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Research Article

ASCERTAINING THE MODE OF ACTION OF PHYTOCOMPOUNDS FROM THE MEDICINAL PLANT *TINOSPORA CORDIFOLIA* USING DOCKING STUDIES

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ABSTRACT
Natural products with medicinal value are progressively gaining importance in clinical research due to their therapeutic uses without any side effects when compared to the available drugs. *Tinospora cordifolia* (Guduchi) is an important herb in folk and Ayurveda systems of medicine which contains various chemical constituents belonging to different classes such as alkaloids, diterpenoid lactones, glycosides and steroids. The plant is reported to have antimicrobial, antioxidant, anticancer and recurring resistant with promising role against HIV. So, the present study was designed to find the mode of action for the phytocompounds present in the plant *T. cordifolia* against Swine flu, AIDS and Tuberculosis through *in silico* studies. Docking studies were performed by using Autodock 4.1 and results were analysed.From the results it was observed that compound columbin exhibited -7.11kcal/mol binding energy, 4 hydrogen bond interactions with ASP25 of the HIV-1 protease receptor. Ecdysterone and Giloin exhibited good binding energy and inhibitory constant against both Neuraminidase and HIV-1 protease receptors.
Keywords: *Tinospora cordifolia*, Swine flu, AIDS, Tuberculosis, phytocompounds.

INTRODUCTION

India is known for its rich repository of medicinal plants, the forest being principal repository of large number of medicinal and aromatic plants. *Tinospora cordifolia* Miers (Menispermaceae) is an important medicinal plant cultivated throughout the Indian subcontinent. In Ayurveda, *T. cordifolia* is a constituent of several preparations used in general debility, dyspepsia, fever and urinary diseases.[1] Stem of *T. cordifolia* is a bitter stomachic, stimulates bile secretion, prevents vomiting and remedy for jaundice, diabetes, piles, respiratory disorders, neurological problems and skin diseases. The juice of *T. cordifolia* is useful in diabetes, vaginal and urethral discharges. The reported medicinal properties are anti-diabetic, anti-periodic, anti-spasmodic, anti-inflammatory, anti-arthritic, anti-oxidant, anti-allergic, anti-stress, anti-leprotic, anti-malarial, hepatoprotective, immunomodulatory and anti-neoplastic activities.[2] Preliminary phytochemical screening showed the presence of terpenoids, alkaloids, lignans, carbohydrates, glycosides, bitters, proteins, tannins, steroids etc.[3] Natural products with medicinal value are progressively gaining importance in clinical research due to their therapeutic uses without any side effects when compared to the available drugs. So the present study was designed with an aim to evaluate the medicinal properties of *T. cordifolia* using *in silico* methods.

Disease selected for Study

Swine flu

Swine influenza virus (SIV) is a strain of the influenza family of viruses that is endemic in pigs. H1N1 virus nucleocapsid is responsible for Swine flu disease.[4] The genome of H1N1 contains eight single (non paired) RNA strands that code for eleven proteins. About 500 molecules of hemagglutinin are needed to make one virion.[5] Neuraminidase, a glycoprotein responsible for viral envelope and are involved in the release of progeny virus from infected cells, by cleaving sugars that bind the mature viral particles. About 100 molecules of neuraminidase are needed to make one virion.[6] Zanamivir, an antiviral agent has side effects such as severe allergic reactions (rash, hives, itching, difficulty breathing, tightness in the chest), abnormal behaviour, confusion, irregular heartbeat, etc.[7]

Acquired Immune Deficiency Syndrome (AIDS)

AIDS is a disease of the human immune system caused by the human immunodeficiency virus (HIV).[8] The virus progressively reduces the effectiveness of the immune system and leaves individuals susceptible to opportunistic infections and tumours. HIV-1 protease is important in HIV replication because it cleaves polypeptide chains to create mature enzymes and structural components of the virus. HIV protein synthesis occurs in the host cell.[9] Amprenavir is an oral medication for HIV which blocks the activity of protease and results in the formation of defective viruses that are unable to infect the body's cells resulting in the decrease of number of viruses in the body. The most frequent side effects are headache, weakness, diarrhoea, nausea and stomach pain. Amprenavir may also cause severe skin reactions and breakdown of red blood cells. The propylene glycol in the oral solution can cause seizures, stupor, increased heart rate, metabolic disturbance, kidney failure, increased body cholesterol and worsening of diabetes.[10]

Tuberculosis

Tuberculosis, deadly infectious disease caused by various strains of mycobacteria, usually *Mycobacterium tuberculosis* in human.[11] The lysX gene encoding the two-domain lysyl-transferase (mprF)-lysyl-tRNAsynthetase (lysU) protein is responsible for L-PG production of *Mycobacterium tuberculosis*. The lysX mutant also showed defective growth in mouse and guinea pig lungs and showed reduced pathology relative to wild type, indicating that LysX activity is required for full virulence.[12] Isoniazid is an antibiotic that is used to treat tuberculosis (TB). It works by killing the

bacteria that causes the disease. The exact mechanism of action of isoniazid is unknown, but it is thought to prevent the tuberculosis bacteria from making substances called mycolic acid, which is needed to form the cell walls of the bacteria. Side effects including allergic reaction (difficulty breathing; closing of the throat; swelling of the lips, tongue, or face or hives), unusual weakness or fatigue, nausea, vomiting, loss of appetite, abdominal pain, yellow skin or eyes, dark urine, numbness or tingling in hands or feet, seizures, blurred vision, confusion or abnormal behaviour.[13]

MATERIALS AND METHODS

Retrieval of the protein

The structures of target proteins were retrieved from the protein data bank. The PDB ID of the target proteins Neuraminidase (Swine flu), HIV-1 Protease (AIDS) and Lysine Biosynthesis Enzyme (Tuberculosis) were 2BAT, 3NU3 and 1UC8, respectively.

Active site analysis using PDBsum

The PDBsumis a pictorial database; it shows the molecule that makeup the structure (i.e. Protein chains, DNA, ligand and metal ions) and schematic diagram of their interactions.

Retrieval of the ligand structures

The reported phytocompounds used for docking were Berberine, Chasmanthin, Columbin, Giloin, Paalmatin, Octacosanol, β-sitosterol, Ecdysterone from *T. cordifolia*. The 3D structure of these phytocompounds were retrieved from the PubChem database in the SDF file format and converted into PDB file format through Open Babel.

Docking analysis

Computer simulated automated docking studies were performed using AutoDock 4.1. All the target proteins were prepared for molecular docking by adding hydrogenatoms using default parameters. The heteroatoms and water molecules were deleted. The binding energy and inhibitory constants were observed and analysed.

RESULTS

The phytocompounds and synthetic drugs were docked in the active site of the respective targets and the results were analysed to identify the natural compounds with good inhibitory activity considering the interactions, binding energy and inhibitory constant.

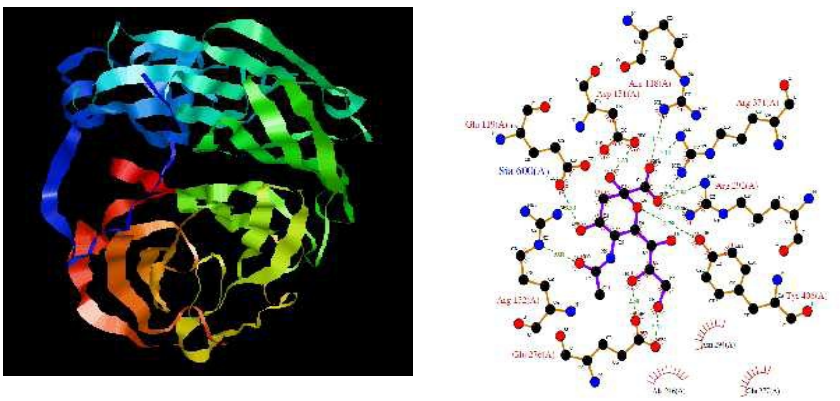


Figure 1:The structure of the complex between influenza virus neuraminidase and sialic acid, the viral receptor with Ligplot result of PDBsum and its Active site residues

The Active site residues are Glu83, Asn86, Arg118, Gly119, Asn146, Asp151, Arg152, Asn200, Asn234, Glu276, Arg292, Arg371 and Tyr406.

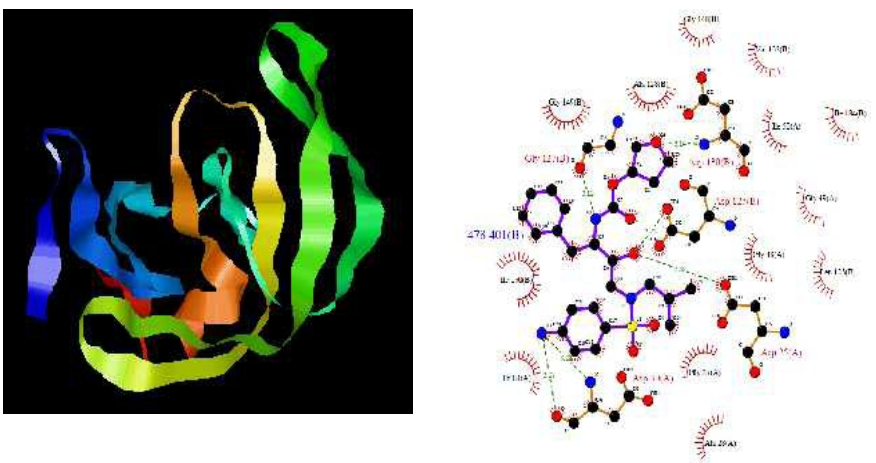


Figure 2: The structure of Wild Type HIV-1 Protease with Antiviral Drug Amprenavir and Ligplot result of PDBsum with active site residues

The active site residues are Asp25, Asp30, Thr112, Asp130, Asp125, Gly127, Glu165, Ala167 and Gly168.

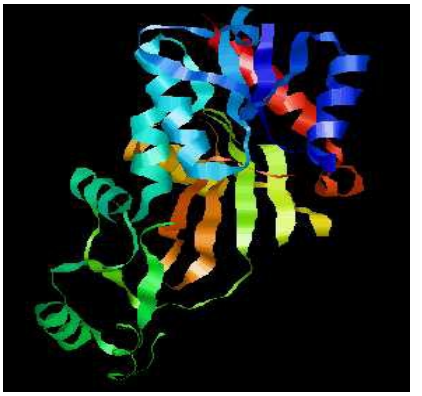
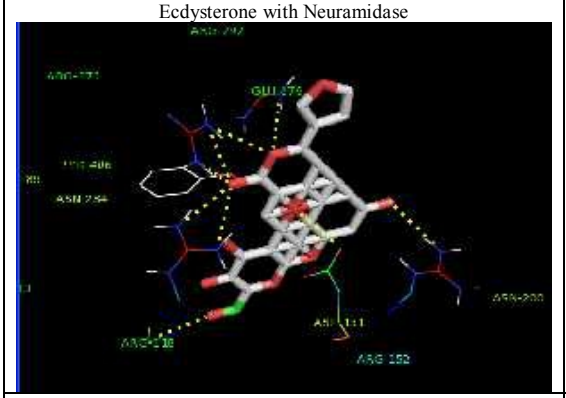
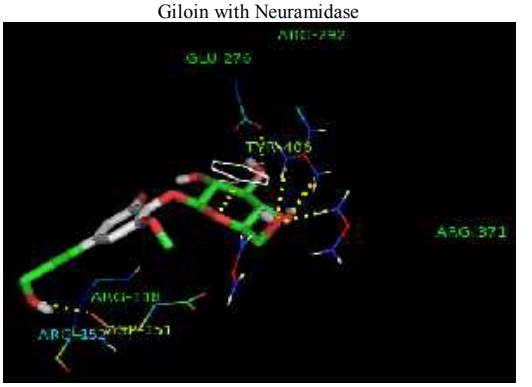
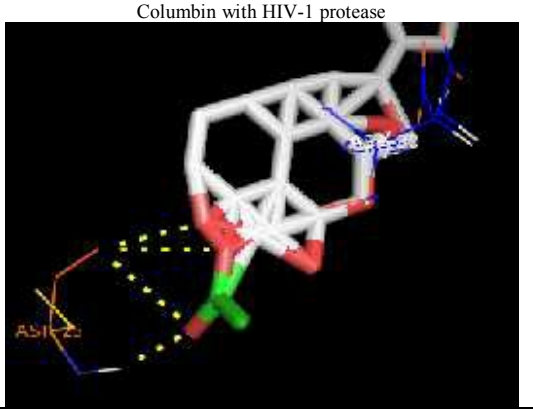
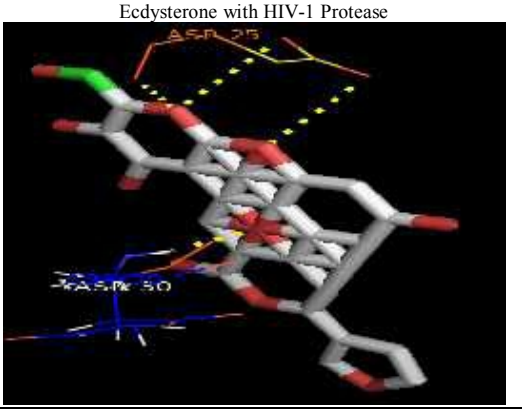
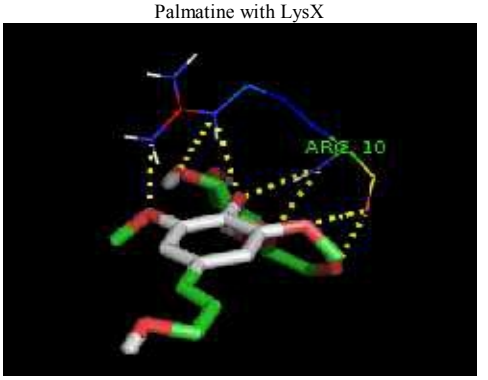
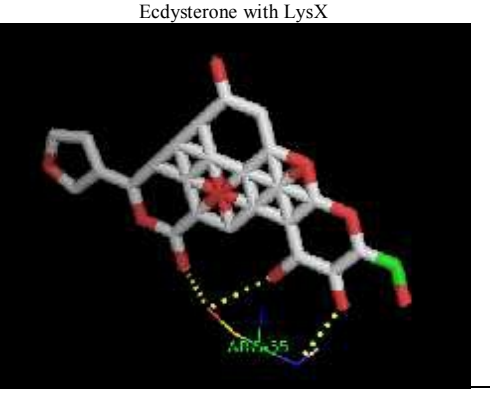


Figure 3: The Crystal structure of a lysine biosynthesis enzyme, LysX, from *Thermus thermophiles* with active site residues

The active sites residues are Arg10, Glu13, Arg55, Arg180, Arg194, Asp237, Asn251, Glu255, Lys257 and Asn258.[14]

Table 1. Interaction of phytocompounds and drugs with target proteins

Ecdysterone with Neuramidase	Giloin with Neuramidase
	
Columbin with HIV-1 protease	Ecdysterone with HIV-1 Protease
	
Palmatine with LysX	Ecdysterone with LysX
	

Interaction of Drug with respective receptor

Zanamavir with Neuramidase | Amprenavir with HIV-1 protease | Isoniazid with LysX

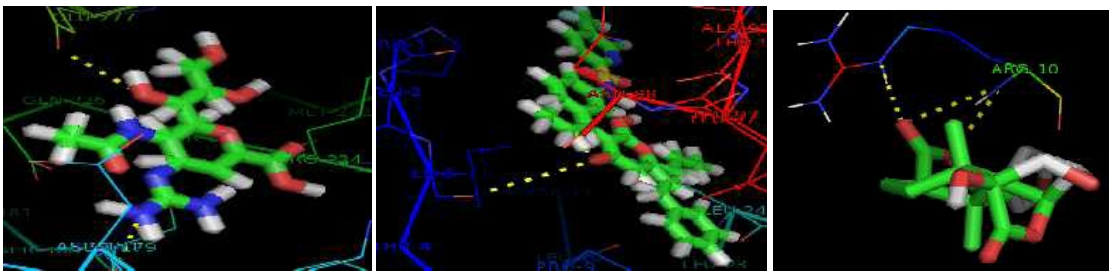


Table 2: Docking with Neuraminidase

Compound name	Binding Energy (kcal/mol)	No. of Hydrogen bonds	Inhibitory constant	Interacting Residues
Ecdysterone	**-6.34**	**9**	**22.34**	**Glu276, Tyr406, Asn 234, Arg118, Asn200, Asp151**
Giloin	**-5.82**	**5**	**54.48**	**Arg152**
β-sitosterol	-4.85	3	277.19	**Glu276, Tyr406,Asn 86**
Octacosanol	-4.61	6	418.68	**Glu276, Arg 371, Tyr 406, Arg118**
Chasmanthin	-4.35	2	645.66	**Arg292**
Columbin	-3.72	2	1.87	**Asp151,Arg152**
Palmatine	3.19	5	4.6	**Glu276,Arg292, Tyr 406, Arg371, Asp151**
Berberine	2.45	2	16.05	**Glu276**
Zanamavir (Drug)	-2.15	2	1.73	Glu277, **Asp151**

Table 3: Docking with HIV-1 Protease

Compound name	Binding Energy (kcal/mol)	No. of Hydrogen bonds	Inhibitory constant	Interacting Residues
Columbin	**-7.11**	**4**	**6.12**	**Asp25**
Ecdysterone	**-6.96**	**4**	**7.98**	**Asp30, Asp25**
Giloin	-5.82	2	54.58	**Asp25**
Chasmanthin	-5.82	3	104.51	**Asp25, Asp30**
Palmatine	-4.86	9	272.88	**Asp25, Asp30**
β-sitosterol	-4.38	2	613.53	**Asp30**
Octacosanol	-3.92	1	1.33	**Asp25**
Berberine	-3.53	2	2.6	**Asp30**
Amprenavir (Drug)	-3.28	2	2.73	Ile3,Asn987

Table 4: Docking with LysX

Compound name	Binding Energy (kcal/mol)	No. of Hydrogen bonds	Inhibitory constant	Interacting Residues
Columbin	**-6.83**	**6**	**8.43**	**Arg10**
Palmatine	**-5.86**	**8**	**43.0**	**Arg10**
Ecdysterone	-5.19	3	747.49	**Arg55**
Chasmanthin	-4.9	3	7.46	**Arg10**
β-sitosterol	-3.93	4	38.8	**Arg10**
Octacosanol	-3.53	1	76.07	**Arg10**
Giloin	-3.21	2	8.11	**Arg10**
Berberine	-2.95	2	200.88	**Arg10**
Isoniazid (Drug)	-2.15	3	2.34	**Arg10**

DISCUSSION

The significance of herbs and herbal products is gaining worldwide recognition. The concept of complementary or alternative medicine is becoming widely accepted and there is an increasing belief in the efficacy of herbal remedies. Clinical researches show the value of herbal medicine in treatment and prevention of diseases. On analyzing the docked results, it was found that all the 8 compounds had interactions with 3 targets such as Neuraminidase, HIV-1 protease and lysX protein. For HIV patients, polyherbal formulation containing *T. cordifolia* was prescribed which had been reported for favourable effects and this plant may also be used in monoherbal preparation.[15] The results of docking phytocompounds with HIV-1 protease for AIDS showed that Columbin, Ecdysterone, Giloin and Chasmanthin exhibited very low binding energy (kcal/mol) such as -7.11, -6.96, -5.82, and 5.82, respectively, when compared with drug amprenavir (-3.28). The mode of action of *T. cordifolia*on HIV may be targeting HIV protease as these compounds exhibited significant binding with the target. The plant preparation "Guduchi" is recommended for swine flu. As *T. cordifolia* has a medicinal use in the treatment of viral diseases like HIV and Hepatitis,[15] the selected compounds were docked with the target for swine flu i.e. neuraminidase and the results were compared with the docking result of the drug Zanamavir. Of the selected compounds, Ecdysterone and Giloin exhibited very low binding energy (kcal/mol) such as -6.34 and -5.82, respectively and the Ecdysterone had 9 hydrogen bond interactions whereas Giloin had 1 interaction. The results of these two docking studies, where the targets

are for the viral diseases, prove the ability of the compounds to act on the viral enzymes. Of these 2 compounds, Ecdysterone obey three of the Rule of 5 with only hydrogen bond donor deviating which was 6, while Giloin obeys all the parameters of Lipinski's rule of five and can be used as lead structures in developing new drugs for those two dreaded viral diseases. One of the various medicinal properties of *T. cordifolia* is to protect against TB infections.[16] The results exhibited that Columbin, Palmatine and Ecdysterone had very low binding energy of -6.83, -5.86 and -5.19, respectively which was better than the drug. The results of the present study validate some of the traditional medicinal properties of *T. cordifolia* in the treatment of HIV, Swine flu and TB. Moreover the mode of action of the important compounds was also determined by the docking analysis. The anti-viral compounds Ecdysterone and Giloin can be used as lead structures in designing new drugs.

CONCLUSION

In future, phytochemicals could be used as promising therapeutic agents for many diseases. Columbin, Ecdysterone and Giloin were found to interact with receptor with low binding energy than the available drug. These can be used as lead structures in synthesizing new drugs for HIV and swine flu.

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