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Computational identification and analysis of Phytoconstituents inhibiting vital proteins in *Mycobacterium tuberculosis*

Gayathri S. G., Vishnu V., Shigil Shibu, Saranya T. S. and Asha Asokan Manakadan*

Department of Pharmaceutical Chemistry, Amrita School of Pharmacy, Amrita Vishwa Vidyapeetham University, AIMS Health Science Campus, Ponekkara, Kochi, Kerala, India

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ABSTRACT

Tuberculosis caused by Mycobacterium tuberculosis is disproportionately one of the major burden in health care system. In the present work we have extensively studied the target proteins Pantothenate synthetase, Thymidylate kinase and Filamentous temperature sensitive protein Z (FtsZ) for its significance in the treatment for tuberculosis. Computer aided drug design (CADD) aided to predict the most effective phytoconstituents for the treatment for tuberculosis among the 28 plant sources used for the study. The phytoconstituents taken up for the investigation included Agnuside, Alloin, Alliin, Alpha-amyrin, Alizarin, Aloe-emodin, Berberine, Coumaric acid, Kaempherol, Kumatakenin, Luteolin, Mimosine, Mangiferin, Myricetin, Niacin, Oleanane, Orientin, Protocatechuic acid, Piperine, Quinoline, Rottlerin, Sesamin, Scopoletin, Serotonin, Taraxerol, Thiamine, Ursolic acid and Xanthone. The computational approach included the prediction of Molecular properties, bioactive scores, the analysis of primary and secondary structures of proteins and the binding interaction calculation using docking principles. The results of in silico study pointed out that the phytoconstituents such as Alizarin, Kaempherol, Myricetin, Alliin, Coumaric acid, Protocatechuic acid, Aloin, Agnuside, Aloe-emodin, Mangiferin and Rottlerin elicits good interaction by inhibiting the selected proteins as compared with the standard drug Isoniazid. Further investigation is essential for the development of potent inhibitors for the control of the disease.

Keywords: Tuberculosis, Pantothenate synthetase, Thymidylate kinase, FtsZ protein, molecular docking

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INTRODUCTION

Tuberculosis (TB) is one of the major deadly infectious disease and a dangerous health threat faced by people globally. As per the WHO estimates, in 2013, 9 million people were confirmed with TB and for about 1.5 million people the disease proved to be fatal. Globally, in 2013, an estimated 480,000 people developed multidrug-resistant TB. Between 1990 and 2013, the TB mortality rate dropped by an estimated 45% [1,2]. *Mycobacterium tuberculosis* is an acid fast bacilli spread by droplet nuclei generated with persons having pulmonary or laryngeal TB [3]. Bacteria enter the lungs through nasal route and affect the alveoli resulting in the release of alveolar macrophages. After the macrophages engulf the microbes, they multiply gradually and modulate the production of enzymes and cytokines which in turn attracts the T-lymphocytes causing cell mediated immunity and formation of granulomas. Lesions begin to develop due to accumulation of T-lymphocytes and macrophages, and creates an environment which destroys macrophages and cause early necrosis. This results in cavity formation in lungs [4,5].

The target proteins utilized for the present computational work were Pantothenate synthetase, Thymidylate kinase and Filamentous temperature sensitive protein Z (FtsZ) protein. Pantothenate synthetase (PS) catalyzes an adenosine triphosphate (ATP)-dependent condensation of D-pantoate and beta-alanine to form pantothenate which is a key precursor for the biosynthesis of coenzyme A (CoA) and acyl carrier protein (ACP) which is implicated in various cell metabolic functions [6]. Apart from that, the enzyme PS is absent in mammals and both CoA and ACP are essential cofactors for bacterial growth, thus PS serves as an attractive chemotherapeutic agent [7].

Thymidylate kinase on the other hand, in the presence of ATP as phosphoryl donor, catalyzes the reaction in which reversible phosphorylation occurs which converts deoxythimidine monophosphate (dTMP) to deoxythimidine diphosphate (dTDP) and adenosine diphosphate (ADP) finally leading to the synthesis of deoxythimidine triphosphate which is essential for cell growth and DNA synthesis [8]. This depicts a way for the design of a specific Thymidylate kinase inhibitors using *in silico* methods.

ATP+dTMP= ADP+dTDP

FtsZ protein is involved in the Polymerisation reaction which occurs in protein in the presence of Guanethidine Triphosphate (GTP) to form a highly active cytokinetic ring (Z ring) which occurs at the septum site. When FtsZ assembly is altered, this result in inhibition of septum formation and Z ring thereby preventing cell division. Thus inhibition of FtsZ protein can prove to be an effective target for the treatment of tuberculosis [9].

Computer aided drug design play an essential role in order to assist in the drug discovery and research. The vital types involved in drug design are structure-based and ligand-based drug designing. In addition they help in the identification of novel lead molecules and play a major role in drug development. *In silico* approaches based on various interactions of phytoconstituents and target proteins have been employed for the present study to check their use for the control of TB [10]. The 28 phytoconstituents taken up for the study includes Agnuside, Alloin, Alliin, Alpha-amyrin, Alizarin, Aloe-emodin, Berberine, Coumaric acid, Kaempherol, Kumatakenin, Luteolin, Mimosine, Mangiferin, Myricetin, Niacin, Oleanane, Orientin, Protocatechuic acid, Piperine, Quinoline, Rottlerin, Sesamin, Scopoletin, Serotonin, Taraxerol, Thiamine, Ursolic acid and Xanthone. Isoniazid was taken up as the reference drug.

The drug likeness parameters calculation was based on a set of rules known as Lipinski rule of five. The molecular descriptors studied were the molecular weight of the ligand, which according to the rule states that the value should not exceed 500 Daltons. The logP values and the number of hydrogen bond donors should not be more than 5. Total number of hydrogen bond acceptors should not be more than 10 [11]. The bioactivity scores were studied using the parameters such as GPCR ligand, Ion-channel modulators, Kinase inhibitors, nuclear receptor ligands, Protease inhibitors and other enzyme targets. The binding energy parameter was measured based on interactional analysis between the proteins and the selected ligand molecules. The target proteins were subjected to primary and secondary structure analysis to determine whether the proteins were stable or not.

EXPERIMENTAL SECTION

The X-ray crystallographic structures of target proteins with PDB ID 3COW, 1G3U and 2Q1Y at resolutions 1.8 Å, 1.95 Å and 2.3 Å respectively were retrieved from the RCSB Protein Data Bank and saved as PDB file [12-14]. The water molecules and other unwanted residues were removed from the protein molecules. Addition of hydrogen atoms to the selected proteins was also performed. The proteins were then subjected to Primary and Secondary structure analysis using online tools ProtParam and SOPMA respectively [15, 16]. The physiochemical parameters of proteins studied were Molecular weight, Extinction coefficient, Theoretical pI, Half life, Grand Average Hydropathicity (GRAVY), Aliphatic index and Instability index [17]. The Extinction-coefficient indicates how much light a protein absorbs at a certain wavelength. They are measured in units of M-1 cm-1 at 280nm in water. The Half-life is the prediction of time it takes for half of the amount for protein to disappear after its synthesis in cell. The ProtParam is based on N-end rule which correlates the half-life of a protein to the identity of N-terminal residue [18]. The Aliphatic index is defined as the relative volume occupied by aliphatic side chains (alanine, valine, isoleucine, leucine) and helps to influence the thermostability of globular proteins. The GRAVY value for a protein is calculated as sum of hydropathy values of all aminoacids divided by number of residues in the sequence.

The ligand structures of naturally occurring compounds like Agnuside, Alloin, Alliin, Alpha-amyrin, Alizarin, Aloe-emodin, Berberine, Coumaric acid, Kaempherol, Kumatakenin, Luteolin, Mimosine, Mangiferin, Myricetin, Niacin, Oleanane, Orientin, Protocatechuic acid, Piperine, Quinoline, Rottlerin, Sesamin, Scopoletin, Serotonin, Taraxerol, Thiamine, Ursolic acid and Xanthone along with the standard drug Isoniazid were generated using ChemSketch and saved in PDB file format [19]. Energy optimization of these ligands was done using the software CORINA [20]. The various molecular properties of ligands were studied using the online tool Molinspiration [21]. The drug likeness was calculated using the following parameters such as total polar surface area (TPSA), number of atoms, molecular weight, number of hydrogen bond acceptors, number of hydrogen bond

donors, number of violations (nviolation) and number of rotatable bonds. The ligands were finally subjected to docking analysis using ArgusLab 4.0.1 software which operates on Lamarckian genetic algorithm [22].

RESULTS AND DISCUSSION

The 28 phytoconstituents with possible anti tubercular activity was generated using ChemSketch and saved in the PDB file format as compiled in Table 1.

Table 1: The phytoconstituents with possible anti tubercular activity

Sl no:	Phytoconstituents/ Reference drug	Botanical source	IUPAC name
1	Agnuside	*Vitex trifolia, Verbenaceae*	[(1S,4aR,5S,7aS)-5-Hydroxy-1-[(2S,3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxy-1,4a,5,7a-tetrahydrocyclopenta[c]pyran-7-yl]methyl 4-hydroxybenzoate
2	Aloin	*Aloe vera , Liliaceae*	(10S)-10-Glucopyranosyl-1,8-dihydroxy-3-(hydroxymethyl)-9(10H)-anthracenone
3	Alliin	*Allium cepa, Liliaceae*	(2R)-2-amino-3-[(S)-prop-2-enylsulfinyl]propanoic acid
4	Alpha-amyrin	*Canscori decussate, Gentianaceae*	α: (3β)-Urs-12-en-3-ol
5	Alizarin	*Morinda citrifolia, Rubiaceae*	1,2-dihydroxy-9,10-anthracenedione
6	Aloe-emodin	*Aloe vera, Xanthorrhoeaceae*	1,8-Dihydroxy-3-(hydroxymethyl)-9,10-anthracenedione
7	Berberine	*Tinospora cordifolia, Curbitaceae*	5,6-dihydro-9,10-dimethoxybenzo[g]-1,3-benzodioxolo[5,6-a]quinolizinium
8	Coumaric acid	*Prunus armeniaca, Rosaceae*	3-(4-hydroxyphenyl)-2-propenoic acid
9	Kaemferol	*Acalypha indica, Euphorbiaceae*	3,5,7-Trihydroxy-2-(4-hydroxyphenyl)-4H-chromen-4-one
10	Kumatakenin	*Parthenium integrifolium, Asteraceae*	5-hydroxy-2-(4-hydroxyphenyl)-3,7-dimethoxychromen-4-one
11	Luteolin	*Vitex trifolia, Verbenaceae*	2-(3,4-Dihydroxyphenyl)- 5,7-dihydroxy-4-chromenone
12	Mimosine	*Mimosa pudica, Mimosaceae*	(2S)-2-Amino-3-(3-hydroxy-4-oxopyridin-1-yl)propanoic acid
13	Mangiferin	*Canscora decussate, Gentianaceae*	(1S)-1,5-Anhydro-1-(1,3,6,7-tetrahydroxy-9-oxo-9H-xanthen-2-yl)-D-glucitol
14	Myricetin	*Myrtus communis, Myrtaceae*	3,5,7-Trihydroxy-2-(3,4,5-trihydroxyphenyl)-4-chromen-4-one
15	Niacin	*Trichosanthes dioica, Cucurbitaeae*	pyridine-3-carboxylic acid
16	Oleanane	*Byrsonima crassa, Malpighiaceae*	Oleanane
17	Orientin	*Vitex trifolia, Verbenaceae*	2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-8-[(2S,3R,4R,5S,6R)-3,4,5-trihydroxy-6-(hydroxyl methyl)oxan-2-yl]chromen-4-one
18	Protocatechuic acid	*Prunus armeniaca, Rosaceae*	3,4-Dihydroxybenzoic acid
19	Piperine	*Piper speies, Piperaceae*	1-[5-(1,3-Benzodioxol-5-yl)-1-oxo-2,4-pentadienyl]piperidine
20	Quinoline	*Acalypha indica, Euphorbiaceae*	2-azabicyclo[4.4.0]deca-1(6),2,4,7,9-pentaene
21	Rottlerin	*Mallotus philippensis, Euphorbiaceae*	(E)-1-[6-[(3-acetyl-2,4,6-trihydroxy-5-methylphenyl)methyl]-5,7-dihydroxy-2,2-dimethylchromen-8-yl]-3-phenylprop-2-en-1-one
22	Sesamin	*Piper species, Piperaceae*	5,5'-(1S,3aR,4S,6aR)-tetrahydro-1H,3H- furo[3,4-c]furan-1,4-diylbis(1,3-benzodioxole)
23	Scopoletin	*Scopolia carniolica, Solanaceae*	7-hydroxy-6-methoxychromen-2-one
24	Serotonin	*Mucuna pruriens, Fabaceae*	5-Hydroxytryptamine
25	Taraxerol	*Myrica cerifera, Myricaceae*	(3*S*,4a*R*,6a*R*,6a*S*,8a*R*,12a*R*,14a*R*,14b*R*)-4,4,6a,6a,8a,11,11,14b-octamethyl-1,2,3,4a,5,6,8,9,10,12,12a,13,14,14a-tetradecahydropicen-3-ol
26	Thiamine	*Trichosanthes dioica, Cucurbitaceae*	3-((4-Amino-2-methyl-5-pyrimidinyl)methyl)- 5-(2-hydroxyethyl)-4-methylthiazolium chloride
27	Ursolic acid	*Ocimum sanctum, Labiatae*	(1*S*,2*R*,4a*S*,6a*R*,6a*S*,6b*R*,8a*R*,10*S*,12a*R*,14b*S*)-10-hydroxy-1,2,6a,6b,9,9,12a-heptamethyl-2,3,4,5,6,6a,7,8,8a,10,11,12,13,14b-tetradecahydro-1*H*-picene-4a-carboxylic acid
28	Xanthone	*Canscora decussate, Gentianaceae*	*9H*-xanthen-9-one
29	Isoniazid		pyridine-4-carbohydrazide

Table 2. Calculation of drug likeness of phytoconstituents

Sl no:	Constituents	milog p	TPSA	n atoms	n ON	n OH NH	n violation	n rotb	MW	Volume
1	Agnuside	0.181	155.14	32	10	5	0	7	466.44	394.43
2	Aloin	0.176	167.9	30	9	7	1	3	418.40	352.43
3	Alliin	-3.39	80.39	11	4	3	0	5	177.22	154.76
4	Alpha-amyrin	6.63	20.23	25	1	1	1	0	426.73	461.05
5	Alizarin	2.9	74.6	18	4	2	0	0	240.21	198.61
6	Aloe-emodin	2.42	94.83	20	5	3	0	1	270.24	223.43
7	Berberine	0.2	40.82	25	5	0	0	2	336.37	296.30
8	Coumaric acid	1.43	57.527	12	3	2	0	2	164.16	146.48
9	Kaemferol	2.172	111.123	21	6	4	0	1	286.24	232.07
10	Kumatakenin	2.98	89.14	23	6	2	0	3	314.29	267.12
11	Luteolin	1.97	111.12	21	6	4	0	1	286.24	232.07
12	Mimosine	-4.16	105.56	14	6	4	0	3	198.18	168.33
13	Mangiferin	0.536	181.041	29	10	7	1	2	422.34	325.80
14	Myricetin	1.39	151.58	23	8	6	1	1	318.24	248.10
15	Niacin	0.27	50.19	9	3	1	0	1	123.11	106.89
16	Oleanane	8.86	0	30	0	0	1	0	412.75	458.87
17	Orientin	0.03	201.27	32	11	8	2	3	448.38	363.22
18	Protocatechuic acid	0.88	77.755	11	4	3	0	1	154.12	127.08
19	Piperine	3.33	38.78	21	4	0	0	3	285.34	267.74
20	Quinoline	1.94	12.89	10	1	0	0	0	129.16	123.88
21	Rottlerin	5.72	144.52	38	8	5	2	6	516.55	457.44
22	Sesamin	3.69	55.4	26	6	0	0	2	354.36	300.51
23	Scopoletin	1.329	59.673	14	4	1	0	1	192.17	162.15
24	Serotonin	0.568	62.042	13	3	4	0	2	176.22	165.93
25	Taraxerol	8.02	20.23	31	1	1	1	0	426.73	460.70
26	Thiamine	-3.45	75.92	18	5	3	0	4	265.36	239.76
27	Ursolic acid	6.79	57.53	33	3	2	1	1	456.71	471.49
28	Xanthone	3.57	30.211	15	2	0	0	0	196.21	172.58
29	Isoniazid	-0.97	68.01	10	4	3	0	1	137.14	122.56

TPSA:total polar surface area; n atoms: number of atoms ; MW: molecular weight; n ON: number of hydrogen bond acceptors ; n OHNH : number of hydrogen bond donors; n rotb: number of rotatable bonds.

The ligand molecules were then subjected to molecular property calculation using online tool Molinspiration. The properties to calculate drug likeness were total polar surface area (TPSA), number of atoms, molecular weight, number of hydrogen bond acceptors, number of hydrogen bond donors, number of violations (nviolation) and number of rotatable bonds as tabulated in Table 2.

According to lipinski rule of five, the logP value should not be greater than 5. Alpha-amyrin, Oleanane, Rottlerin, Taraxerol, Ursolic acid showed slight variation in log P values whereas all other phytoconstituents were satisfactory as their log P values are less than 5, which points out the good permeability of these constituents across the cell membrane or moderate blood-brain barrier penetration. The molecular weight as per the lipinski rule states that, it should not be more than 500 daltons, all phytoconstituents followed the lipinski rule of five except Rottlerin whose molecular weight was more than 500 daltons. If the TPSA is below 160Å and nviolation =1 or <0 the compounds will bind to the receptors readily. Aloin, Mangiferin and Orientin have higher TPSA values while Orientin and Rottlerin have more than 1 violations. All the phytoconstituents have number of rotatable bonds less than 10, hence they satisfy the lipinski rule of five. The number of H-bond acceptors should be < or = 10 and the selected phytoconstituents follow the rule except Orientin which have a slight higher value. The number of H-bond donors should be < or =5 but Aloin, Mangiferin, Myricetin and Orientin have slight variation compared to the remaining phytoconstituents which follow the rule. The molecular properties were calculated and compared with that of standard drug Isoniazid. The bioactivity score was calculated using the same tool as compiled in Table 3.

Table 3. Calculation of Bioactivity score of phytoconstituents

Sl no:	Constituents	GPCR ligand	Ion channel modulator	Kinase inhibitor	Nuclear receptor ligand	Protease inhibitor	Enzyme inhibitor
1	Agnuside	0.05	0.11	-0.13	0.07	0.2	0.31
2	Aloin	0.18	0.08	0.14	0.33	0.08	0.35
3	Alliin	-0.59	-0.38	-1.42	-1.01	-0.3	0.07
4	Alpha-amyrin	0.24	0.11	-0.19	0.56	0.02	0.42
5	Alizarin	-0.26	-0.15	-0.01	-0.08	-0.38	0.21
6	Aloe-emodin	-0.02	0.02	0.12	0.24	0.04	0.38
7	Berberine	-0.11	0.71	-0.27	-0.78	-0.35	0.82
8	Coumaric acid	-0.56	-0.25	-0.91	-0.12	-0.85	-0.15
9	Kaemferol	-0.1	-0.21	0.21	0.31	-0.27	0.26
10	Kumatakenin	-0.1	-0.21	0.13	0.22	-0.26	0.17
11	Luteolin	-0.02	-0.07	0.26	0.39	-0.22	0.28
12	Mimosine	-0.39	0.09	-0.44	-0.51	-0.07	0.42
13	Mangiferin	0.18	-0.07	0.19	0.3	0.03	0.58
14	Myricetin	-0.06	-0.18	0.28	0.32	-0.2	0.3
15	Niacin	-2.01	-1.31	-2.1	-2.07	-2.22	-1.34
16	Oleanane	0.21	0.22	-0.20	0.54	0	0.49
17	Orientin	0.12	-0.14	0.19	0.2	0.01	0.45
18	Protocatechuic acid	-0.88	-0.35	-1.1	-0.58	-1.09	0.34
19	Piperine	0.15	-0.18	-0.13	-0.13	-0.1	0.04
20	Quinoline	-0.81	-0.15	-0.61	-1.26	-1.18	-0.35
21	Rottlerin	0.08	-0.17	-0.08	0.3	0.11	0.27
22	Sesamin	0.02	-0.31	-0.27	-0.08	-0.15	0.03
23	Scopoletin	-1	-0.65	-0.95	-0.81	-1.16	-0.24
24	Serotonin	0.14	0.33	0.12	-0.61	-0.38	0.2
25	Taraxerol	0.21	0.02	-0.2	0.54	0	0.49
26	Thiamine	0.26	0.01	-0.37	-1.72	-0.64	1.12
27	Ursolic acid	0.28	-0.03	-0.5	0.89	0.23	0.69
28	Xanthone	-0.59	-0.34	-0.54	-0.51	-0.71	-0.09
29	Isoniazid	-1.39	-1.45	-1.05	-2.033	-1.23	-0.66

The bioactivity score was calculated for various parameters like GPCR ligand, ion channel modulator, kinase inhibitor, nuclear receptor ligand, protease inhibitor and enzyme inhibitor. The bioactivity score was compared with the values of standard drug Isoniazid. The phytoconstituents were comparable with that of the reference drug.

The *in silico* analysis was done to analyse parameters like primary and secondary structure of target proteins 3COW, 2Q1Y and 1G3U. The total number of aminoacids for pantothenate synthetase enzyme 3COW was calculated to be 602. The molecular weight was found to be 63060.5g and the theoretical pI 5.76. The total number of negatively charged residues (Asp+Glu) was 62 and total number of positively charged residues (Arg+Lys) was 54. The total number of atoms was computed to be 8969. The extinction coefficient was found to be 20860 at absorbance 0.1%(=1g/l) 0.331. The N-terminal of the sequence is considered as A (ala). The estimated half-life was calculated as 4.4 hours (mammalian reticulocytes, in vitro) , >20 hours (yeast , in vivo), >10 hours (Escherichia coli, in vivo). The instability index was 26.66, which helped to classify the protein as stable. The aliphatic index was found to be 104.78 therefore the protein demonstrates considerable thermostability whereas the Grand Average of Hydropathicity was found to be 0.223. The secondary structure analysis of pantothenate synthetase was performed. The alpha helix was found to be 238 (39.53%); 3_{10} helix (Gg), Pi helix (Ii), Beta bridge (Bb), Bend region (Ss) was 0%; the extended strand (Ee) was 107 (17.77%); the Beta turn (Tt) was found to be 72 (11.96%) and the random coil (Cc) as 185 (30.73%).

The total number of aminoacids for Thymidylate kinase enzyme 1G3U was calculated to be 404. The molecular weight of the protein was found to be 45792.1 and the theoretical pI was 8.89. The total number of negatively charged residues was found to be 38 and the total number of positively charged residues was 44. The total number of atoms was 6423 whereas the extinction coefficient was found to be 35090. The N terminal sequence was considered as G (Gly).The instability index was found to be 21.63 which classifies the protein as stable. The aliphatic index was found to be 79.21 with considerable thermostability. The estimated half life was calculated as 30 hours (mammalian reticulocytes , in vitro), >20 hours (yeast, in vivo), >10 hours (Escherichia coli, in vivo). The Grand average of hydropathicity was found to be -0.269 which indicates that the protein was hydrophilic in nature. The alpha helix (Hh) was found to be 113 (53.80%), 3_{10} helix (Gg), Pi helix (Ii), Beta bridge (Bb), Bend region (Ss) was 0%. The extended strand (Ee) was 26 (12.15%), the Beta turn (Tt) was found to be 23 (10.75%) and the random coil (Cc) as 52 (24.30%).

The total number of aminoacids for FtsZ protein 2Q1Y was found to be 214. The molecular weight was calculated to be 22634.5, the theoretical Pi was 6.92; the total number of negatively charged residues was computed to be 24 and the total number of positively charged residues was found to be 24. The total number of atoms was 3181 with the Extinction coefficient as 32430. The N terminal sequence considered as M (Met). The instability index was found to be 25.33 which classifies the protein as stable. The aliphatic index was found to be 92.34 with considerable thermostability. The estimated half life was calculated as 30 (mammalian reticulocytes, in vitro), >20 hours (yeast, in vivo), >10 hours (Escherichia coli, in vivo). The Grand average hydropathicity was found to be -0.067, the protein being hydrophilic in nature. The alpha helix (Hh) was found to be 154 (38.12%), 3_{10} helix (Gg), Pi helix (Ii), Beta bridge (Bb), Bend region (Ss) was 0%. The extended strand (Ee) was 104 (25.74%). The Beta turn (Tt) was found to be 38 (9.41%) and the random coil (Cc) as 108 (26.73%).

The target proteins were then subjected to interactional analysis along with the phytoconstituents as the ligand molecules. The Pantothenate synthetase enzyme 3COW was docked with the phytoconstituents and it was observed that Agnuside, Alizarin, Kaempherol and Myricetin displayed good interaction with the protein molecule (results complied in Table 4).

Table 4. Docking analysis of Pantothenate synthetase 3COW

Sl no:	Phytoconstituents	Docking Score (kcal/mol)
1	Agnuside	-9.20907
2	Aloin	-8.83332
3	Alliin	-6.41706
4	Alizarin	-9.38951
5	Aloe-emodin	-7.86179
6	Berberine	-6.84281
7	Coumaric acid	-7.73076
8	Kaempherol	-9.23243
9	Kumatakenin	-7.52182
10	Luteolin	-7.94638
11	Myricetin	-9.40155
12	Niacin	1.72507
13	Orientin	-8.61962
14	Protocatechuic acid	-6.95997
15	Piperine	-7.29103
16	Quinoline	-7.85259
17	Sesamin	-7.81743
18	Scopoletin	-7.58885
19	Serotonin	-7.63947
20	Thiamine	-7.55403
21	Xanthone	-8.52801
22	Isoniazid	-7.20731

The Thymidylate kinase enzyme 1G3U was docked with the phytoconstituents and it was observed that Alliin, Coumaric acid, Protocatechuic acid and Serotonin showed good interaction with the protein molecule as in Table 5.

Table 5. Docking analysis of Thymidylate kinase 1G3U

Sl no:	Constituents	Docking Score (kcal/mol)
1	Alliin	-6.30577
2	Coumaric acid	-6.7028
3	Niacin	-5.68265
4	Protocatechuic acid	-6.49111
5	Quinoline	-5.98143
6	Scopoletin	26.2123
7	Serotonin	-6.38362
8	Thiamine	-5.64063
9	Xanthone	-5.89264
10	Isoniazid	-6.61723

The FtsZ protein 2Q1Y was docked with the phytoconstituents and it was observed that Aloin, Agnuside, Aloe-emodin, Mangiferin and Rottlerin pointed out considerable interaction with the protein molecule as in compiled in Table 6

Table 6. Docking(2Q1Y); FtsZ protein

Sl no:	Constituents	Docking Score (kcal/mol)
1	Agnuside	-9.11566
2	Aloin	-10.1576
3	Alliin	-6.61515
4	Alizarin	-8.39*708*
5	Aloe-emodin	-9.08172
6	Berberine	-6.57969
7	Coumaric acid	-7.74146
8	Kaemferol	-7.6837
9	Kumatakenin	-7.17538
10	Luteolin	-7.86693
11	Mangiferin	-9.4309
12	Myricetin	-8.59046
13	Niacin	-6.09658
14	Oleanane	-8.22058
15	Protocatechuic acid	-7.35478
16	Piperine	-6.81955
17	Quinoline	-7.54807
18	Rottlerin	-9.71537
19	Sesamin	-7.43762
20	Scopoletin	-7.64953
21	Serotonin	-7.893
22	Taraxerol	-7.98929
23	Xanthone	-7.43
24	Isoniazid	-7.38149

CONCLUSION

The computational analysis proved to be helpful to evaluate drug likeness of various phytoconstituents. Multiple targets were taken and docked with the same phytoconstituents for the comparison of the interactional energies. It was observed that phytoconstituents such as Agnuside, Alizarin, Kaempherol and Myricetin when docked with Pantothenate synthetase enzyme 3COW displayed significant interaction. The Thymidylate kinase enzyme 1G3U was docked with the phytoconstituents and it was observed that the compounds such as Alliin, Coumaric acid, Protocatechuic acid and Serotonin proved to be useful. The FtsZ protein 2Q1Y was docked with the phytoconstituents and was observed that Aloin, Agnuside, Aloe-emodin, Mangiferin and Rottlerin elicited out good binding interaction. Thus the selection of natural compounds for inhibiting enzymes such as Pantothenate synthetase, Thymidylate kinase, Filamentous Temperature Sensitive protein Z could prove to be effective in the treatment of TB when compared with that of standard drug Isoniazid. Further investigation is essential for the development of potent inhibitors for the control of the disease.

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