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Research Article

MOLECULAR DOCKING OF ANTITRYPANOSOMAL INHIBITORS FROM *EUCALYPTUS TERETICORNIS* FOR SLEEPING SICKNESS

AARTHI RASHMI B*, HARISHCHANDER A, PRIYANKA K, VASANTH NIRMAL BOSCO

Department of Bioinformatics, Sri Krishna Arts and Science College, Coimbatore, Tamil Nadu, India. Email: aarthirashmi@skasc.ac.in

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ABSTRACT

Objectives: This study aims to investigate the antitrypanosomal inhibitors of *Eucalyptus tereticornis* for sleeping sickness through molecular docking and studies on Absorption distribution metabolism excursion and toxicology (ADMET).

Methods: *In silico* molecular docking in ArgusLab software and ADMET analysis in AdmetSAR software was performed for the antitrypanosomal inhibitors of *E. tereticornis* for sleeping sickness.

Results: Interactions were studied for the ten proteins responsible for sleeping sickness with the 50 antitrypanosomal inhibitors of *E. tereticornis*. Docking was performed to see the interaction and the best binding energy of compounds with the proteins involved in sleeping sickness. The docking scores were highest for betulonic acid with −15.66 kcal/mol followed by euglobal with −12.24 kcal/mol, B-pinene with −10.313 kcal/mol, A-pinene with −10.3418 kcal/mol, and the least docking score for P-cymene with −10.6045 kcal/mol. Docking results showed that only betulonic acid and euglobal showed that hydrogen bond interaction was as b-pinene, a-pinene, and p-cymene yielded no hydrogen bond interactions so we will be taking the former docking results for further studies. The best docking result was shown by betulonic acid with trypanothione reductase giving binding energy of −15.66 kcal/mol with hydrogen bond interaction of 2.9, so this result was taken for further analysis.

Conclusion: The results of the compound extracted from *E. tereticornis* will become physiological relevant only when (i) the pure compounds of this plant is available in large quantities; (ii) the *Eucalyptus* is biochemically stabilized to avoid degradation and enhance absorption in the gastrointestinal tract; and (iii) special delivery methods for this drug to reach the areas of treatment. In this work, the efficacy of *E. tereticornis* to act against trypanosomal protein was initiated and thus further research in this process would help us to take full advantage of the remedial effects of the compounds extracted from this plant.

Keywords: Antitrypanosomal Inhibitors, *Eucalyptus tereticornis*, Sleeping sickness, Molecular Docking, ADMET studies.

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INTRODUCTION

Human trypanosomiasis, also known as "sleeping sickness," is caused by microscopic parasites of the species *Trypanosoma brucei.* At present, about 10,000 new cases each year are reported to the World Health Organization occurred in India in 2007 and re-emerging these days; however, it is believed that many cases go undiagnosed and unreported. Sleeping sickness is curable with medication but is fatal if left untreated [1]. A report on a case of a 37-day-old infant from Uttar Pradesh who was presented with fever, lethargy, and convulsions, and who had a history of painful insect bite the day before admission [2]. In some cases, a pregnant woman can pass the infection to her fetus. In theory, the transmission of infection can also be by the transfusion of blood or sexual contact, but such cases were rarely documented [3]. The course of untreated infection rarely lasts longer than 6–7 years and more often kills in about 3 years [4]. The widely used criteria for defining the second stage in disease are the observation of trypanosomes in CSF or a white blood cell count of six or higher. Other indications of the second-stage disease include elevated protein and an increase in nonspecific immunoglobulin M in CSF [5]. Medicinal plants have served as raw materials for natural product isolation and screening. *Cetraria islandica* is a well-known medicinal plant commonly referred to as Iceland moss [6]. Mosses have yielded very interesting bioactive compounds in the past. Previous studies have yielded from *C. islandica*, usnic acid and isousnic acid, protolichesteric acid, myelochroic acid, lichesterinic acid, praesorediosic acid, phaseolin acid, and dihydropertusaric acid, also the naphthazarin, hybocarpone, naphthoquinones, anthraquinones, and dibenzofurans. The present study demonstrates *in vitro* antitrypanosomal activity of four isolated phytoconstituents from lichen *C. islandica* and supporting evidence with the help of docking studies [7]. Antimicrobial activity was studied on the basis of the phytochemical analysis of methanolic extracts from the bark and leaf of *Eucalyptus tereticornis* [8].

The goal of the current study was to identify the protein targets that the medicinal plants target selectivity for phytochemical classes. In doing so, we have theoretically identified the strongly interacting plant chemicals and their biomolecular targets. These results should lead to further research to verify the efficacy of phytochemical agents [8]. *In silico* screening of small molecules has been at the forefront of drug discovery in recent years. There are various drug targets in *T. brucei*. These include trypanothione reductase (TR), rhodesain, triosephosphate isomerase (TIM), and farnesyl diphosphate synthase, in line with the fact that target-based drug discovery efforts remain a front runner in lead identification [9,10]. The clinical significance of the young febrile infant was malaria, bacterial sepsis, or viral fever. The clinical diagnosis of trypanosomiasis was surprising and incidental because this parasitic infection in humans is very rare in India. The characteristic morphology and the polymerase chain reaction made the diagnosis unequivocal. However, a causal association between the parasite and the febrile illness is difficult to establish [11]. The patient was treated with suramin, a drug used for the treatment of human African trypanosomiasis. The authors hypothesized that the patient was infected by a wound in the index finger while delivering an infected cattle or a bite by the flies of *Tabanidstriatus* to transmit infection in animals. Subsequently, a serologic study was conducted in the same village, and it illustrated that the sera of 81 of 1806 people (4.5%)

were seropositive for *Trypanosoma evansi* infection by the card agglutination test but none had parasitemia on peripheral blood [11].

METHODS

Bioinformatics is vital to significantly improve the position and function of molecules in binding and simulation. In bioinformatics, the process of computer-aided drug design (CADD) exists as a specialized discipline to use the computational [12] methods to simulate the interactions between a drug and a receptor. CADD methods are heavily dependent on bioinformatics tools, applications, and databases. The small molecules used in this study have been taken from literature survey; they have been selected on the criteria that these ligands have not been used prior used as antitrypanosomal studies. The structures of the ligand were downloaded directly from PubChem.

Retrieval of the target protein

The 3D structures of the target trypanosomal proteins were downloaded from the Protein data bank database (PDB) in.pdb format.

Protein preparation

Protein-ligand docking studies [13] were carried out based on the crystal structures of *T. brucei* adenosine kinase, TbAK (PDB 2xtb and PDB 3otx), *T. brucei* pteridine reductase 1 (TbPTR1), TbPTR1 (PDB 3jq7), *T. brucei* dihydrofolate reductase (TbDHFR), TbDHFR (PDB 3rg9 and PDB 3qfx), *T. brucei* trypanothione reductase, *T. brucei* cathepsin B, *T. brucei* heat shock protein 90 (TbHSP90), TbHSP90 (PDB 3omu and PDB 3opd), *T. brucei* sterol 14α-demethylase, *T. brucei* nucleoside hydrolase (TbNH), TbNH (PDB 3fz0), *T. brucei* TIM (TbTIM), and TbTIM (PDB 1iih, *T. brucei* nucleoside 2-deoxyribosyltransferase, and *T. brucei* ornithine decarboxylase, TbODCPDB 1njj). The solvent molecules and the cocrystallized ligands were removed from the crystal structure. To be used as a receptor for docking, protein structures should be processed. Some of the typical operations include (i) addition of hydrogen atoms, (ii) elimination of water molecules that are not involved in ligand binding, and (iii) making binding groups. This was done in ArgusLab.

Protein-ligand interaction using ArgusLab

The compounds isolated from the plants were docked against the proteins using ArgusLab, to find the reasonable binding geometries and explore the protein-ligand interactions. Docking of the protein-ligand complex was mainly targeted to the predicted active site only. The selected residues of the receptor were defined to be a part of the binding site. All the compounds in the dataset were docked into the active site of the protein following the same procedure. After docking, the docked protein (protein-ligand complex) was analyzed to investigate the type of interactions. The poses of docking were saved for each compound and ranked according to their function. The pose having the highest dock score was selected for further analysis [14] (QSAR studies).

RESULTS

This study was conducted to understand the interactions between the proteins and the ligand to discover their binding affinity. This docking study was executed using ArgusLab. The 3D structure of the trypanosomal protein was downloaded from PDB and used as a target for docking. The results are as follows.

Protein's binding site prediction

CASTp was used for predicting the binding site of the protein. The active site of protein comprises of amino acid for 3OTX, 3QFX, 3JQ7, 3FZO, 2XTB, 1NJJ, 3RG9, and 1IIH is listed in Tables 1-8.

In Table 1, the position of amino acids in the active sites of the protein with PDB id 3OTX was analyzed by CASTp server.

In Table 2, the position of amino acids in the active sites of the protein with PDB id 3QFX was analyzed by CASTp server.

In Table 3, the position of amino acids in the active sites of the protein with PDB id 3JQ7 was analyzed by CASTp server.

Table 1: Active sites of the protein with PDB id 3OTX

S. No.	Amino acid	Active sites
1	Cystine	12, 123, 239
2	Arginine	7, 34, 58, 70, 94, 132, 156, 223, 245, 265, 316, 332
3	Asparagine	13, 56, 67, 195, 222, 231, 295
4	Leucine	15, 16, 39, 134, 138, 286
5	Aspergin	17, 92, 238, 266, 287, 289, 293, 299
6	Serine	19, 64, 197, 269
7	Alanine	20, 37, 78, 102, 111, 136, 153, 198, 157, 221, 297, 300, 326
8	Histidine	21, 105, 114, 224, 323
9	Glucine	33, 101, 104, 106, 131, 160, 225, 279, 268, 241, 228, 328, 339
10	Glycine	35, 62, 63, 81, 107, 129, 298, 296
11	Threonine	36, 85, 172, 264, 270, 280, 325
12	Isoleucine	38, 90, 108, 127, 267, 292, 330
13	Proline	55, 61, 199, 282, 284, 338
14	Valine	57, 60, 68, 71, 98, 109, 283, 240, 125, 278, 291, 329
15	Tyrosine	59, 79, 95, 165
16	Glutamine	73, 77, 203, 285, 288, 327
17	Trypsin	74
18	Lysins	80, 82, 97, 100, 130, 227, 340
19	Methionine	110, 294, 302
20	Phenylalanine	169, 200, 301

Table 2: Active sites of the protein with PDB id 3QFX

S. No.	Amino acid	Active sites
1	Arginine	59, 84, 95, 100, 107, 183
2	Leucine	90, 97, 105
3	Aspergin	43, 45, 54, 88, 120
4	Serine	89, 98, 106, 108, 192, 216
5	Alanine	34, 226
6	Histidine	182
7	Glycine	42, 44, 45, 83, 136, 161, 162, 163
8	Threonine	46, 86, 164, 184
9	Isoleucine	41, 47, 51, 118, 160, 165
10	Proline	48, 52, 91, 92,119
11	Valine	32, 33, 195
12	Glutamine	50, 234
13	Trypsin	57, 166
14	Lysins	85, 93, 123, 235
15	Methionine	55, 82
16	Phenylalanine	58, 94, 233

Table 3: Active sites of the protein with PDB Id 3JQ7

S. No.	Amino acid	Active sites
1	Arginine	89, 98, 106, 108, 192, 216
2	Leucine	90, 97, 105, 137, 168
3	Aspergin	46, 86, 164, 184
4	Serine	43, 45, 54, 88, 120
5	Alanine	34, 226
6	Histidine	52, 60, 101
7	Glycine	42, 44, 45, 83, 136, 161, 162, 163
8	Threonine	46, 86, 164, 184
9	Isoleucine	41, 47, 51, 118, 160, 165
10	Glutamine	50, 234
11	Trypsin	30, 168
12	Lysins	85, 93, 123, 235
13	Methionine	55, 90, 100, 105
14	Phenylalanine	55, 98, 133

In Table 4, the position of amino acids in the active sites of the protein with PDB id 3FZO was analyzed by CASTp server.

In Table 5, the position of amino acids in the active sites of the protein with PDB id 2XTB was analyzed by CASTp server.

Table 4: Active sites of the protein with PDB id 3FZO

S. No.	Amino acid	Active sites
1	Serine	20, 56, 88
2	Alanine	34, 56, 123, 226
3	Histidine	136, 161, 162, 163
4	Glycine	42, 44, 45, 83
5	Threonine	46, 86, 164, 184
6	Isoleucine	28, 51, 118, 160, 165
7	Glutamine	52, 60, 10150, 234
8	Trypsin	30, 123, 235
9	Lysins	85, 93, 168
10	Methionine	155, 290, 305
11	Phenylalanine	133

Table 5: Active sites of the protein with PDB id 2XTB

S. No.	Amino acid	Active sites
1	Cystine	25, 33, 140
2	Arginine	57, 60, 68, 71, 98, 109, 283, 240
3	Asparagine	125, 278, 291, 329
4	Alanine	19, 64, 197, 269
5	Histidine	21, 105, 114, 224, 323
6	Glucine	33, 101, 104, 106, 131, 160, 225, 279, 268, 241, 228, 328, 339
7	Glycine	35, 62, 63, 81, 107, 129, 298, 296
8	Threonine	36, 85, 172, 264, 270, 280, 325
9	Isoleucine	38, 90, 108, 330125, 278, 291, 329
10	Proline	55, 61, 199, 282, 284, 338127, 267, 292
11	Valine	57, 60, 68, 71, 98, 109, 283, 240
12	Tyrosine	59, 79, 95, 165
13	Glutamine	73, 77, 203, 82, 97, 100, 130, 227
14	Trypsin	301
15	Lysins	80, 285, 288, 327340
16	Methionine	200, 74
17	Phenylalanine	169, 110, 294, 302

Table 6: Active sites of the protein with PDB id 1NJJ

S. No.	Amino acid	Active sites
1	Arginine	33, 35, 106, 108, 192, 216, 289
2	Leucine	105, 137, 168, 190, 197, 206
3	Aspergin	46, 86, 164, 184
4	Serine	43, 45, 54, 88, 120
5	Alanine	34, 226
6	Histidine	52, 60, 101
7	Glycine	42, 44, 45, 83, 136, 161, 162, 163
8	Threonine	46, 86, 164, 184
9	Isoleucine	50, 41, 47, 51
10	Glutamine	234
11	Trypsin	30, 168
12	Lysins	55, 98, 133
13	Methionine	55, 90, 100, 105
14	Phenylalanine	85, 93, 123, 235

In Table 6, the position of amino acids in the active sites of the protein with PDB id 1NJJ was analyzed by CASTp server.

In Table 7, the position of amino acids in the active sites of the protein with PDB id 3RG9 was analyzed by CASTp server.

In Table 8, the position of amino acids in the active sites of the protein with PDB id 1IIH was analyzed by CASTp server.

Docking of proteins with plant compounds

In this study, the interactions between the ligands and various trypanosomal proteins were explored to check their binding affinity; docking study was performed using ArgusLab. The interaction between the protein and ligand was analyzed on the basis of binding energy and the results are compiled in Tables 9-17.

Table 7: Active sites of the protein with PDB id 3RG9

S. No.	Amino acid	Active sites
1	Cystine	20, 37, 78, 102, 111, 136, 153, 198, 157, 221, 297, 300, 326
2	Arginine	223, 245, 265, 316, 332
3	Asparagine	13, 56, 67, 195, 222, 231, 295
4	Leucine	7, 34, 58 70, 94, 132, 156
5	Aspergin	17, 92, 238, 266, 287, 289, 293, 299
6	Serine	15, 16, 39, 134, 138
7	Alanine	28619, 64, 197, 269, 12, 123, 239
8	Histidine	21, 105, 114, 224, 323
9	Glucine	33, 101, 104, 106, 131, 160, 225, 279, 268, 241, 228, 328, 339
10	Glycine	35, 62, 63, 81, 107, 129, 298, 296
11	Threonine	36, 85, 172, 264, 270, 280, 325
12	Isoleucine	38, 90, 108, 127, 267, 292, 330
13	Proline	55, 61, 199, 282, 284, 338
14	Valine	57, 60, 68, 71, 98, 109, 283
15	Tyrosine	59, 79, 95, 165, 240, 125, 278, 291, 329
16	Glutamine	73, 77, 203, 285, 288, 327
17	Trypsin	294, 302
18	Lysins	80, 82, 97, 100, 130, 227, 340
19	Methionine	111, 265
20	Phenylalanine	169, 200, 301

Table 8: Active sites of the protein with PDB id 1IIH

S. No.	Amino acid	Active sites
1	Cystine	25, 33, 140
2	Asparagine	125, 278, 291, 329
3	Arginine	57, 60, 68, 71, 98, 109, 283
4	Alanine	19, 64, 197, 269
5	Histidine	21, 105, 114, 224, 323
6	Glucine	160, 225, 279, 268, 241, 228, 328, 339
7	Glycine	35, 62, 63, 81, 107, 129, 298, 296
8	Threonine	36, 85, 172, 264, 270, 280, 325
9	Isoleucine	33, 101, 104, 106, 131
10	Proline	33, 81, 27, 267, 292
11	Valine	57, 60, 68, 71, 98, 109, 283, 240
12	Tyrosine	59, 79, 95, 165
13	Glutamine	73, 77, 203, 82, 97, 100, 130, 227
14	Trypsin	11, 33, 62
15	Lysins	80, 285, 288, 32, 73, 40

Table 9: Binding of ligands with the pterine reductase

S. No.	Protein	Ligand	Binding enery
1	Pterine reductase	Betulonic acid	−7.698 kcal/mol
2	Pterine reductase	Euglobal	−8.24 kcal/mol
3	Pterine reductase	Beta pinene	−8.313 kcal/mol
4	Pterine reductase	Alpha-pinene	−10.18 kcal/mol
5	Pterine reductase	P-Cymene	−8.6045 kcal/mol

Table 10: Binding of ligands with adenosine monophosphate

S. No.	Protein	Ligand	Binding energy
1	Adenosine monophosphate	Betulonic acid	−9.544 kcal/mol
2	Adenosine monophosphate	Euglobal	−9.042 kcal/mol
3	Adenosine monophosphate	Beta pinene	−10.31 kcal/mol
4	Adenosine monophosphate	Alpha pinene	−8.844 kcal/mol
5	Adenosine monophosphate	P-Cymene	−8.386 kcal/mol

In Table 9, alpha-pinene has the minimum binding energy with pterine reductase.

In Table 10, beta-pinene has the minimum binding energy with adenosine monophosphate.

Table 11: Binding of ligands with ornithine decarboxylase

S. No.	Protein	Ligand	Binding energy
1	Ornithine decarboxylase	Betulonic acid	−8.386 kcal/mol
2	Ornithine decarboxylase	Euglobal	−7.24 kcal/mol
3	Ornithine decarboxylase	Beta-pinene	−9.313 kcal/mol
4	Ornithine decarboxylase	Alpha-pinene	−10.18 kcal/mol
5	Ornithine decarboxylase	P-Cymene	−10.31 kcal/mol

Table 12: Binding of ligands with dihydrofolate reductase

S. No.	Protein	Ligand	Binding energy
1	Dihydrofolate reductase	Betulonic acid	−8.313 kcal/mol
2	Dihydrofolate reductase	Euglobal	−9.313 kcal/mol
3	Dihydrofolate reductase	Beta-pinene	−8.6045 kcal/mol
4	Dihydrofolate reductase	Alpha-pinene	−10.41 kcal/mol
5	Dihydrofolate reductase	P-Cymene	−10.15 kcal/mol

Table 13: Binding of ligands with dihydroorotate dehydrogenase

S. No.	Protein	Ligand	Binding energy
1	Dihydroorotate dehydrogenase	Betulonic acid	−8.14 kcal/mol
2	Dihydroorotate dehydrogenase	Euglobal	−10.24 kcal/mol
3	Dihydroorotate dehydrogenase	Beta-pinene	−9.16 kcal/mol
4	Dihydroorotate dehydrogenase	Alpha-pinene	−8.18 kcal/mol
5	Dihydroorotate dehydrogenase	P-Cymene	−9.28 kcal/mol

Table 14: Binding of ligands with TR

S. No.	Protein	Ligand	Binding energy
1	TR	Betulonic acid	−15.66 kcal/mol
2	TR	Euglobal	−9.24 kcal/mol
3	TR	Beta-pinene	−9.10 kcal/mol
4	TR	Alpha-pinene	−9.121 kcal/mol
5	TR	P-Cymene	−9.01 kcal/mol

TR: Trypanothione reductase

Table 15: Binding of ligands with HSP90

S. No.	Protein	Ligand	Binding energy
1	HSP90	Betulonic acid	−6.325 kcal/mol
2	HSP90	Euglobal	−6.589 kcal/mol
3	HSP90	Beta-pinene	−7.546 kcal/mol
4	HSP90	Alpha-pinene	−9.122 kcal/mol
5	HSP90	P-Cymene	−8.045 kcal/mol

Table 16: Binding of ligands with adenosine kinase

S. No.	Protein	Ligand	Binding energy
1	Adenosine kinase	Betulonic acid	−7.66 kcal/mol
2	Adenosine kinase	Euglobal	−12.24 kcal/mol
3	Adenosine kinase	Beta-pinene	−10.313 kcal/mol
4	Adenosine kinase	Alpha-pinene	−10.3418 kcal/mol
5	Adenosine kinase	P-Cymene	−10.6045 kcal/mol

In Table 11, P-Cymene has the minimum binding energy with ornithine decarboxylase.

In Table 12, alpha-pinene has the minimum binding energy with dihydrofolate reductase.

Table 17: Binding of ligands with triosephosphate isomerase

S. No.	Protein	Ligand	Binding energy
1	Triosephosphate isomerase	Betulonic acid	−7.38 kcal/mol
2	Triosephosphate isomerase	Euglobal	−7.20 kcal/mol
3	Triosephosphate isomerase	Beta pinene	−9.114 kcal/mol
4	Triosephosphate isomerase	Alpha pinene	−9.364 kcal/mol
5	Triosephosphate isomerase	P-Cymene	−8.857 kcal/mol

Table 18: Binding of ligands with tyrosine kinase

S. No.	Protein	Ligand	Binding energy
1	Tyrosine kinase	Betulonic acid	−10.12 kcal/mol
2	Tyrosine kinase	Euglobal	−9.982 kcal/mol
3	Tyrosine kinase	Beta pinene	−9.642 kcal/mol
4	Tyrosine kinase	Alpha pinene	−10.3418 kcal/mol
5	Tyrosine kinase	P-Cymene	−9.954 Kcal/mol

In Table 13, Euglobal has the minimum binding energy with dihydrofolate reductase.

In Table 14, betulonic acid has the minimum binding energy with TR.

In Table 15, alpha-pinene has the minimum binding energy with HSP90.

In Table 16, P-Cymene has the minimum binding energy with adenosine kinase.

In Table 17, alpha-pinene has the minimum binding energy with triosephosphate isomerase.

In Table 18, betulonic acid has the minimum binding energy with tyrosine kinase.

Five compounds were selected from the extract of *Eucalyptus tereticornis* and subjected to interaction studies with the ten proteins associated with the sleeping sickness disease. The results of docking were compared to see the interaction, and the best binding energy is given in Table 19. Among 50 compounds, the best compound (shown in Table 19) was taken for further studies in QSAR.

In Table 19, betulonic acid has the minimum binding energy with TR (3QFX).

The docking scores were highest for betulonic acid with −15.66 kcal/mol followed by euglobal with −12.24 kcal/mol, B-pinene with −10.313 kcal/mol, A-pinene with −10.3418 kcal/mol, and the least docking score for P-cymene with −10.6045 kcal/mol. Docking results showed that only betulonic acid and euglobal showed that hydrogen bond interaction was as b-pinene, a-pinene, and p-cymene yielded no hydrogen bond interactions. The best docking result was shown by betulonic acid with TR with a binding energy of −15.66 kcal/mol with a hydrogen bond interaction of 2.9 angstroms, and this result was taken for further analysis in QSAR.

DISCUSSION

In this study, the molecular docking was applied to discover the binding [15] mechanism and to correlate its docking score with the activity of plant-derived compounds. The TR was subjected to molecular docking study with all the five *E. tereticornis* compounds that can act as antitrypanosomiasis drugs (either directly or indirectly). The studies by applying the docking technique yielded

Table 19: Selected compounds with the least binding energy

S. No	Protein	Ligand	Binding energy (kcal/mol)	No. of h-bond interactions (angstroms)
1	Trypanothione reductase (3QFX)	Betulonic acid	−15.66	1 (2.90000)
2	Adenosine kinase (3OTX)	Euglobal	−12.24	3 (2.715618, 2.947590, and 2.454139)
3	Adenosine kinase (3OTX)	B-pinene	−10.31	0
4	Adenosine kinase (3OTX)	A-pinene	−10.3418	0
5	Adenosine kinase (3OTX)	P-cymene	−10.6045	0

crucial information concerning the orientation of the inhibitors in the binding pocket of the target protein. Several potential inhibitors have been identified. The results of our present study suggested that it can be used for the design and development of novel compounds having better inhibitory activity against several types of trypanosomiasis. These potential drug candidates can be further be validated in wet lab studies for its proper function. In other words, the results of the present study can be concluded in the following points. Betulonic acid was better ligands of choice that inhibits TR antitrypanosomal protein than other ligands showing the best affinity to bind with the protein and showed outstanding score and energy when compared to all other compounds. By applying QSAR studies to the best-scored compounds, it also stands for both properties (drug likeness and orally bioavailability).

CONCLUSION

Human trypanosomiasis or sleeping sickness currently is a common disease among the rural regions though found rare in the city sides causing deaths in humans as well as livestock. Although continuing to decline in the city sides, yet incidence rates remain level in rural regions following an increase in India since 2007. Trends in human trypanosomiasis related death trends due to livestock and causative agents over the past several decades. In this field of structure-based drug designing, there is a growing interest in the human trypanosomiasis protein study for the screening of putative leaf compounds. This approach involves the structure-based study of trypanosoma proteins and the antitrypanosomal properties of the selected plant compounds based on the literature available. The active sites of the trypanosoma proteins were found out and the molecular docking of the plant compounds was performed. The five compounds were docked, from based on the binding energy and the number of hydrogen bonds. Among them betulonic acid, a compound in *E. tereticornis* is found to have the best binding affinity and strong hydrogen bond interaction with trypanosoma protein. Euglobal, B-pinene, A-pinene, and P-cymene also gave good scores.

The results of the compound extracted from *E. tereticornis* will become physiological relevant only when (i) the pure compounds of this plant are available in large quantities; (ii) the *Eucalyptus* is biochemically stabilized to avoid degradation and enhance absorption in the gastrointestinal tract; and (iii) special delivery methods for this drug to reach the areas of treatment. In this work, the efficacy of *E. tereticornis* to act against trypanosomal protein was initiated and thus further research in this process would help us to take full advantage of the remedial effects of the compounds extracted from this plant. Solving these issues in the future would help the *in vitro* and *in vivo* studies to enhance the possibility of using *Eucalyptus* in clinical practice. The below mentioned wet-lab studies were possible that can be carried out in future can be listed as below: (i) The synergistic effect of TR can be tested with other compounds; (ii) to test how far these are useful in combinatory chemotherapies; (iii) its role in targeting multiple arms of the immune system machinery; (iv) identification and effect of structurally modified *E. tereticornis* compounds as trypanosomal inhibitors; and (v) real-life challenges and possibility in bringing up these inhibitors as orally available drugs or even as energy drinks.

AUTHORS' CONTRIBUTIONS

Aarthi Rashmi B guided the research. Vasanth Nirmal Bosco supervised the research. Priyanka K interpreted the results. Harishchander A prepared the manuscript with a highlight on critical points.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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