Ag85C, FtsZ, PanK and DprE1.

The antigen 85C represents protein C in Ag85 antigenic protein complex containing Ag85A, Ag85B and Ag85C which catalyze the synthesis of trehalose 6, 6'-dimycolate or cord factor which is considered to be one of the major toxic components of cell wall and is present only in virulent strains of *Mycobacterium tuberculosis*. The protein, Ag85C is also known as FbpC as it has high affinity with fibronectin, which facilitates the attachment of *M. tuberculosis* to murine alveolar macrophages[22] and also it contributes to the low permeability of the cell wall[23]. The protein consists of 39 % helices, 21 % beta sheets, 38 % coils and 19 % beta turns with mean hydrogen bond distance of 2.2 Å and mean hydrogen bond energy -1.8 kJ/mol and about 80 % of the residues form hydrogen bonds. The theoretical isoelectric point and average hydropathicity values were 4.99 and -0.417 respectively. The X-ray crystal structure of protein possess only a single chain with two unique ligands namely (4R)-2-methylpentane-2,4-diol and diethyl phosphonate without H-bonds. The catalytic residues of the protein include Ser124, Glu228 and His260.

FtsZ, a bacterial tubulin homolog plays a critical role in cell division by the formation of Z ring and recruiting other proteins for septum formation leading to a new cell wall between dividing cells which is conserved in most prokaryotes and several organelles[24,25]. These proteins consists of 41 % helices, 29 % beta sheets, 28 % coils and 10 % with a mean H-bond distance of 2.2 Å and mean H-bond energy -1.8 kj/mol. The theoretical isoelectric point and average hydropathicity appears to be 4.50 and 0.080 respectively. The crystal structure holds citric acid as the ligand molecule having five H-bonds with four residues including Gly105, Thr106, Gly18 and Gly19.

PanK, pantothenase kinase catalyses the phosphorylation of pantothenate, the first committed and rate limiting step in the biosynthesis of coenzyme A, which is an essential acyl group carrier indispensable for respiration

and lipid metabolism. Based on the differences in biochemical and structural characteristics, three types-PanK type I, II and III were present, among which PanK type I is essential for the growth of bacteria *in vitro* and *in vivo*[26]. The structure contains 43 % helices, 23 % beta sheets, 33 % coils and 25 % turns with a mean H-bond distance and mean H-bond energy 2.2Å and -1.7 kj/mol respectively. The theoretical isoelectric point of the protein was 7.47 and average hydropathicity -0.249. The crystal structure consist of N-[1-(5-{[2-(4-fluorophenoxy) ethyl]sulfanyl}-4-methyl-4h-1,2,4-triazol-3-yl) ethyl]-2-(trigluoro-methyl) benzamide that forms H-bonds with Tyr235 and Asn277 respectively.

DprE1, Decaprenylphosphoryl-β-D-ribose oxidase belongs to DprE1-DprE2 complex, catalyses the formation of decaprenyl-phospho-arabinose (DPA), which is the sole arabinosyl donor of mycobacterium cell wall through epimerization reaction[27]. DprE1 is a flavin adenine dinucleotide (FAD) dependent enzyme. The enzyme DprE1 first epimerizes decaprenylphosphoryl-β-D-ribofuranose (DPR) to a keto intermediate decaprenylphosphoryl-D-2-keto-erythro-pentofuranose (DPX) which is then catalysed by DprE2 to decaprenylphosphoryl-β-D-arabinofuranose (DPA). The structures consists of 28 % helices, 33 % beta sheets, 38 % coil, 20 % turns with mean H-bond distance and mean H-bond energy of 2.2 Å and -1.7 kj/mol respectively. The protein possesses a theoretical isoelectric point of 7.86 and average hydropathicity of -0.147. The 3-nitro-N-[(1R)-1-phenylethyl]-5-(trifluoromethyl) benzamide (His132 and Asn385-hydrogen bonding residues) and flavin-adenine dinucleotide (Gly117, His132, Tyr415, Gly125, Gly55, Gly57, Asn63, Ala53, Ile184, Thr122, Ser59, Leu56-hydrogen bonding residues) were the unique ligands present in the crystal structure. PDB format of the target proteins were converted to PDBQT using AutoDock tools.

Considering the ligands molecules, out of 243 phytochemicals selected for the study, the details of 219 molecules were procured from various literature and open access databases and remaining 24 phytochemicals were identified from the fruit of *P. granatum* through GC-MS analysis, of which oleic acid, beta carotene and methyl 3-bromo-1-adamentacetate were already reported. Lists of compounds identified through GC-MS analysis were depicted in Table 1.

The analysis of docked results between the targets

TABLE 1: COMPOUNDS IDENTIFIED FROM THE FRUIT OF *Punica granatum* THROUGH GC-MS ANALYSIS

Name of the extract	RT	Name of the compound	Peak area %
Chloroform	21.026	Dodecane, 1-chloro-	6.004
	21.446	14-heptadecenal	9.333
	21.551	Tetradecane, 1-chloro-	10.239
	21.736	Oleic acid	2.795
	22.526	10,12-tricosadiynoic acid	12.093
	22.916	Beta Carotene	2.864
	23.587	Pentanoic acid, 3-[(adamantan-1-ylmethyl)carbamoyl]-4-phenyl	2.132
	24.637	1-methylene-2b-hydroxymethyl-3,3-dimethyl-4b-(3-methylbut-2-enyl)-cyclohexane	8.594
	25.177	Methyl 3-bromo-1-adamantaneacetate	2.704
	26.973	Pregn-5-en-20-one, 3-(acetyloxy)-16-bromo-, (3.beta.,16.alpha.)-	30.044
Hexane	3.209	Hexane, 2-chloro	3.110
	3.259	Pentafluoropropionic acid, hexyl ester	3.526
	3.379	1,1-dimethylethylamine, n-methoxycarbonyloxy	4.364
	22.997	1,2-benzenedicarboxylic acid, diisooctyl ester	71.247
	24.637	Psi-psi-carotene, 7,7',8,8',11,11',12,12',15,15'-decahydro-	2.945
Methanol	9.111	3-amino-2-oxazolidinone	4.057
	11.162	Cyclohexanone, 3-hydroxy	8.407
	13.408	4h-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl	19.957
	14.948	4-hepten-3-one, 5-methyl	26.949
	14.948	T-butyl cyclopentaneperoxycarboxylate	12.312
	18.605	Alpha.-d-glucopyrnoside, o-.alpha.-d-glucopyranosyl-(1.fwdarw.3)-.beta.-d-fructofuranosyl	10.438
	19.190	Pentanoic acid, 2-(amino_oxy)-	4.492
	20.015	3-deoxy-d-mannoic lactone	12.442
	22.631	1,10-hexadecanediol	0.946

viz Ag85C/FbpC, FtsZ, PanK and DprE1 and phytochemicals from *Punica granatum* through virtual screening revealed that out of 243 phytochemicals screened 126 have inhibitory activity (ΔGbind≤-6 kcal/mol) on all the above targets. In many reports[28,29] minimum free energy level of the active/hit molecules' was considered as ≤-5 kcal/mol, however, as suggested by Shityakov[30] minimum free energy of binding of active/hit molecules was considered here as ≤-6 kcal/mol. The number of active/hit molecules (ΔGbind≤-6 kcal/mol) obtained against each target protein such as Ag85C/FbpC, FtsZ, PanK and DprE1 was 155, 146, 166 and 176 respectively. The top five hit molecules obtained against Ag85C/FbpC based on least binding energy in the order of merit was pomegranatate (-9.5 kcal/mol), icariside D1 (-9.3 kcal/mol), luteolin-3'-o-β-D-glucoside (-9.3 kcal/mol), 1-2-4-tri-o-galloyl-β-gluco-pyranose (-9.1 kcal/mol) and epigallocatechin-3-o-rhamnoside (-9.1 kcal/mol). Similarly, the compounds tercatain (-10.2 kcal/mol), granatin A (-9.4 kcal/mol), 1-2-4-tri-o-galloyl-β-gluco-pyranose (-9.7 kcal/mol), granatin B (-8.8 kcal/mol) and luteolin-3'-o-β-D-glucoside (-8.8 kcal/mol) were the top ranked hit molecules against FtsZ and the compounds lupenone (-11.2 kcal/mol), terminalin (-10.9 kcal/mol), gallocatechin-(4β→8)-catechin (-10.8 kcal/mol), kaempferol-3-o-rhamnoglucoside (-10.8 kcal/mol) and pomegranatate (-10.3 kcal/mol) were the top ranked hits against PanK. L-malic acid (-13.0 kcal/mol), (+)-gallocatechin-(4α→8)-(+)-catechin (-12.5 kcal/mol), serraten (-12.0 kcal/mol), 1,2,4,6-tetra-o-galloyl-β-D-glucose(-11.8 kcal/mol), procyanidin B1 (-11.8 kcal/mol) and pomegranatate (-11.6 kcal/mol) were identified as top ranked hits against DprE1. The docked results of phytochemicals that can inhibit all the four target proteins were depicted in Table 2.

TABLE 2: COMPOUNDS QUALIFIED AS HIT MOLECULES (BINDING ENERGY ≤-6 kcal/mol) FROM *Punica granatum* AGAINST ALL THE SELECTED TARGETS SUCH AS AG85C, DPRE1, FTSZ AND PANK.

Punica granatum					
		Targets			
Sl. No.	Phytomolecule name	Ag85C (kcal/mol)	DprE1 (kcal/mol)	FtsZ (kcal/mol)	PanK (kcal/mol)
	(2E,6E)-9-(3,3'-Dimethyl-2-oxiranyl)-3-7-dimethyl-2-6-nonadienyl phenyl sulfide	-7.7	-8.6	-6.3	-7.2
	1,2,3,4,6-penta-o-galloyl- ß d glucose	-7.1	-10.9	-7.7	-7.8
	1,2,3-tri-o-galloyl- ß -4c-1-glucopyranose	-8.2	-11.1	-7.9	-9.5
	1,2,3-tri-o-galloyl- ß -4c1-glucose	-7.0	-11.2	-7.5	-9.1
	1,2,3-tri-o-galloyl- ß -d-glucose	-7.9	-11.0	-8.1	-9.0
	1,2,4,6-tetra-o-galloyl-ß-D-glucose	-7.1	-11.8	-7.9	-7.6
	1,2,4 tri-o-galloyl-ß-glucopyranose	-9.1	-11.3	-8.8	-9.7
	2,3-(S)-Hexahydroxydiphenoyl-D-glucose	-7.7	-10.5	-7.3	-8.8
	2-hydroxycyclopentadecanone	-6.9	-8.2	-6.5	-7.9
	3,3,4-tri-O-Methylellagicacid	-7.4	-8.7	-7.1	-8.0
	3-3'-di-O-methylellagic acid	-7.8	-9.2	-7.5	-8.4
	3,4,8,9,10-pentahydroxydibenzo(b-d)-pyran-6-one	-7.6	-8.8	-7.4	-7.5
	3-o-methylellagic acid	-7.6	-9.4	-7.3	-8.0
	17alpha estradiol	-7.2	-8.7	-7.0	-8.4
	17- ß estradiol	-7.2	-8.7	-7.0	-8.4
	17- ß estriol	-7.1	-9.0	-7.2	-8.7
	Alpha tocopherol	-8.1	-8.8	-6.4	-8.5
	Amurensin	-7.7	-10.7	-8.3	-8.4
	Antirrhinin	-8.5	-10.8	-8.5	-9.0
	Apigenin	-8.7	-9.5	-7.1	-7.7
	Apigenin 4'-o-beta-glucopyranoside	-8.1	-10.0	-7.1	-8.3
	Apigenin 7-O-glucoside	-8.7	-10.1	-7.5	-8.6
	Astragalin	-8.1	-10.5	-7.6	-7.9
	ß -Eleostearic acid	-6.6	-6.8	-6.1	-6.7
	ß -sitosterol	-6.4	-9.7	-7.0	-8.4

Betulic acid	-6.6	-9.2	-7.2	-9.9
Betulinic acid	-6.4	-9.3	-7.2	-9.9
Brevifolin-carboxylic-acid-10-monosulphate	-7.1	-8.6	-7.9	-7.1
Brevifolin-carboxylicacid	-7.1	-8.5	-7.9	-7.6
Caffeic acid	-6.4	-6.7	-6.1	-6.0
Callistephin	-8.1	-9.8	-7.7	-8.4
Campesterol	-6.0	-10.0	-6.7	-8.9
Catechin	-8.6	-9.0	-7.2	-7.8
catechin-(4ß→8)-gallo-catechin	-7.6	-12.5	-7.7	-7.9
Chlorogenic acid	-7.7	-9.2	-6.7	-7.9
Chrysanthemin	-8.5	-10.1	-8.1	-8.9
Conidendrin	-7.7	-9.3	-8.3	-8.7
Corilagin	-8.5	-9.9	-7.9	-9.8
Coumestrol	-8.0	-9.2	-6.6	-8.2
Coutaric acid	-7.4	-8.1	-7.0	-6.4
Cyanidin	-8.6	-9.0	-7.3	-8.1
Cyanidin-3-5-diglucoside	-7.1	-10.7	-7.7	-6.0
Cyanidin-3-glucoside	-8.3	-10.3	-7.7	-8.3
Cyanidin-3-rutinoside	-8.2	-10.8	-8.5	-9.1
Cyanin	-7.1	-10.6	-7.3	-9.0
Cycloartenol acetate	-7.0	-10.2	-6.1	-9.5
Cynaroside	-7.9	-10.3	-8.6	-9.4
Daidzein	-7.6	-8.8	-6.7	-7.5
Daucosterol	-6.8	-9.8	-8.0	-8.2
Delphinidin	-8.2	-9.2	-7.4	-8.0
Delphinidin-3-5-di-o-glucoside	-7.8	-10.9	-7.3	-9.2
Ellagic acid	-8.0	-9.6	-7.6	-8.1
Epicatechin gallate	-8.9	-10.9	-7.9	-8.6
Epicatechin	-8.5	-8.8	-7.3	-7.8
Epigallocatechin-3-o-gallate	-9.1	-11.0	-8.1	-8.2
Eschweilenol C	-8.8	-11.2	-8.7	-9.6
Esterone	-7.6	-8.8	-7.4	-8.6
Estradiol	-7.2	-8.7	-7.0	-8.4
Estriol	-7.4	-9.0	-6.8	-8.4
Ethyl-brevifolin-carboxylate	-7.5	-8.9	-7.6	-7.7
Ferulic acid	-6.4	-7.1	-6.1	-6.2
Flavan-3-ol	-8.0	-8.9	-6.3	-7.6
Flavogallol	-8.4	-10.3	-8.1	-10.1
Gallic acid 3-O-Beta-D-(6'-O-galloyl)-glucopyranoside	-8.4	-9.8	-7.7	-8.2
Gallocatechin (4 ß →8)-catechin	-6.7	-11.6	-8.2	-10.8
Gamma Sitosterol	-6.7	-10.1	-7.1	-8.9
Gemin D	-6.7	-7.3	-8.2	-8.6
Genistein	-7.8	-9.2	-6.8	-7.6
Hirsutrin	-8.9	-11.1	-8.3	-9.0
Hovetrichoside C	-8.0	-10.6	-6.9	-8.6
Icariside D1	-9.3	-9.6	-7.3	-8.5
Isocorilagin	-7.7	-10.0	-8.2	-10.0
Isohydroxymatairesinol	-8.0	-9.3	-6.7	-7.8

Isolariciresinol	-6.9	-8.6	-6.9	-8.0
Isoquercetrin	-8.9	-10.6	-8.1	-8.9
Kaempferol	-8.7	-9.3	-7.2	-7.7
Kaempferol-3-o-glycoside	-8.6	-9.3	-8.0	-8.9
Kaempferol-3-o-rhamnoglucoside	-8.6	-10.2	-8.7	-10.8
Lupenone	-7.0	-11.3	-8.5	-11.2
Luteolin 3'-o-ß-xylopyranoside	-8.8	-11.0	-7.3	-8.8
Luteolin	-8.9	-9.5	-7.2	-8.0
luteolin-3'-o-ß-D-glucoside	-9.3	-10.9	-8.8	-8.8
Luteolin-4'-o- ß -glucopyranoside	-8.1	-10.5	-8.2	-9.0
Matairesinol	-7.4	-9.5	-7.4	-8.0
Medioresinol	-7.5	-9.4	-7.3	-8.7
Melatonin	-7.3	-7.6	-6.6	-6.8
Mirtillin	-8.3	-10.5	-7.8	-8.7
Myricetin	-8.6	-9.5	-7.4	-8.2
Naringin	-8.2	-10.3	-7.1	-9.2
Neochlorogenic acid	-8.0	-9.1	-7.2	-7.7
Oleanolic acid	-6.0	-11.0	-7.4	-6.8
Oxandrolone	-7.4	-9.6	-7.1	-8.8
Pelargonidin	-8.4	-8.9	-7.2	-7.4
Phellatin	-8.2	-10.3	-7.5	-8.6
Phenethyl rutinoside	-7.7	-9.8	-7.3	-8.0
Phloretin	-7.6	-8.5	-6.8	-6.9
Phlorizin	-8.0	-10.2	-8.2	-8.6
Phyllanthusiin E	-7.7	-9.9	-7.9	-7.7
Phylligenin	-7.7	-9.5	-7.1	-8.1
Pinocembrin	-8.6	-9.3	-7.3	-8.1
Pinoresinol	-8.2	-9.1	-6.2	-8.3
Pomegralignan	-8.3	-9.8	-8.6	-8.8
Pomegranatate	-9.5	-11.6	-7.7	-10.3
Procyanidin B1	-7.8	-11.8	-7.7	-10.3
Procyanidin B3	-7.5	-11.3	-8.2	-9.0
Procyanidin-B2	-7.8	-11.6	-8.6	-10.1
Prodelphinidin B	-6.9	-9.2	-8.6	-6.6
Prodelphinidin C	-7.0	-9.7	-8.3	-9.6
Punicafolin	-6.1	-10.2	-7.4	-6.3
Punicanolic acid	-6.1	-10.3	-6.8	-7.8
Punigluconin	-8.3	-10.5	-8.6	-9.7
Quercetin 3-O-rhamnoside	-9.0	-10.8	-8.1	-9.1
Quercetin	-8.7	-9.5	-7.5	-7.9
Quercetin-3-o-rutinoside	-8.3	-11.6	-7.8	-8.8
Quercimeritrin	-8.3	-10.5	-6.4	-8.7
Rutin	-8.3	-11.0	-7.8	-8.9
Secoisolariciresinol	-7.9	-8.3	-6.9	-7.2
Serraten	-6.1	-12.0	-7.5	-8.6
Stigmasterol	-7.6	-10.1	-7.6	-9.4
Strictinin	-8.0	-11.0	-8.1	-10.0
Terminalin	-8.9	-6.5	-7.8	-10.9

Tricetin	-8.7	-9.3	-7.3	-8.1
Tricin	-8.5	-9.3	-7.0	-8.0
Ursolic acid	-6.9	-11.1	-6.6	-8.4
Valoneic acid dilactone	-8.3	-11.2	-8.7	-9.6
1-2-benzenedicarboxylic acid -mono(2-ethylhexyl) ester	-6.6	-7.2	-6.3	-6.7

To observe the molecular interaction and to avoid the selection of false positive ranked molecules as lead, the docked structures were analyzed using the Hbind tool which can precisely demonstrate H-bond interaction with residues, its accurate distance and angle and donor-acceptor criterion by avoiding false scoring. Raschka *et al.*[15] reported that generally natural compounds avoid the presence of chemical groups bearing both H-bond donor and acceptor capacity in the binding site of protein or ligand lead to non-selective ligand binding and false positive scoring/ranking. But proteins selectively donate H-bonds (instead of donate and accept) to small molecules avoid false positive scoring/ranking and better binding. The Hbind tool follows the analysis of molecular interaction based on the forgoing principle. The analysis of the docked structures using Hbind tool revealed that among the selected top ranked hit molecules against Ag85C except epigallocatechin-3-o-gallate and luteolin-3-o-β-glucoside all others gave true positive scoring while against FtsZ only two compounds such as tercatain and 1,2,4-tri-o-galloyl-β-gluco-pyranose showed true positive scoring. In the case of PanK, except the compound terminalin, all other top ranked hit molecules showed positive scoring and against DprE1 only two compounds *viz* catechin-(4β→8)-gallo-catechin and 1,2,4,6-tetra-o-galloyl-β-D-glucose showed positive scoring.

It was noted that although 126 phytochemicals have inhibitory effect on all the selected targets none of the lead molecule identified from top ranked five hit molecules through the forgoing process could inhibit all targets. Therefore, to identify common lead molecule against all the four selected targets the phytochemicals with free energy of binding ≤-8.6 kcal/mol against Ag85C, <-7.7 kcal/mol against FtsZ, <-9.7 kcal/mol against PanK and ≤-10.2 kca/mol against DprE1 were further analysed using Hbind tool since the number of active molecules with free energy of binding level depend on each target protein (Table 3). Analysis of molecular interaction revealed that pomegranate showed hydrogen bonding with one of the catalytic triad, Ser124 whereas the compound showed hydrogen bonding with His132, the residue in which the natural inhibitor bound with the protein, DprE1. In case of 1,2,4-tri-o-galloyl-β-gluco-pyranose, two hydrogen bonds were formed with the critical residue, Tyr235 of PanK protein whereas with DprE1 protein, one hydrogen formed with critical residue, His 132. Kaempferol-3-o-rhamnoglucoside showed interaction with critical residue, Tyr235 with PanK protein only. Thus the compounds pomegranate, kaempferol-3-o-rhamnoglucoside and 1,2,4-tri-o-galloyl-β-gluco-pyranose could inhibit all the selected target proteins.

True hit molecules against each target and the three common hit molecules identified against all the four targets were subjected to molecular property analysis using the tool OSIRIS property explorer which indicated that the compound tercatain showed all kind of tested toxicity such as tumerogenic, irritant, mutagenic and reproductive effect (Table 4).

The ADMET analysis using the tool pkCSM (Table 5 & Table 6) revealed that the phytochemical, pomegranatate can be considered as the best lead against transferase antigen 85C protein and pantothenate kinase protein whereas 1,2,4-tri-o-galloyl-β-glucopyranose can be considered as the best lead molecule against filamentous temperature-sensitive protein Z and decaprenyl phosphoryl β-D-ribose 2'-epimerase protein. Both phytochemicals act as common lead against all the target proteins. In addition, kaempferol-3-o-rhamnoglucoside present in fruit[31] can be recommended as a common lead since its binding energy level and ADMET properties were on par with other two molecules.

In order to check the accuracy and determining the best lead, the selected three lead molecules were docked with each of the four targets using Glide (Table 7) and ADMET properties of these molecules were analysed using QikProp (Schrödinger, LLC, New York, NY, USA) (Table 8). The comparative analysis of the results revealed that among the three common leads, the compound pomegranatate derived from the flowers[32] could be considered as the best one since it showed least violation in both QikProp and pkCSM analysis. The docked structure of pomogranatate with its target proteins are depicted in fig. 2. Besides, the difference in binding interaction and binding energy level with all the four targets was insignificant.

TABLE 3: HYDROGEN BOND AND HYDROPHOBIC INTERACTION OF THE TOP FIVE LEAD MOLECULES AGAINST SELECTED TARGETS

H bond interactions – *Punica granatum*-1DQY									
Sl. No	Name of the ligand & target protein	Atom No.	Atom Type Ligand	Residue & No.	Atom Type Protein	Distance	Angle	Interaction	Hydro-phobic interact-tions
1	Pomegranatate & Ag85C/FbpC	8	O.3	TRP262	NE1	2.998	150.0	Acceptor - Donor	42
		10	O.3	ARG41	NE	2.944	156.5	Acceptor - Donor	
		10	O.2	ARG41	NH2	3.132	146.2	Acceptor - Donor	
		16	O.2	LEU40	N	3.221	147.1	Acceptor - Donor	
		16	O.2	SER124	OG	3.051	170.3	Acceptor - Donor	
		17	O.2	MET125	N	3.028	120.4	Acceptor - Donor	
		24	O.3	ARG41	NH2	2.806	156.5	Acceptor - Donor	
2	Icariside D1& Ag85C/ FbpC	6	O.2	LEU40	N	3.117	152.1	Acceptor - Donor	74
		11	O.2	ARG41	N	3.085	171.3	Acceptor - Donor	
		14	O.2	TRP262	NE1	3.052	153.8	Acceptor - Donor	
		17	O.3	ASN52	ND2	3.139	127.4	Acceptor - Donor	
		17	O.3	TRP262	NE1	3.097	128.6	Acceptor - Donor	
		20	O.3	ASN52	ND2	2.916	155.0	Acceptor - Donor	
		21	O.2	ARG41	NH2	3.069	122.0	Acceptor - Donor	
3	1,2,4-tri-o-galloyl-ß-gluco-pyranose & Ag85C/FbpC	16	O.3	ASN52	ND2	3.233	139.0	Acceptor - Donor	67
		18	O.3	ASN52	ND2	3.193	131.8	Acceptor - Donor	
		20	O.3	ARG41	NE	2.591	172.7	Acceptor - Donor	
		20	O.3	ARG41	NH2	3.255	131.9	Acceptor - Donor	
		34	O.3	ARG41	NH2	3.232	138.6	Acceptor - Donor	
4	Apigenin & Ag85C/ FbpC	4	O.2	TRP262	NE1	2.961	158.1	Acceptor - Donor	54
		11	O.2	ARG41	N	3.351	158.7	Acceptor - Donor	
		20	O.3	ASN52	ND2	2.892	132.5	Acceptor - Donor	
		20	O.3	TRP262	NE1	3.060	125.5	Acceptor - Donor	
5	Kaempferol 3-o-rhanoglucoside & Ag85C/FbpC	21	O.3	GLN43	N	3.115	126.3	Acceptor - Donor	51
		32	O.2	ARG41	NE	2.856	155.3	Acceptor - Donor	
		32	O.2	ARG41	NH2	3.196	139.4	Acceptor - Donor	
		41	O.2	LEU40	N	2.877	140.1	Acceptor - Donor	
6	Pinocembrin_ Ag85C/ FbpC	1	O.3	ARG41	N	3.368	156.5	Acceptor - Donor	45
		2	O.2	TRP262	NE1	3.162	157.1	Acceptor - Donor	
		18	O.3	ASN52	ND2	2.854	134.7	Acceptor - Donor	
7	1,2,4 tri-o-galloyl-ß-glucopyranose & FtsZ	10	O.2	ARG140	NH2	3.150	139.9	Acceptor - Donor	63
		16	O.3	THR130	OG1	2.828	178.0	Acceptor - Donor	
		16	O.3	ASN163	ND2	2.831	161.4	Acceptor - Donor	
		18	O.3	THR130	OG1	3.123	155.7	Acceptor - Donor	
		33	O.3	ARG140	NE	2.918	163.9	Acceptor - Donor	
		33	O.3	ARG 140	NH2	3.227	145.0	Acceptor - Donor	
8	Kaempferol-3-o rhamnoglucoside & FtsZ	39	O.2	ASN163	ND2	2.801	141.2	Acceptor - Donor	70
		42	O.2	ARG140	NH1	2.756	159.1	Acceptor - Donor	
		42	O.2	ARG140	NH2	3.074	141.2	Acceptor - Donor	
9	Tercatain & FtsZ	16	O.2	ARG140	NE	3.007	122.9	Acceptor - Donor	70
		27	O.2	ARG139	NH1	3.200	143.3	Acceptor - Donor	
		30	O.2	ARG139	NH1	3.014	123.9	Acceptor - Donor	
		42	O.3	ASN22	ND2	3.042	151.1	Acceptor - Donor	
		11	O.3	SER228	OG	3.023	147.3	Doneptor -Doneptor	

		2	O.2	LYS103	NZ	3.225	169.0	Acceptor - Donor	
		7	O.2	TYR153	OH	2.738	144.4	Acceptor - Donor	
		22	O.2	HIS179	NE2	2.942	134.2	Acceptor - Donor	
10	1,2,4-tri-o-galloyl-ß-gluco-pyranose & PanK	28	O.3	TYR235	OH	3.116	135.3	Acceptor - Donor	61
		30	O.3	TYR235	OH	2.935	154.9	Acceptor - Donor	
		33	O.3	THR128	OG1	3.208	172.6	Acceptor - Donor	
		36	O.2	TYR153	OH	3.172	166.6	Acceptor - Donor	
		44	O.3	TYR182	OH	2.715	171.1	Acceptor - Donor	
11	Betulic_acid & PanK	28	O.2	TYR235	OH	2.746	156.7	Acceptor - Donor	97
12	Betulinic_acid & PanK	28	O.2	TYR235	OH	2.738	156.5	Acceptor - Donor	97
13	Cycloartenol acetate & PanK	31	O.3	HIS179	NE2	2.854	156.6	Acceptor - Donor	32
14	Gallocatechin-(4ß→8)-catechin & PanK	10	O.3	TYR153	OH	2.695	145.8	Acceptor - Donor	70
		20	O.3	ARG238	NH1	3.269	153.5	Acceptor - Donor	
15	Kaempferol3-o-rhamnoglucoside& PanK	14	O.2	TYR257	OH	3.126	178.1	Acceptor - Donor	
		18	O.2	TYR257	OH	2.788	147.1	Acceptor - Donor	70
		39	O.2	LYS103	NZ	2.962	179.9	Acceptor - Donor	
16	Pomegranatate & PanK	7	O.3	THR128	OG1	3.021	123.8	Acceptor - Donor	
		7	O.3	TYR153	OH	2.802	162.3	Acceptor - Donor	31
		16	O.2	TYR153	OH	2.807	126.1	Acceptor - Donor	
17	1,2,4-tri-o-galloyl -ß-gluco-pyranose & DprE1	18	O.2	ARG58	NE	3.265	124.6	Acceptor - Donor	
		18	O.2	ARG58	NH2	2.785	135.5	Acceptor - Donor	
		28	O.3	HIS 132	N	3.079	168.7	Acceptor - Donor	
		28	O.3	TYR415	OH	2.936	179.4	Acceptor - Donor	74
		30	O.3	TYR415	OH	3.001	170.7	Acceptor - Donor	
		36	O.2	LYS 418	NZ	2.821	175.1	Acceptor - Donor	
18	1,2,4,6- Tetra_O_ galloyl-beta-D-glucose & DprE1	12	O.2	HIS132	N	3.088	147.5	Acceptor - Donor	
		12	O.2	TYR415	OH	3.123	160.1	Acceptor - Donor	
		32	O.3	ARG58	NE	3.078	142.7	Acceptor - Donor	94
		32	O.3	ARG58	NH2	3.101	142.0	Acceptor - Donor	
19	Catechin-(4ß→8)-gallocatechin & DprE1	12	O.2	HIS132	N	3.088	147.5	Acceptor - Donor	
		12	O.2	TYR415	OH	3.123	160.1	Acceptor - Donor	
		32	O.3	ARG58	NE	3.078	142.7	Acceptor - Donor	94
		32	O.3	ARG58	NH2	3.101	142.0	Acceptor - Donor	
		43	O.3	TYR60	N	3.409	128.9	Acceptor - Donor	
20	Kaempferol 3-o-rhamnoglucoside & DprE1	39	O.2	ASN163	ND2	2.801	141.2	Acceptor - Donor	
		42	O.2	ARG140	NH1	2.756	159.1	Acceptor - Donor	64
		42	O.2	ARG140	NH2	3.074	141.2	Acceptor - Donor	
21	Pomegranatate & DprE1	7	O.3	TYR415	OH	2.965	161.8	Acceptor - Donor	
		10	O.2	TYR60	OH	3.216	174.8	Acceptor - Donor	38
		17	O.2	HIS132	NE2	3.321	130.4	Acceptor - Donor	
22	L-malic acid & DprE1	24	O.3	TYR60	OH	3.006	146.9	Acceptor - Donor	
		4	O.2	SER228	OG	2.794	122.8	Acceptor - Donor	
		5	O.3	LYS134	NZ	3.252	164.7	Acceptor - Donor	
		5	O.3	PHE313	O	2.855	138.1	Donor - Acceptor	18
		5	O.3	HIS 315	O	3.118	162.3	Donor - Acceptor	
		9	O.3	ALA244	O	2.712	159.6	Donor - Acceptor	
		11	O.3	SER228	O	3.020	128.3	Donor - Acceptor	
saltb	1 4 O.2 -- LYS A 134 NZ 3.705 N/A Acceptor - Donor								

TABLE 4: MOLECULAR PROPERTY ANALYSIS USING OSIRIS DATAWARRIOR

Name	Molecular weight	LOGP	Rotatable Bonds	Acceptors	Donors	Total Polar Surface Area	Mutagenic	Tumerogenic	Irrirant	Reproductive effective	Drug likeness score	Drug score
1,2,4-tri-o-galloyl-ß-gluco-pyranose	636.471	-0.28	7	18	11	249.5	N	N	N	N	1.8	0.48
Apigenin	270.248	2.69	1	4	3	112.519	Y	N	N	N	1.21	0.47
Icariside D1	410.375	0.63	8	10	6	165.315	N	N	N	N	-1.26	0.41
Kaempferol-3-o-rhamnoglucoside	586.458	4.03	5	15	10	234.318	N	N	N	N	-1.34	0.24
Pinocembrin	256.257	2.80	1	4	2	109.441	N	N	N	N	1.95	0.83
Pomegranatate	392.316	0.54	0	9	4	154.646	N	N	N	Y	-9.4	0.26
Tercatain	812.768	-0.08	4	19	15	319.296	Y	Y	Y	Y	-0.66	0.15
Betulic acid	456.711	7.09	2	2	2	201.354	N	N	N	N	-21.49	0.15
Cycloartenolacetate	468.766	8.74	5	2	0	209.609	N	N	Y	N	-4.2	0.07
1,2,4,6-Catechin -(4 ß→8)-gallaocatechin	592.509	4.62	2	13	11	241.371	N	N	N	N	-0.17	0.67
L malic_acid	134.087	-1.09	3	3	3	50.54	N	N	N	N	0.71	0.83

TABLE 5: ADSORPTION, DISTRIBUTION AND METABOLISM ANALYSIS OF SELECTED MOLECULES USING PKCSM SERVER

	Absorption						Distribution			Metabolism						
Compound	Caco2 permeability	Intestinal absorption (human)	Skin Permeability	P-glycoprotein substrate	P-glycoprotein I inhibitor	P-glycoprotein II inhibitor	VDss (human)	BBB permeability	CNS permeability	CYP2D6 substrate	CYP3A4 substrate	CYP1A2 inhibitior	CYP2C19 inhibitior	CYP2C9 inhibitior	CYP2D6 inhibitior	CYP3A4 inhibitior
1,2,4-tri-o-galloyl-ß-gluco-pyranose	-1.99	11.66	-2.74	Y	Y	Y	0.60	-3.22	-5.38	N	N	N	N	N	N	N
Apigenin	1.05	91.02	-2.74	Y	N	N	0.09	-0.90	-2.10	N	N	Y	Y	Y	N	N
Icariside D1	-0.55	22.47	-2.74	Y	Y	N	0.07	-1.68	-4.03	N	N	N	N	N	N	N
Pinocembrin	0.95	92.30	-3.00	Y	N	N	-0.26	0.42	-2.12	N	Y	Y	Y	N	N	N
Pomegranatate	0.51	59.53	-2.74	Y	N	N	1.18	-1.17	-3.44	N	N	N	N	N	N	N
Kaempferol 3-o-rhamnoglucoside	-0.31	46.16	-2.74	Y	Y	N	2.10	-2.58	-4.10	N	N	N	N	N	N	N
Tercatain	-2.37	0.00	-2.74	Y	N	N	-0.61	-4.28	-6.27	N	N	N	N	N	N	N
Betulic_acid	1.26	99.23	-2.74	N	N	N	-1.32	-0.27	-1.30	N	Y	N	N	N	N	N
Cycloartenol acetate	1.20	97.98	-2.74	N	Y	Y	-0.14	0.72	-1.75	N	Y	N	N	N	N	N
1,2,4,6-Catechin(4 ß→8)- gallocatechin	-1.48	44.82	-2.74	Y	Y	Y	0.36	-2.70	-3.75	N	N	N	N	N	N	N
L-malic acid	-0.39	14.72	-2.74	N	N	N	-0.88	-0.77	-3.52	N	N	N	N	N	N	N

The *in silico* screening results revealed that phytochemiccals present in *Punica granatum* have significant inhibitory activity on the growth and multiplication of *Mycobacterium tuberculosis*. Among such compounds, pomegranate can be recommended as the best one for further investigation. However, the compound kaempferol-3-o-rhamnoglucoside also known as nicotiflorin or kaempferol 3-rutinoside an equally competent compound determined through this investigation has already been approved by Food and Drug Administration (FDA) as a druggable compound[33]. In this backdrop, both compounds have

TABLE 6: EXCRETION AND TOXICITY ANALYSIS OF SELECTED MOLECULES USING PKCSM SERVER

	Excretion				Toxicity						
Compound	Caco2 permeability	Intestinal absorption (human)	Renal OCT2 substrate	Max. tolerated dose (human)	AMES toxicity	hERG I inhibitor	hERG II inhibitor	Oral Rat Acute Toxicity (LD50)	Oral Rat Chronic Toxicity (LOAEL)	Hepatotoxicity	Skin Sensitisation
1,2,4-tri-o-galloyl-ß-gluco-pyranose	-1.99	11.66	N	0.44	N	N	Y	2.48	6.11	N	N
Apigenin	1.05	91.02	N	0.34	N	N	N	2.46	1.57	N	N
Icariside D1	-0.55	22.47	N	0.50	N	N	N	3.21	4.36	N	N
Pinocembrin	0.95	92.30	N	0.07	N	N	N	1.75	1.97	N	N
Pomegranatate	0.51	59.53	N	0.09	N	N	N	2.88	2.64	N	N
Kaempferol 3-o-rhamnoglucoside	-0.31	46.16	N	0.49	N	N	Y	2.51	3.48	N	N
Tercatain	-2.37	0.00	N	0.53	N	N	Y	2.58	3.72	N	N
Betulic acid	1.26	99.23	N	0.14	N	N	N	2.31	2.15	Y	N
Cycloartenol acetate	1.20	97.98	N	-0.32	N	N	Y	2.61	2.32	N	N
1,2,4,6-Catechin(4 ß→8)-gallocatechin	-1.48	44.82	N	0.44	N	N	Y	2.49	3.64	N	N
L-malic acid	-0.39	14.72	N	0.57	N	N	N	1.49	3.10	N	N

TABLE 7: GLIDE XP DOCKING SCORES OF THE TOP HIT MOLECULES

	Phytochemicals		
Name of the target protein	Kaempferol-3-o-rhamnoglucoside (kcal/mol)	Pomegranatate (kcal/mol)	1,2,4-tri-o-galloyl-ß-gluco-pyranose (kcal/mol)
Mycolyl transferase antigen 85C protein (Ag85C)	-6.394	-5.304	-5.747
Filamentous temperature sensitive protein Z (FtsZ)	-6.046	-5.723	-6.337
Pantothenate kinase (PanK)	-6.799	-6.845	-6.843
Decaprenyl phosphoryl beta D-ribose 2'-epimerase (DprE1)	-6.171	**-8.8**	-5.405

TABLE 8: ANALYSIS OF TOP LEADS USING QIKPROP

Variant	Kaempferol-3-o rhamnoside	Pomegranatate	1,2,4-tri-o-galloyl-ß-gluco-pyranose
#stars	7	1	10
#amine	0	0	0
#amidine	0	0	0
#acid	0	0	0
#amide	0	0	0
#rotor	14	4	18
#rtvFG	2	2	4
CNS	-2	-2	-2
mol MW	594.525	392.316	636.476
dipole	8.098	0.001	10.121
SASA	735.895	449.818	909.134
FOSA	152.343	0	63.095
FISA	394.923	327.304	612.828
PISA	188.629	122.514	233.211
WPSA	0	0	0
Volume-1	1502.62	766.726	1673.1
doNrHB	8	4	11
accptHB	19.8	8	17.85
dip^2/V	0.04364	0	0.06123
QPpolrz	46.793	23.132	51.473

QPlogPC16	17.92	9.574	22.596
QPlogPoct	39.676	18.332	45.393
QPlogPw	33.591	16.717	37.384
QPlogPo/w	-2.08	-1.342	-2.861
QPlogS	-1.91	-1.85	-3.26
CIQPlogS	-4.208	-3.214	-5.44
QPlogHERG	-4.662	-3.724	-6.694
QPPCaco	1.782	7.8	0.015
QPlogBB	-3.822	-2.368	-7.791
QPPMDCK	0.528	2.606	0.003
QPlogKp	-6.789	-6.736	-10.264
#metab	9	4	11
QPlogKhsa	-1.088	-0.662	-1.273
HumaOralAbsorption	1	2	1
PercentHumaOralAbsorption	0	35.052	0
SAfluorine	0	0	0
SAamideO	0	0	0
PSA	249.574	164.745	345.451
#NandO	15	8	18
RuleOfFive	3	0	3
RuleOfThree	2	1	2

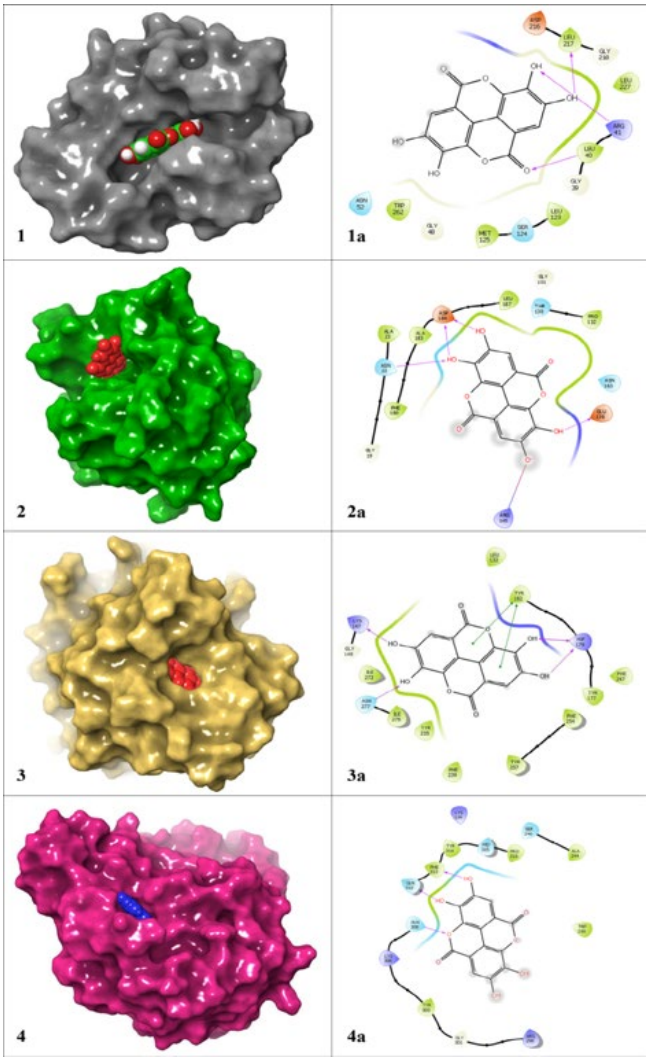

Fig. 2: Docked images of pomegranate with four target proteins created using\Maestro 11.9: 1. Complex with Ag85C, 1a. Ligand interaction with Ag85C; 2. Complex with FtsZ, 2a. Ligand interaction with FtsZ; 3. Complex with PanK, 3a. Ligand interaction with PanK; 4. Complex with DprE1, 4a. Ligand interaction with DprE1.

been recommended for further study leading to the discovery of novel drug against tuberculosis.

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Conflict of interest:

The author(s) confirm that this article content has no conflict of interest.

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