Original Article

Computational approaches for screening for bacterial inhibitors from the plant Malabar nut

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Abstract

Bacterial infections are a growing world-wide problem. Bacterial infections are now known to assassinate annually more people than malaria. One of the best practices of the ancient time for the treatment of bacterial infection was Ayurvedic. On analyzing the latest survey about the plant possessing the antibacterial activity, it was identified that the plants Malabar nut possess various pharmacological properties. The native of the plant was India and was commonly called as Adusa which belongs to the family of Acanthaceae. In the current research work, totally 102 phytochemical compounds were retrieved from the plant Malabar nut through literature survey. Using PubChem databases, the two dimensional structure were identified only for 84 compounds. Virtual screening was carried out for these compounds and the result predicted that only 28 compounds were screened to be active drug molecules. The bacterial protein Cytochrome P450 14-alpha-sterol demethylases (CYP51) was responsible for most of the bacterial disease caused to human. Using PDB databases, the three dimensional structure of the protein were retrieved which were further used for docking process. Out of 28 compounds only 1 compound predicted the best binding interaction with the protein using docking software.

Keywords: Malabar nut, Antibacterial activity, Phytochemical, Protein Cytochrome P450 14-alpha-serol demethylases, Docking

INTRODUCTION

Bacterial pathogens have a significant impact on public health. Any part of the body can develop disease, and it can be brought on by the organism itself or by the body's reaction to its existence[1]. Humans can contract bacteria via the air, water, food, or living things. The main means of bacterial infection transmission are contact, aerosol, droplet, vectors, and moving vehicles[2]. Morbidity and mortality are significantly impacted by preventive strategies. As we encroach into new environments, new species and novel variations of well-known species continue to be discovered[3]. Disease-causing pathogenic microorganisms have properties that enable them to sidestep the body's defenses and utilize its resources4. Last but not least, virulence refers to an organism's potential to spread disease through traits including invasiveness and toxin production[5]. In determining whether a disease will spread after a bacterial agent has been transmitted, host factors are crucial[6]. These variables include genetic make-up, nutritional status, age, length of organism exposure, and co-occurring diseases. Antibiotic resistance among bacteria is a rising issue that necessitates careful usage of antibiotics[7,8].

Adhatoda vasica Nees (Acanthaceae), sometimes referred to as Adusa or the Malabar nut tree[9]. The Assam, Bangladesh, India, Nepal, and Sri Lankan subcontinents, Laos, and Myanmar make up the natural range of the Malabar nut[10]. Many alkaloids, phenolics, flavonoids, sterols, and their glycoside derivatives have been identified from the well-known medicinal herb Malabar nut. Its wide range of therapeutic effects include hepatoprotective, antimicrobial, antitussive, abortifacient, antitubercular, antimutagenic, antiulcer, antiasthmatic and cardiovascular protection[11,12]. It is commonly used in South-East Asian traditional and folk medical systems, and it is also a well-known plant medication in Ayurvedic and Unani medicine[13].

The protein that was found to cause of the bacterial infections in human was Cytochrome P450 14-alpha-sterol demethylases (CYP51) are important enzymes in sterol in plants biosynthesis and cholesterol biosynthesis in humans[14]. In

the pathways leading to the synthesis of ergosterol in fungus, various forms of sterols in plants, and cholesterol in humans, the demethylated products of the CYP51 reaction are essential intermediates[15]. These sterols are found in cells plasma membranes, where they serve a crucial structural function in the control of membrane permeability and fluidity[16]. They also have an impact on the activity of enzymes, ion channels, and other embedded cell components. In an effort to find novel ways to cure bacterial infections, drug researchers have started focusing on the 14-demethylase enzyme in bacteria[17]. By inhibiting the bacteria's production of ergosterol, the pathogen is killed since the breakdown of the plasma membrane causes cellular leakage[18]. The CYP51 family is an intriguing subject for fundamental P450 structure/function studies and is also an important clinical drug target[19].

In silico screening, large libraries of drug-like compounds that are commercially accessible are computationally screened against targets of known structure, and those that are predicted to bind well are experimentally tested[20]. Virtual Screening of the compound was excluded by Molinspiration software[21]. The software predicts the calculations of leading molecular properties as well as calculation of bioactivity score for the most important drug targets[22]. Docking method is one of the most important and frequently used methods in structural-based drug designing, which predict the binding affinity of small molecules to their applicable target binding sites there by inhibiting the target functions[23].

MATERIALS AND METHODS:

Ligand selection:

Through literature study, 102 phytochemicals were identified from the plant Malabar nut for computational analysis. From the Pubchem database, 2D structures were retrieved for 84 compounds and for remaining 18 compounds, 2D structures was not available in the database.

Prediction of oral drug activity using Molinspiration server:

By using Molinspiration server, the drug likeness filter test was conducted for above 84 phytochemicals. Molinspiration expressed 6 rules (logP, Topological Polar Surface Area (TPSA), Molecular weight (MW), number of hydrogen bond acceptor (No.HA), number of hydrogen bond acceptor No.HD, and volume (Vol)) to determine whether a compound could be orally consummate. The compounds which clear the oral drug properties were further, analyzed for its biological properties using Molinspiration bioactivity server. Bioactivity predicts the 6 properties including GPCR ligand, Ion channel modulator, Kinase inhibitor, nuclear receptor ligand, Protease inhibitor, Enzyme inhibitor. The compound which satisfies all the biological properties could be taken for further docking process.

Accession of target protein:

The protein cytochrome P450 14-alpha-sterol demethylases (CYP51) were found to be most important bacterial protein. The three dimensional structure (1EA1) of that protein was retrieved using Protein Databank database which was resolute by experimental studies through X-Ray Diffraction with the Resolution of 2.21 Å.

Analysis of target active binding sites:

The Binding sites of small molecules present in the protein structure were recognized by SCFBio - Supercomputing Facility for Bioinformatics and Computational Biology (http://www.scfbio-iitd.res.in/dock/ActiveSite.jsp). SCFBioActive Site prediction server which will predict the active site in the 3 dimensional structure of the protein for the binding of the ligand.

Molecular Docking Interactions using Argus Lab:

The docking studies were performed to analyze the structural relationship between receptor and ligand using Argus Lab 4.0.1 docking software program28. The following factors were set for docking process: Population size, Grid resolution, Binding site box size, Maximum generation, Crossover rate, Mutation rate, Elitism, Dock engine used Lamarckian Genetic Algorithm. For the docking calculations, docking mode should be was set as “Dock” and “Flexible” ligand for each docking runs.

Visualization of docking interaction using PyMol:

The best docking result were analyzed using PyMol which is an open source molecular visualization tool to view the hydrogen bond interactions between the protein and ligand and also predict the distance of hydrogen formation between them. The predicted distance reveal that binding interaction was stable one and small molecule could inhibit the function of protein37.

RESULTS AND DISCUSSIONS:

Preparation of small molecules:

Through literature survey, totally 102 phytoconstituents were identified from plant Malabar nut. The virtual screening was carried out for 84 phytoconstituents using Molinspiration server. From the result of Drug likeness properties revealed that only 38 compounds pass the Drug likeness screening test (Table 1). Further these compound were screened for its bioactivity test, the result proved that out of 38, only 28 compounds satisfies the oral drug properties (Table 2).

Table 1: The phytochemical compounds which satisfy the drug likeness properties

Compound	Pubchem ID	LogP	TPSA	MW	No. HA	No. HD	Vol
2-Glycylaminobutyric acid	86878	2.62	92	160	5	4	149
Anisotine	442884	2.99	73	349	6	1	312
Apigenin	5280443	2.46	90	270	5	3	224
Betaine	247	5.41	40	117	3	0	119
42752-07-8	206	2.64	110	180	6	5	151
Deoxyvasicine Hydrochloride	3046290	1.95	15	172	2	0	165
Deoxyvasicinone	68261	1.47	34	186	3	0	167
2-Aminoacetic acid	750	2.55	63	75	3	3	67
4-Heptanone	31246	2.36	17	114	1	0	131
Hydroxychalcone	97765	3.57	37	224	2	1	209
Kaempferol	5280863	2.17	111	286	6	4	232
178740-33-5	21769547	0.49	64	232	5	1	201
77500-13-1	110349	3.76	66	319	5	2	344
147-85-3	145742	1.72	49	115	3	2	108
Quercetin	5280343	1.68	131	302	7	5	240
2-Amino-3-hydroxypropanoic acid	617	1.67	83	105	4	4	92
2-amino-3-methylbutanoic acid	1182	1.91	63	117	3	3	117
Peganine	72610	1.04	35	188	3	1	173
Vasicinone	442935	0.48	55	202	4	1	175
Benzenamine	626005	3.33	18	291	3	0	282
Vasicolinon	627712	2.91	38	305	4	0	285
2-(3'-hydroxy-2'-oxopyrrolidinomethyl) aniline	14285793	0.02	66	206	4	3	191
Vasicinolone	13970119	0.03	75	218	5	2	183
Vasicinol	4486241	0.53	56	204	4	2	181
Vasnetine	9997066	3.03	73	335	6	1	296
Erythorbic acid	54675810	1.40	107	176	6	4	139
2,4-Dimethyl-2,4-Heptadiene	572949	3.93	10	124	0	0	150
Methyl 4-hydroxycinnamate	5319562	2.05	46	178	3	1	164
5,5-Dimethyl-1,3-Dioxane-2-Ethanol, Tert-Butyldimethylsilyl Ether	91714410	4.24	27	274	3	0	291
5-Isopropyl-1,3-Cyclohexanedione	566106	1.57	34	154	2	0	156
16688-21-4	595376	1.72	67	351	6	1	318
STK766913	5292275	4.53	72	370	6	1	288
Syringic Acid	10742	1.20	76	198	5	2	170
Methyl 4-hydroxy-3-methoxy-cinnamate	16830	1.86	55	208	4	1	189
1151-93-5	2825054	3.55	29	255	3	0	245
1-Cyclohexylsilatrane	208266	1.87	30	257	4	0	251
Hydroquinone	785	0.98	40	110	2	2	100
Dibutyl Phthalate	3026	4.43	52	278	4	0	273

Table 2: The phytochemical compounds which satisfy the bioactivity properties

S.No	Pubchem ID	GPCR Ligand	Ion Channel Modulator	Kinase Inhibitor	Nuclear Receptor Ligand	Protease Inhibitor	Enzyme Inhibitor
1.	86878	-0.30	0.05	-1.25	-1.16	0.04	-0.02
2.	442884	-0.14	-0.31	-0.19	-0.37	-0.41	-0.08
3.	247	-2.53	-1.79	-3.50	-3.75	-3.47	-2.12
4.	3046290	-0.26	0.09	-0.83	-1.22	-0.91	0.03
5.	68261	-0.98	-0.92	-0.75	-1.39	-1.22	-0.09
6.	750	-3.45	-3.11	-3.71	-3.55	-2.95	-3.29
7	31246	-3.17	-2.75	-3.66	-2.91	-3.06	-2.63
8.	97765	-0.34	-0.17	-0.54	-0.30	-0.51	-0.05
9.	21769547	-0.24	-0.43	-0.44	-0.67	-0.86	0.07
10.	145742	-2.15	-1.54	-3.08	-3.07	-1.72	-1.85
11.	617	-2.66	-2.54	-3.34	3.34	-2.36	-2.38
12.	1182	-2.66	-2.11	-3.40	-3.04	-2.12	-2.18
13.	627712	-0.40	-0.35	-0.53	-0.85	-0.97	0.03
14.	442935	-0.04	-0.26	-0.21	-0.35	-0.48	-0.01
15.	14285793	-0.13	0.04	-0.29	-0.52	0.14	0.16
16.	13970119	-0.26	-0.25	-0.33	-0.51	-0.87	0.15
17.	9997066	-0.24	-0.40	-0.26	-0.43	-0.50	-0.08
18.	54675810	-0.53	-0.24	-1.09	-1.01	-0.81	0.20
19.	572949	-2.13	-1.14	-2.53	-1.39	-2.35	-1.03
20.	5319562	-0.71	-0.35	-0.92	-0.30	-0.87	-0.28
21.	566106	-0.91	-0.38	-1.74	-0.67	-0.73	-0.32
22.	595376	0.01	-0.29	-0.32	-0.12	-0.36	0.12
23.	5292275	-0.58	-1.01	-0.37	-0.99	-0.66	-0.46
24.	10742	-0.65	-0.28	-0.69	-0.44	-0.82	-0.15
25.	16830	-0.58	-0.35	-0.72	-0.26	-0.78	-0.21
26.	2825054	-0.14	-0.13	-0.06	0.00	-0.39	-0.04
27.	785	-3.02	-2.48	-3.07	-2.84	-3.20	-2.66
28.	3026	-0.16	-0.09	-0.27	-0.12	-0.25	-0.07

Preparation of protein:

The 3D structure of protein (1EA1) was retrieved using Protein Data Bank database. The possible binding site sites of the protein Cytochrome P450 14-alpha-sterol demethylases (CYP51) were predicted using SCFBio. The secondary structure, name, position and of the amino acid present in the protein are as follows: Alpha Helix - SER260, THR261 and PHE399: Coil- GLY94, PRO319, ILE322, GLY388, ARG391, CYS394 and ALA397.

Docking analysis:

The predicted 10 active residues were used as the binding sites for 28 natural compounds from the plant Malabar nut for docking studies. The results of the binding interaction between the active site residues of target protein Cytochrome P450 14-alpha-sterol demethylases (CYP51) and 28 compounds were shown in the Table 3.

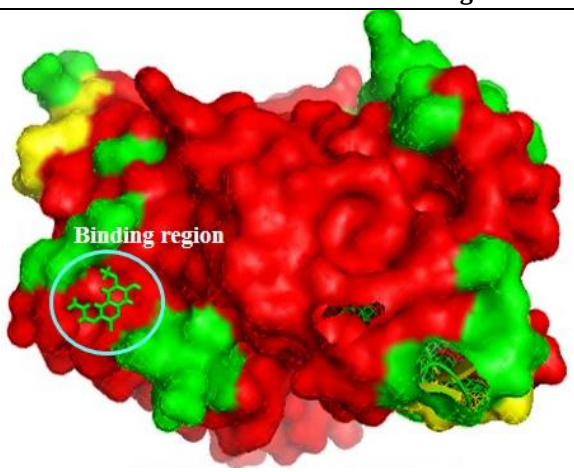
Table 3: Molecular Docking between Phytochemical Compounds and the Protein (1EA1)

Compound	Binding Affinity	Compound	Binding Affinity
Hydroxychalcone	-8.9 Kcal/Mol	Dibutyl Phthalate	-7.4 Kcal/Mol
Methanone [4-(Dimethylamino)Phenyl] (4-Methoxyphenyl)	-8.7 Kcal/Mol	Methyl 3-(4-Hydroxy-3-Methoxyphenyl)Prop-2-Enoate	-7 Kcal/Mol
5-Isopropyl-1,3-Cyclohexanedione	-7 Kcal/Mol	2,4-Dimethyl-2,4-Heptadiene	-5.5 Kcal/Mol
Syringic Acid	-6.5 Kcal/Mol	Hydroquinone	-5.3 Kcal/Mol
4-Heptanone	-5.2 Kcal/Mol	Amino-N Butyric Acid	-5.1 Kcal/Mol

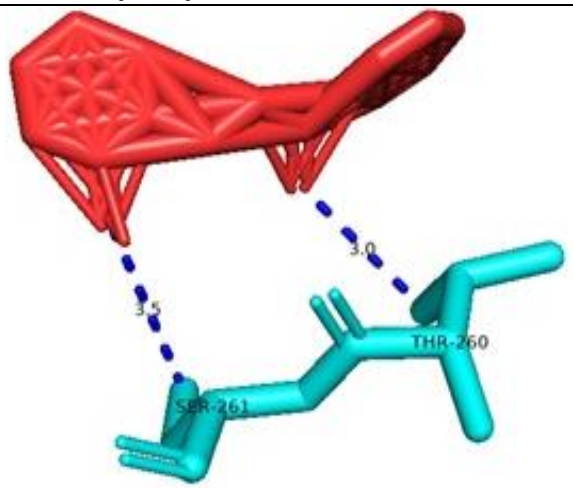
D-Erythro-Hex-2-Enonic Acid, Gamma-Lactone	-4.7 Kcal/Mol	Glycine	-4 Kcal/Mol

By analyzing the docking result, 2 compounds: Hydroxychalcone and Methanone [4-(Dimethylamino) Phenyl] (4-Methoxyphenyl) were found to have the highest inhibitory activity of -8.9 Kcal/Mol and -8.7 Kcal/Mol when compared with 10 other compounds which showed the inhibitory activity of < -8 Kcal/mol. The remaining 16 compounds do not exhibit any inhibitory activity between protein Cytochrome P450 14-alpha-sterol demethylases (CYP51).

Table 4: Docking interactions between hydroxychalcone – 1EA1



Best docking interactions between Hydroxychalcone - 1EA1



Hydrogen distance between Hydroxychalcone - 1EA1

The latest docking study on 1EA1 protein revealed that same binding inhibitory activity towards compounds Emodin when compared with the existing drugs[24]. From the current docking results, it was clearly conveyed that among the 28 plant compounds, Hydroxychalcone exhibits the best binding interaction with the Protein Cytochrome P450 14-alpha-sterol demethylases (CYP51) and further this study could be useful in designing of new preventive and therapeutic drug against bacterial infection.

CONCLUSION

The development of novel natural compounds with pharmacological activity is an urgent need in designing effective drug towards bacterial infections. In the present study, docking results revealed the binding interactions between protein Cytochrome P450 14-alpha-sterol demethylases (CYP51) and 28 natural flavonoid compounds. Among those compounds, Hydroxychalcone were found to have the best binding energy of -8.9 Kcal/mol and also satisfies the pharmacological properties which provide the strong recommendation for the compound to be considered for an oral drug. On the whole, this study could be concluded that compound Hydroxychalcone could be potent anti-bacterial drugs activity against the protein Cytochrome P450 14-alpha-sterol demethylases (CYP51). Further In vivo investigations are needed to enrich the bacterial activity of the Hydroxychalcone compound from Malabar nut to determine the dosage for the safety levels.

CONFLICT OF INTEREST:

The authors declare they have no competing interests.

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