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Validation of Anti-Tuberculosis Activity and Identification of Leads in *Alstonia scholaris* L. (R.Br.)

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Abstract:*The anti-tuberculosis activity of Alstonia scholaris was evaluated through in silico method and identified potential lead molecules against the target proteins viz. the filamentous temperature sensitive protein (FtsZ) and Decaprenyl phosphoryl-beta D ribofuranose 2 epimerase (DprE1). The three dimensional (3D) structures of these proteins were retrieved from Protein Data Bank (PDB). A total of 152 phytochemicals present in A. scholaris were used as ligand molecules. The canonical SMILES of 70 phytochemicals were downloaded from open access chemical databases and others were drawn using ChemSketch. The 3D structure in .pdb format of all ligand molecules were generated using CORINA. The structural details of protein molecules were analyzed using Protparam and active site were detected using Q-SiteFinder. Docking was carried out using the tool AutoDock 4.2 and to avoid errors in lead identification the top five ranked hit molecules obtained in Autodock were again docked using four different docking tools viz: HexServer, PatchDock, SwissDock and iGEMDOCK. The results obtained in different docking tools were analysed by consensus scoring and RankSum technique. The results revealed that the compound 19-epischolaricine and beta amyrin present in the leaves and flowers of the plant have strong inhibitory activity on the targets, FtsZ and DprE1 respectively. The overall results substantiate the anti-tuberculosis activity of this plant in Indian Systems of Medicine and gave an insight to develop novel drug.*

Keywords*: Alstonia scholaris, Docking, DprE1, FtsZ, Tuberculosis.*

I. Introduction

Tuberculosis (TB) is an infectious disease caused by *Mycobacterium tuberculosis*. It is an ancient disease reported in Egyptian mummies (1550-1080 B.C) [1]. Despite the use of attenuated live vaccine and antibiotics, it still remains as the top second killer disease in the world [2]. According to World Health Organization (2012) TB kills 1.4 million people every year, over 3800 every day, one person every second and 8.0 million new TB patients per year. The standard therapy for the treatment of tuberculosis is the administration of more than one antibiotics for a long period (several months/years) which induce serious side effects to the patient, favors the emergence of multidrug-resistant (MDR) and extensively drug resistant (XDR) TB. Combined treatment in patients' co-infected with HIV may cause drug interaction and subsequent adverse effect to immune system. The available drugs are ineffective against the MDR and XDR strains of *M. tuberculosis.* In these backdrops, discovery of novel, faster and better drug for TB attain *prima face* importance. However, pharmaceutical companies are least interested in this line mainly because of limited commercial return as 95% of TB patients are in poor underdeveloped and developing countries like India and requires long term expensive research input.

Nature produces diverse range of bioactive molecules, which are being used in the traditional systems of medicine for a variety of diseases. About 25% of the drugs used in modern medicine were derived from plants [3]. Unlike synthetic compounds plant derived compounds are synthesized within the living system in accordance with environmental and other stimuli including the attack of pathogens/diseases and those molecules are biologically validated, therefore it may induce less or no side effects. Several medicinal plants have been used against tuberculosis in the traditional systems of medicine in all over the world particularly in India [4]. However, its efficacy and mode of drug action are seldom scientifically validated due to several reasons such as lack of efficient screening methods, high expense, slow and difficulty in executing the experimental work, lack of model organism for testing etc. [5].

Alstonia scholaris L. (R.Br.), belonging to the family Apocynaceae, is a tall evergreen tree cultivated throughout India [6]. It has been used in the traditional systems of medicine to treat various ailments such as asthma, malaria, leprosy, tuberculosis, skin diseases, congestion of liver, dropsy, ulcers, tumours, etc. [7]. Ayush 64, an anti-malarial ayurvedic preparation containing the extracts of *A. scholaris* is marketing by National Research Development Corporation. The leaves and latex of this plant have been used against beriberi, ulcers, tumors, rheumatoid arthritis, blood purifier [8] and as a potent anti-bactericide [9]. Stem bark is used in phosphaturia [10]. The plant has rich source of phytochemicals, with alkaloids being the major constituent which were reported from different parts *viz* bark, leaves, flowers and seeds [11]. The major chemical

compounds include echitamine, picrinine, lupeol, scholaricine and its derivatives, indole alkaloids, triterpenoids, secoiridoide glucosides etc. [12]. The main objective of the present investigation was to validate the anti-tuberculosis activity and identification of lead molecules in *Alstonia scholaris* through *in silico* screening method.

II. Materials And Methods

2.1 Preparation of Target Macromolecules

The three dimensional (3D) structures of the receptor proteins *Filamentous temperature sensitive protein (FtsZ)* (PDB id: 2Q1X) and *Decaprenyl phosphoryl-beta D ribofuranose 2 epimerase* (DprE1) (PDB id:4FDO) were retrieved from the Protein Data Bank (PDB)[13]. The physical and chemical parameters of the receptor proteins were analyzed using the tool ProtParam [14]. The sequence data of both the target proteins were subjected to BLASTp against human genome [15]. The citrate ligand bound to the FtsZ protein and the 3-nitro-N-[(1R)-1-phenylethyl]-5-(trifluoromethyl) benzamide and flavin-adenine dinucleotide present in DprE1were removed prior to docking. The active site of the protein molecules were predicted using Q SiteFinder [16].

2.2 Preparation of Ligands

Perusal of the literature and search on open access chemical databases, information on 152 chemical molecules present in *Alstonia scholaris* were collected (TABLE: 1). Of these, canonical SMILES of 70 molecules were obtained from the chemical database Pubchem [17]. Structures of remaining 82 molecules were drawn using the tool ChemSketch. The 3D structures in .pdb format of all phytochemicals were generated using the tool CORINA [18].

2.3 Docking

Docking experiments were carried out using an automated molecular docking software package AutoDock 4.2, following the standard procedure [19]. Water molecules present in the protein molecules were removed and polar hydrogen added. Root of each ligand molecule was automatically detected and the torsions were selected. All torsions of the ligand were allowed to rotate. In the protein molecules torsions were checked for the selected residues. Pre-calculated grid maps were required for running the program, which were calculated using the Autogrid program. The grid was positioned at the macromolecule with XYZ co-ordinates set at -6.164, 53.402,-0.146 respectively and grid point spacing of 0.375 A^o for FtsZ protein. Similarly for DprE1 protein, the XYZ co-ordinates were set at 3.028, 3.333, -0.278 respectively and grid point spacing of 0.375 A^o. Grid calculations were used to determine the total interaction energy for a ligand with a macromolecule. The docking calculations were done by keeping all the docking parameters at default value. A total of 10 GA runs were done with a population size of 150 and 25x105 evaluations were done with 27000 iterations. The mutation rate and cross over rate were set at 0.02 and 0.80 respectively. A global local search method based on Lamarckian Genetic Algorithm (LGA) was selected to calculate the best conformers. LGA is the most efficient, reliable and successful when compared to the other algorithms. The docked structure binding affinity and possible orientations were ranked based on lowest binding energy through cluster analysis. The molecules having free energy of binding less than or equal to -7 Kcal/mol were considered as the hit molecules.

2.4 Consensus Scoring And Ranksum Technique

The top ranked five hit molecules obtained from AutoDock were further docked with other four different docking tools *viz* HexServer, PatchDock, SwissDock and iGEMDOCK. The results including Autodock were documented in .xls spread sheet file format and uploaded on the website http://allamapparao.org/dst/ application tool. The uploaded data were parsed and stored in 2D array and subsequently analyzed as follows (1) divide the data into 4 classes; (2) get results from Rank Sum Technique; (3) get results from DST unweighted; (4) get results from DST weighted; (5) get results from Zhang Rule. The top ranked molecules obtained from 2-5 procedures were selected as best lead molecules for further investigation [20].

III. Results And Discussion

3.1 Docking of FtsZ With Phytochemicals From *Alstonia scholaris*

FtsZ, a bacterial tubulin homolog [21] which play key role in bacterial cell division [22, 23]. In the presence of Guanosine triphosphate (GTP), FtsZ polymerizes bidirectionally at the centre of the cell on the inner membrane to form a highly dynamic helical structure known as the Z-ring. The recruitment of several other cell division proteins lead to Z-ring formation and eventually cell division [21]. Unlike other bacteria, the mechanism of septum formation in *Mycobacterium tuberculosis* (Mtb) was not well demonstrated. Septum formation in *Mtb* is much slower than other species [24]. Many of the genes that encode proteins involved in the

regulation of septum formation and cell division in other bacterial species like *E.coli* are not annotated in Mtb [25]. Therefore it may possess unique processes for regulation of septum formation. However, when FtsZ inhibitors were used, cell division was arrested in Mtb. It indicates that, FtsZ is a potential target to block the propagation of Mtb [21].

Analysis of target protein sequence using ProtParam tool showed that the protein FtsZ consists of 379 amino acids with a molecular weight of 38755.8 Da and theoretical pI value of 4.55. FtsZ is a stable protein with instability index of 29.27. The protein has an aliphatic index of 97.57, consists of 14 helices and 11 strands. The predicted active site of FtsZ has a volume of 323 cubic Angstrom which includes 15 amino acid residues.
The docking results between the target protein FtsZ and 152 phytochemicals derived from *Alstonia scholaris* in Autodock revealed that the compounds, 19-epischolaricine, lupeol, alpha amyrin acetate, echitamidine, and alpha amyrin (Fig.1) showed high binding affinity with a binding score of -9.09, -8.53, -8.45, -8.41 and -8.19 respectively (TABLE. 2) In general, molecules having binding energy less than or equal to -5kcal/mol can be considered as hit molecule. The results showed that out of 152 phytomolecules 111of them showed binding energy $\leq$-5kcal/mol and therefore the compounds having binding energy less than -8.0 were taken as hit molecules. The molecular interaction of 19-epischolaricine with FtsZ protein showed six hydrogen bonds, three hydrogen bonds formed with ASN 22 residue, one hydrogen bond each at ARG 25, LYS 12 and ARG 91 (Fig.2). Lupeol showed two hydrogen bond interactions with GLY 34 and ILU11, echitamidine showed four hydrogen bond interactions, two hydrogen bonds with ASN22 and one each with LYS12 and ARG91. Alpha amyrin and alpha amyrin acetate showed no hydrogen bond interaction. An alkaloid 19 epischolaricine present in the leaves of the plant formed six hydrogen bonds with the protein FtsZ. It indicated that the molecule was strongly bound within the active site of the protein molecule and can be considered as a promising lead compound. However, in order to minimize errors in lead selection the five selected hit molecules were again docked with other four different docking tools such as HexServer, PatchDock, SwissDock and iGEMDOCK. Analysis of the results from all docking tools by DST method revealed that the compound 19-epischolaricine can be recommended as the best lead molecule (TABLE 3).

3.2 Docking of DprE1 With Phytochemicals From *Alstonia scholaris*

Decaprenylphosphoryl-beta-D-ribose epimerase (DprE1), a key enzyme involved in the synthesis of virulent factor arabinan [26] in Mtb, together with DprE2 is involved in the epimerization of decaprenylphosphoryl-beta-D-ribose (DPR) to decaprenylphosphoryl-beta-D-arabinofuranose (DPA). DPA is a precursor of mycobacterial cell wall content arabinan and the sole known donor substrate for a series of membrane embedded arabinosyltransferases [26]. These two enzymes have been suggested to be essential for the *in vitro* growth of bacteria as determined by transposon site hybridization (TraSH) [27].While DprE2 is also necessary for the epimerisation reaction, there is evidence of redundancy at this step, making DprE2 a less attractive target for drug intervention [28]. Recent studies showed that the protein DprE1 was inhibited by Benzothiazinones [29].

The protein DprE1 composed of 461 amino acids with a molecular weight of 50163.18 Da and theoretical pI values of 7.17. DprE1 belongs to a stable protein class with instability index of 28.31. The protein consists of 20 helices and 22 beta strands. The protein is having an aliphatic index of 83.99. The active site of the protein was predicted using the interaction energy between the protein and a simple van der Waals probe to locate energetically favorable binding sites. The predicted site of DprE1 has a volume of 685 cubic Angstroms with 35 amino acid residues. Sequence similarity search was done to check the similarity of bacterial proteins with proteins present in the human system, which gave a negative result.

Docking of phytochemicals with the target protein DprE1 revealed that the compounds beta amyrin, yohimbine, betulinic acid, alstonidine and scholaricine A (Fig.3) showed high binding affinity with a promising score of -11.37, -9.11, -9.24,-7.77 and -7.6 respectively (TABLE 4). A total of 103 phytomolecules showed binding energy less than -5kcal/mol and therefore molecules showed binding energy less than -7.0 only considered as hit molecules. The hydrogen bond interactions of beta amyrin with DprE1 showed one hydrogen bond interaction with ARG58 (Fig.4) and yohimbine showed three hydrogen bond interactions with ARG58, LEU56 and THR122. The compound alstonidine has two hydrogen bond interactions with HIS 132. The DST analysis of docked results in different tools as mentioned earlier revealed that the compound beta amyrin can be recommended as a best lead molecule among the 152 molecules screened (TABLE 5). The application of consensus scoring method in both cases may reduce false positive results. It may reduce errors that may occur in single scoring systems and increases the probability of identifying correct ligand molecule [30].

IV. Conclusion

The foregoing *in silico* screening results revealed that the compounds 19-epischolaricine present in the leaves and beta amyrin present in the flowers of *Alstonia scholaris* can inhibit the activity of FtsZ protein leading to bacterial death and DprE1 leading to cease the synthesis of virulent factor arabinan respectively.

These results strongly substantiate the potentiality of anti-tuberculosis activity of the plant. However, *in vitro* and *in vivo* experimental demonstrations are to be inevitable to develop these molecules as drug.

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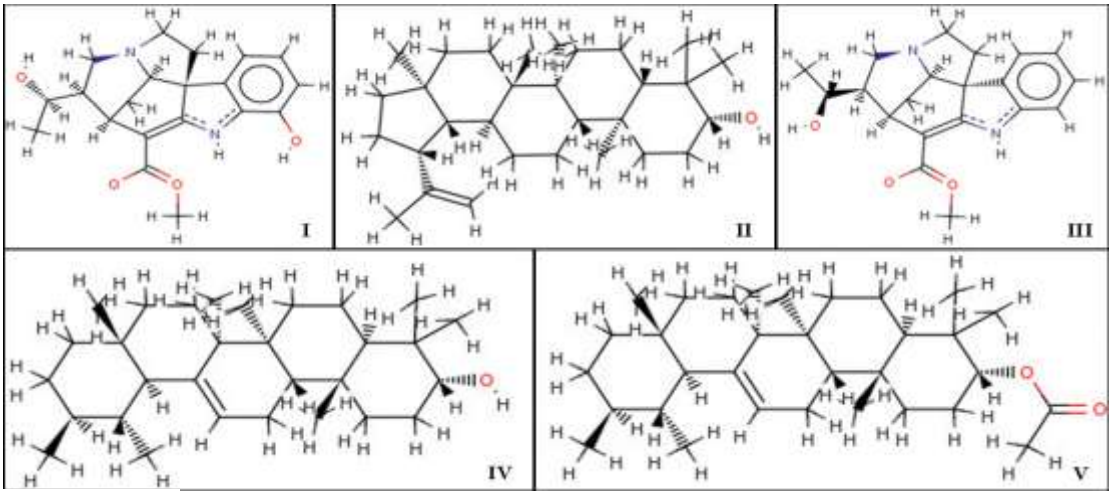

Figure 1: 2-D structures of lead molecules identified from *Alstonia scholaris* against FtsZ: (I) 19-Epischolaricine, (II) Lupeol, (III) Echitamidine, (IV) Alpha Amyrin (V) Alpha Amyrin Acetate

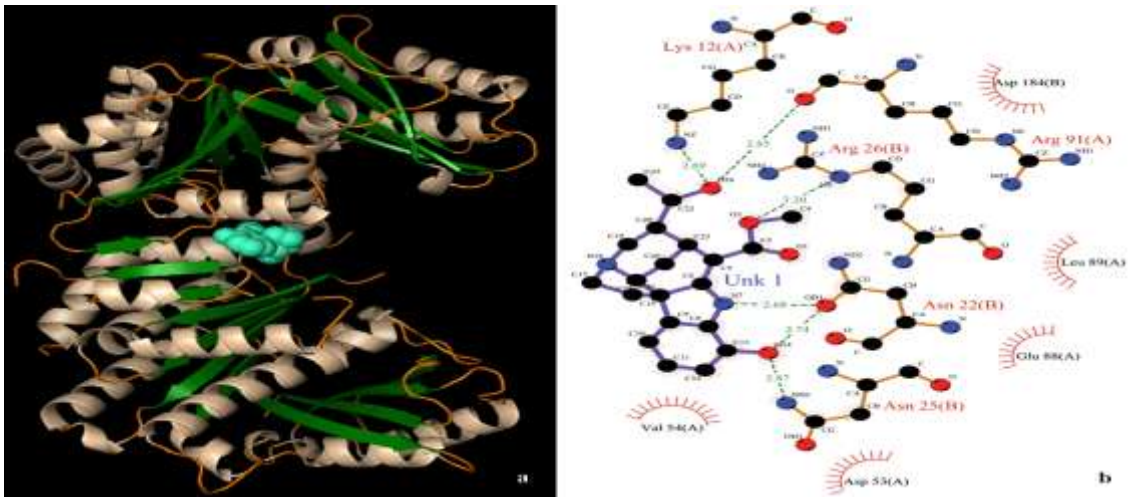

Figure 2: (a) Interaction of FtsZ with 19-epischolaricine. (b) Hydrogen bond interaction of 19-epischolaricine with active site residues.

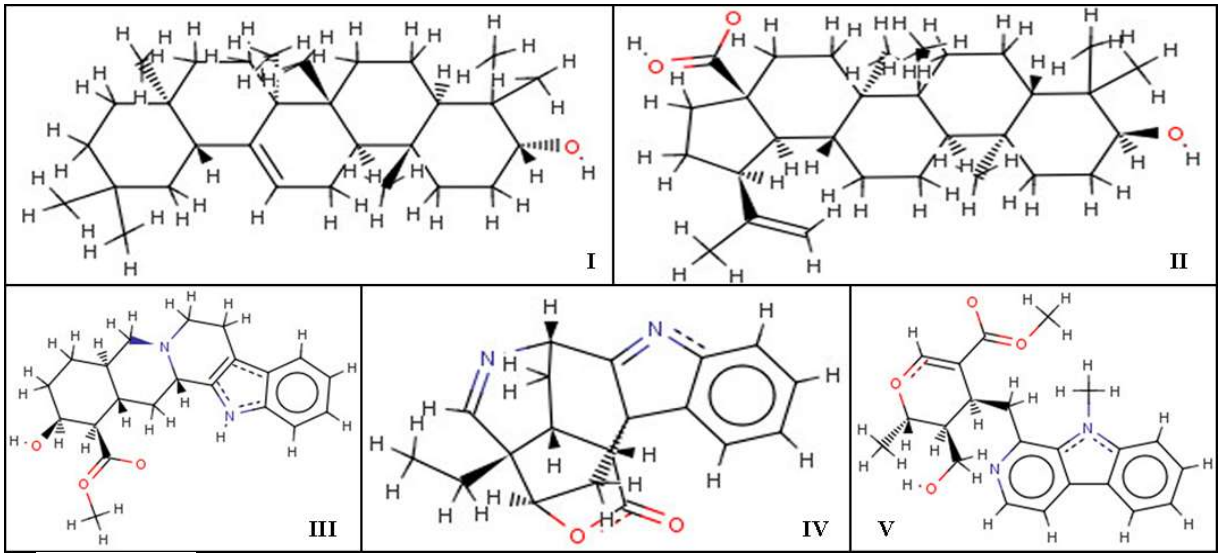

Figure 3: 2-D structures of lead molecules identified from *Alstonia scholaris* against DprE1: (I) Beta Amyrin (II) Betulinic Acid, (III) Yohimbine, (IV) Scholaricine A (V) Alstonidine D

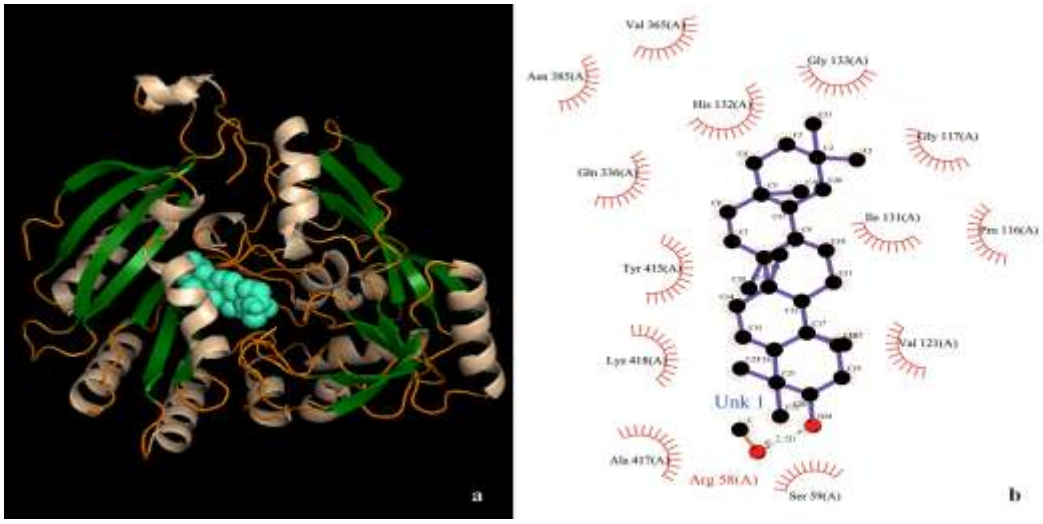

Figure 4. (a) Interaction of DprE1 with Beta amyrin. (b) Hydrogen bond interaction of Beta amyrin with active site residues.

TABLE: 1 List of chemical molecules selected from *Alstonia scholaris* with molecular formula and molecular weight for docking

1	(+)-Lochneridine,$C_{20}H_{24}N_2O_3$ (340.416)*	77	Akuammicine n_b –oxide, $C_{20}H_{25}N_2O_3$ (341.42)
2	1-Ethyldecyl acrylate $C_{15}H_{28}O_2$ (240.38)*	78	Akuammidine / rhazine, $C_{21}H_{24}N_2O_3$ (352.42)*
3	2-Dodecyloxirane, $C_{14}H_{28}O$ (212.37)*	79	Alpha amyrin acetate, $C_{32}H_{52}O_2$ (468.75)*
4	2,6,10,15-tetramethylheptadecane,$C_{21}H_{44}$ (296.57)	80	Alpha amyrin linoleate, $C_{48}H_{80}O_2$ (689.14)
5	(19,20) e-alstoscholarine , $C_{22}H_{20}N_2O_3$ (360.40)	81	Alpha terpineol $C_{10}H_{18}O$, (154.24)
6	(19,20) z-alstoscholarine, $C_{22}H_{20}N_2O_3$ (360.40)	82	Alpha tocopherolquinone, $C_{26}H_{44}O_3$ (404.63)
7	12-methoxyechitamidine/scholarine, $C_{21}H_{26}N_2O_4$ (370.442)	83	Alpha-amyrin , $C_{30}H_{50}O$ (426.71)
8	17-o-acetylechitamine, $C_{24}H_{31}N_2O_5$ (427.513)	84	Alschomine , $C_{21}H_{24}N_2O_5$ (384.42)
9	19,20-e-vallesamine, $C_{20}H_{24}N_2O_3$ (340.41)	85	Alstonic acid a, $C_{30}H_{48}O_3$ (456.7)
10	19-e-akuammidine ,$C_{21}H_{24}N_2O_3$ (352.43)	86	Alstonic acid b, $C_{30}H_{46}O_3$ (454.7)
11	19-e-picrinine, $C_{20}H_{22}N_2O_3$ (338.40)	87	Alstonidine , $C_{22}H_{24}N_2O_4$ (380.43)
12	19-epischolaricine, $C_{20}H_{24}N_2O_4$ (356.42)	88	Alstonine / chlorogenine , $C_{21}H_{20}N_2O_3$ (348.39)*
13	19-e-vallesamine , $C_{20}H_{24}N_2O_3$ (340.42)	89	Alstovenine, $C_{22}H_{28}N_2O_4$ (384.46)*
14	19-s-scholaricine, $C_{20}H_{24}N_2O_4$ (356.41)*	90	Alyxialactone, $C_{10}H_{16}O_4$ (200.2)*
15	1-hydroxy-3,5-dimethoxyxanthone, $C_{15}H_{12}O_5$ (272.25)	91	Angustilobine b acid, $C_{19}H_{20}N_2O_3$ (324.37)
16	20(s)-tubotaiwine, $C_{20}H_{24}N_2O_2$ (324.41)	92	Beta amyrin, $C_{30}H_{50}O$ (426.71)*
17	2-Methylenecholestan-3-ol, $C_{28}H_{48}O$ (400.68)	93	Beta amyrin-3-palmitate,$C_{46}H_{80}O_2$ (665.12)
18	2-phenylethyl acetate , $C_{10}H_{12}O_2$ (164.201 Da)	94	Beta sitosterol, $C_{29}H_{50}O$ (414.70)*
19	5-methoxystrictamine , $C_{21}H_{24}N_2O_3$ (352.4)	95	Betulin, $C_{30}H_{50}O_2$ (442.71)*
20	6,7-seco- 19,20-epoxyyanggustibobine b, $C_{20}H_{24}N_2O_4$ (356.41)	96	Betulinic acid, $C_{30}H_{48}O_3$ (456.70)*
21	6,7-seco- angustilobine b, $C_{20}H_{24}N_2O_3$ (340.41)	97	Bis(2-ethylhexyl)phthalate, $C_{24}H_{38}O_4$ (390.55)
22	7-Hexylicosane, $C_{26}H_{54}$ (366.70)*	98	Capric ether ,$C_{20}H_{42}O$ (298.54)*
23	7,3',4'-trimethoxyl-5-hydroxyflavone, $C_{18}H_{16}O_6$ (328.31)	99	Chlorogenic acid, $C_{16}H_{18}O_9$ (354.30)*
24	7-megastigmene-3,6,9-triol, $C_{13}H_{24}O_2$ (212.32)	100	Chlorogenin, $C_{27}H_{44}O_4$ (432.63)*
25	Akuammicine, $C_{20}H_{22}N_2O_2$ (322.40)	101	Cis linalool oxide, $C_{10}H_{18}O_2$ (170.24)*
26	Cis Myrtanol, $C_{10}H_{18}O$ (154.249)*	102	Linalool $C_{10}H_{18}O$ (154.24)*
27	Corialstonidine , $C_{22}H_{26}N_2O_5$ (398.45)	103	Linoleic acid $C_{18}H_{32}O_2$ (280.44)*
28	Corialstonine , $C_{23}H_{26}N_2O_5$ (410.46)*	104	Loganin $C_{17}H_{26}O_{10}$ (390.38)*
29	Cycloeucalenol, $C_{30}H_{50}O$ (426.71)*	105	Losbanine (6,7-seco-6-nor- angustilobine b), $C_{19}H_{24}N_2O_3$ (328.40)*
30	Dibutyl phthalate , $C_{16}H_{22}O_4$ (278.34)*	106	Lupen-3-palmitate, $C_{46}H_{80}O_2$ (665.12)
31	Ditamine, $C_{22}H_{30}N_2O_4$ (386.48)	107	Lupeol acetate, $C_{32}H_{52}O_2$ (468.75)
32	E-alstoscholarine, $C_{22}H_{20}N_2O_3$ (360.40)	108	Lupeol, $C_{30}H_{50}O$ (426.71)*
33	Echicaoutchin, $C_{25}H_{40}O_2$ (372.58)	109	Lupeol linoleate, $C_{48}H_{80}O_2$ (689.14)*
34	Echicerin, $C_{30}H_{48}O_2$ (440.70)	110	Manilamine , $C_{18}H_{22}N_2O$ (282.38)*

35	Echitamidine ,$C_{20}H_{24}N_2O_3$ (340.41)	111	N(4)-demethyl echitamine, $C_{21}H_{26}N_2O_4$ (370.44)
36	Echitamidine-n-oxide 19-o- β-d-glucopyranoside, $C_{26}H_{34}N_2O_9$ (518.22)	112	N(b)demethylalstogustine, $C_{20}H_{26}N_2O_3$ (342.43)
37	Echitamidine-n-oxide, $C_{20}H_{24}N_2O_4$ (356.41)	113	N1-methoxymethyl picrinine , $C_{22}H_{26}N_2O_4$ (382.45)
38	Echitamine ,$C_{22}H_{29}N_2O_4^+$ (385.47)*	114	Leuconoxine, $C_{19}H_{22}N_2O_2$ (310.39)
39	Echitamine chloride,$C_{22}H_{29}N_2O_4$ (385.47)	115	Nareline, $C_{20}H_{20}N_2O_4$ (352.38)
40	Echitaminic acid, $C_{20}H_{24}N_2O_4$ (356.41)	116	N-demethylalstogustine n-oxide, $C_{20}H_{27}N_2O_4$ (359.43)
41	Echitin ,$C_{42}H_{70}O_2$ (607.00)	117	N-hexacosane , $C_{26}H_{54}$ (366.70)
42	Formononetin-7-o-β-d-apiofuranosyl-(1→6)- β-d-glucopyranoside, $C_{27}H_{30}O_{13}$ (562.16)	118	nerolidyl acetate ,$C_{17}H_{28}O$ (264.40)
43	Imidazole-2-carboxylic acid, $C_4H_4N_2O_2$ (112.08)	119	Oleic acid, $C_{18}H_{34}O_2$ (282.46)*
44	Isoboonein, $C_9H_{14}O_3$ (170.2)	120	Palmitic acid, $C_{16}H_{32}O_2$ (256.42)*
45	Isookanin-7-o-alpha-l- rhamnopyranoside, $C_{21}H_{22}O_{10}$ (434.40)	121	Picralinal, $C_{21}H_{22}N_2O_4$ (366.41)*
46	Isookaninrhamnoside ,$C_{21}H_{22}O_{10}$ (434.39)	122	Picrinine , $C_{20}H_{22}N_2O_3$ (338.40)*
47	Isorhamnetin, $C_{16}H_{12}O_7$ (316.26)*	123	Pleiocarpamine $C_{20}H_{22}N_2O_2$ (322.40)
48	Kaempferol, $C_{15}H_{10}O_6$ (286.23)*	124	Quercetin ,$C_{15}H_{10}O_7$ (302.23)*
49	Lagunamine (19-hydroxytubotaiwine) ,$C_{20}H_{24}N_2O_3$ (340.41)	125	Leuconolam, $C_{19}H_{22}N_2O_3$ (326.38)
50	Quercetin-3-o-β-d-galactopyranoside, $C_{21}H_{20}O_{12}$ (464.37)	126	Vallesamine-n-oxide, $C_{20}H_{24}N_2O_4$ (356.41)
51	Rhazimanine ,$C_{21}H_{26}N_2O_3$ (354.44)	127	Venenatine, $C_{22}H_{28}N_2O_4$ (384.46)*
52	Rserpine ,$C_{33}H_{40}N_2O_9$ (608.67)*	128	Villalstonine ,$C_{41}H_{48}N_4O_4$ (660.84)*
53	Scholarein a, $C_9H_{16}O_2$ (156.22)	129	Yohimbine, $C_{21}H_{26}N_2O_3$ (354.44)*
54	Scholarein c, $C_9H_{16}O_3$, (172.22)	130	Z-alstoscholarine ,$C_{22}H_{20}N_2O_3$ (360.40)
55	Scholarein d, $C_{10}H_{18}O_3$ (186.24)	131	Ψ-akuammigine, $C_{21}H_{24}N_2O_3$ (352.42)
56	Scholaricine, $C_{20}H_{26}N_2O_4$ (358.431)	132	Scholarisines e, $C_{21}H_{24}N_2O_5$ (384.42)
57	Scholarisine a, $C_{19}H_{18}N_2O_2$ (306.35)*	133	Scholarisines f, $C_{21}H_{26}N_2O_4$ (370.44)
58	Scholarisine ii,$C_{17}H_{15}NO_3$ (281.30)	134	Scolarisine I, $C_{17}H_{13}NO_2$ (263.29)
59	Scholarisines b, $C_{23}H_{26}N_2O_5$ (410.46)*	135	Spinacene, $C_{30}H_{50}$ (410.71)*
60	Scholarisines c, $C_{21}H_{26}N_2O_4$ (370.44)	136	Squalene ,$C_{30}H_{50}$ (410.71)
61	Scholarisines d,$C_{21}H_{26}N_2O_4$ (370.44)	137	Stearic acid, $C_{18}H_{36}O_2$ (284.47)*
62	Strictamine, $C_{20}H_{22}N_2O_2$ (322.40)*	138	Terpinen-4-ol, $C_{10}H_{18}O$ (154.29)*
63	Sweroside, $C_{16}H_{22}O_9$ (358.34)	139	Terpinyl acetate, $C_{12}H_{20}O_2$ (196.28)*
64	Tetrahydroalstonine , $C_{21}H_{24}N_2O_3$ (352.42)*	140	Tubotaiwine ,$C_{20}H_{26}N_2O_2$ (326.43)*
65	Trans linalool oxide, $C_{10}H_{18}O_2$ (170.24)*	141	Tubotaiwine oxide, $C_{20}H_{27}N_2O_3$ (343.43)*
66	Ursolic acid, $C_{30}H_{48}O_3$ (456.74)*	142	Tritetracontan, $C_{43}H_{88}$ (605.15)*
67	Trimethylsilyl {3-[(trimethylsilyl)oxy]phenyl}acetate, $C_{14}H_{24}O_3Si_2$, (296.51)*	143	Rhamnitol, 1-O-octyl-, $C_{14}H_{30}O_5$ (278.3)*
68	N,N'-Diacetylethylenediamine, $C_6H_{12}N_2O_2$ (144.17)*	144	Methyl tridecanoate, $C_{14}H_{28}O_2$ (228.37)*
69	Oxirane, 2,2-dimethyl-3-(3,7,12,16,20-pentamethyl-3,7,11,15,19-heneicosapentaenyl)-, (all-E), $C_{30}H_{50}$ (426.71)*	145	Methyl 10-methylundecanoate, $C_{13}H_{26}O_2$ (214.34)*
70	Eugenol, $C_{10}H_{12}O_2$ (164.20)*	146	E-2-Tetradecen-1-ol, $C_{14}H_{28}O$ (212.37)*
71	Dithioerythritol, O,O',S,S'-tetrakis(trimethylsilyl), $C_{16}H_{42}O_2S_2Si_4$ (442.97)*	147	Dihydroartemisinin, 10-O-(t-butyloxy), $C_{19}H_{32}O_6$ (356.45)*
72	Bis[2-(trimethylsilyl)ethyl] malonate,$C_{13}H_{28}O_4Si_2$ (304.53)*	148	Benzene, 1,2-dimethoxy-4-(2-propenyl)-, $C_{11}H_{14}O_2$ (178.22)
73	10-Henicosene, $C_{21}H_{42}$ (294.55)*	149	9-Methyl-5-methylene-8-decen-2-one, $C_{12}H_{20}O$ (180.28)*
74	6,11-Dimethyl-2,6,10-dodecatrien-1-ol, $C_{14}H_{24}O$ (208.34)*	150	4-(1,3,2-Dioxaborinan-2-yl)-2-nitrobenzoic acid, $C_{10}H_{10}BNO_6$ (251.00)*
75	1,3-Dioxolane,2-(5-bromo pentyl), $C_8H_{15}BrO_2$ (223.10)	151	2,3-Bis(acetyloxy)propyl laurate , $C_{19}H_{34}O_6$ (358.46)*
76	2,4-Bis(dimethylbenzyl)phenol, $C_{24}H_{26}O$ (330.46)*	152	2,6-Dimethyl-2,6-undecadien-10-ol, $C_{13}H_{24}O$ (196.32)*

*obtained from chemical database

TABLE 2: Top five phytochemicals from *Alstonia scholaris* showed lowest free energy of binding with FtsZ in autodock

Lead Molecule	Binding Energy (Kcal/mol)	Inhibition constant	H-Bond
19-epischolaricine	-9.09	218.66nM	UNK1: H35::ASN22:O1 ASN22:H21 :: UNK1:O1,O3 UNK1:H46: ARG91: O ASN25:H22 ::UNK1:O14 UNK1:H31 :: ASN22:O1
Lupeol	-8.53	563.43nM	UNK: 1: H75: GLY34: O ILE11:H1::UNK1:O29
Alpha Amyrin Acetate	-8.45	644.07nM	NO HYDROGEN BOND
Echitamidine	-8.41	685.14nM	UNK1:H41::ASN22:O1 ASN22:H21::UNK1:O22,23 UNK1:H49: ARG91: O
Alpha Amyrin	-8.19	986.26nM	NO HYDROGEN BOND

TABLE 3: Binding energy of top five hits molecules obtained from *Alstonia scholaris* when docked with FtsZ protein using different docking tools and RankSum analysis.

Sl NO:	Ligand ID	AutoDock	PatchDock	iGemDock	Hex	SwissDock	RankSum
1	19-epischolaricine	-9.09 (4)*	4742 (1)	-95.22 (4)	-295.89 (4)	-6.76 (2)	15
2	Lupeol	-8.53 (2)	5540 (3)	-68.18 (1)	-200.1 (1)	-6.07 (1)	8
3	Alpha Amyrin Acetate	-8.45 (2)	6110 (4)	-70.29 (1)	-240.3 (2)	-6.57 (2)	11
4	Echitamidine	-8.41 (1)	4610 (1)	-75.71 (2)	-304.13 (4)	-755 (4)	12
5	Alpha Amyrin	-8.19 (1)	5964 (4)	-71.59 (1)	-214.73 (1)	-7.44 (4)	11

**rank assigned by Dempster Shafer Theory*

TABLE 4: Top five phytochemicals from *Alstonia scholaris* showed lowest free energy of binding with DprE1 in autodock

Lead Molecule	Binding Energy (kcal/mol)	Inhibition constant	H-Bond
Beta amyrin	-11.37	4.66nM	UNK: H63::ARG58: O
Yohimbine		208.3nM	UNK:1 : O3::ARG 58:HN UNK:1 : O1::THR122:HN UNK:1:O1::LEU56:HN
Betulinic Acid	-9.24	168.96nM	NO HYDROGEN BOND
Alstonidine	-7.77	2.02uM	HIS132:HN::UNK1:O1 LYS18:H1::UNK1:O30 UNK1:H32:: HIS132:O
Scholaricine A	-7.6	15.29uM	NO HYDROGEN BOND

TABLE 5: Binding energy of top five hits molecules obtained from *Alstonia scholaris* when docked with DprE1 using different docking tools and RankSum analysis.

Sl NO:	Ligand ID	**AutoDock**	**PatchDock**	**iGemDock**	**Hex**	**SwissDock**	**RankSum**
1	Beta Amyrin	-11.37 (4)	-68.87 (4)	-94.3 (4)	-239.39 (3)	-7.23 (2)	17
2	Yohimbine	-9.11 (3)	-46.92 (1)	-90.97 (4)	-257.22 (4)	-6.72 (2)	14
3	Betulinic Acid	-9.24 (2)	-55.02 (2)	-74.9 (1)	-246.09 (4)	-5.66 (1)	10
4	Alstonidine	-7.77 (1)	-44.54 (1)	-88.54 (3)	-258.36 (4)	-8.96 (4)	13
5	Scholaricine A	-7.6 (1)	-60.9 (3)	-83.58 (2)	-201.57 (1)	-6.95 (2)	9