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To ELUCIDATE THE ROLE OF METABOLITES PRESENT IN SIDDHA HERBAL PREPARATION MV KASHAYAM IN PREVENTING THE ENTRY AND MULTIPLICATION OF HIV

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Abstract

Aims and objectives

To do insilico screening of phytochemicals present in herbal preparation MV Kashayam using docking method against antiretroviral drug targets used against HIV (Human immunodeficiency virus)

Method

Phytochemical ligands of the herbs were docked against HIV Reverse transcriptase structures 6KDJ and 1S1X and Protease structure 3WJS down loaded from RCSB-PDB site using Auto-Dock Vina PyRX screening software and the docking scores were compared against docking scores of antiretroviral drug ligands lamivudine, Nevirapine and Indanavir.

Results and conclusion

Amentoflavone, Cordifolide, Neoandrographolide, Queretaroic acid, Amritoside showed good docking scores in active site of 6KDJ. Tinosporide, Piperlonguminine, Serratin, Tricostachine, Tinosporaside showed good docking scores in active site of 1S1X. Palmatoside, Cordifolide, Neoandrographolide, Tinasporaside, Amritoside showed good docking scores in active site of 3WJS.

Keywords: Docking – Siddha herbal- MV Kashayam- HIV- anti retroviral drugs

Introduction

The human immunodeficiency viruses (HIV) are two species of *Lentivirus* (a subgroup of retrovirus) that infect humans. Over time, they cause acquired immunodeficiency syndrome (AIDS)[1, 2], a condition in which progressive failure of the immune system allows life-threatening opportunistic infections and cancers to thrive[3].Without treatment, average survival time after infection with HIV is estimated to be 9 to 11 years, depending on the HIV subtype[4]. HIV infects vital cells in the human immune system, such as helper T cells (specifically $CD4^+$ T cells), macrophages, and dendritic cells[5]. HIV infection leads to low levels of $CD4^+$ T cells .When $CD4^+$ T cell numbers decline below a critical level, cell-mediated immunity is lost, and the body becomes progressively more susceptible to opportunistic infections, leading to the development of AIDS. Some authors consider HIV/AIDS a global pandemic. As of 2016 approximately 36.7 million people worldwide have HIV, the number of new infections that year being about 1.8 million. South & South East Asia is the second most affected; in 2010 this region contained an estimated 4 million cases or 12% of all people living with HIV resulting in approximately 250,000 deaths. Approximately 2.4 million of these cases are in India.[6, 7, 8, 9]

Anti retro viral therapy mechanism

Nucleoside reverse-transcriptase inhibitors (NRTI) and nucleotide reverse-transcriptase inhibitors (NtRTI) are nucleoside and nucleotide analogues which inhibit reverse transcription. HIV is an RNA virus, so it cannot be integrated into the DNA in the nucleus of the human cell unless it is first "reverse" transcribed into DNA. Since the conversion of RNA to DNA is not naturally done in the mammalian cell, it is performed by a viral protein, reverse transcriptase, which makes it a selective target for inhibition[10]. Non-nucleoside reverse-transcriptase inhibitors (NNRTI) inhibit reverse transcriptase by binding to an allosteric site of the enzyme; NNRTIs act as non-competitive inhibitors of reverse transcriptase. NNRTIs affect the handling of substrate (nucleotides) by reverse transcriptase by binding near the active site[11]. Protease inhibitors block the viral protease enzyme necessary to produce mature virions upon budding from the host membrane. Particularly, these drugs prevent the cleavage of gag and gag/pol precursor proteins.[12]

Aim of the study

At present, there is no specific medicine to treat the HIV and hence this study aimed to analyze the possible antiviral activity of Herbal preparation M V Kashayam by docking the constituent chemicals of herbs present in the kashayam to various drug targets for HIV.

Materials and methods

Literature search was done for individual plants to identify their constituent phytochemicals. The structure of these phytochemicals were drawn using canonical smiles obtained from Pubchem using Chemsketch software

and stored as .mol files which were later converted to pdb format using Argus lab software. Three target proteins of existing antiretroviral drugs were chosen for the study

1. 6KDJ- Reverse transcriptase with Nucleoside reverse-transcriptase inhibitor Lamivudine[13].
2. 1S1X- Reverse transcriptase with Non-nucleoside reverse-transcriptase inhibito nevirapine[14].
3. 3WJS-Protease with inhibitor indanavir[15].

The structures were downloaded as pdb files from RCSB PDB site. The structures of protein was loaded to CAST-p website (job id- J_61D28DEB3EB1D for 6KDJ, J_61D2952DC2267 for 1S1X, J_61D290CAEC743 for 3WJS) and active sites were identified. The protein structures were subjected to removal of water molecules and addition of hydrogen atoms. The target molecule and the ligands were loaded in the PyRx virtual screening software which uses Auto dock Vina[16] for docking. The protein molecules and the ligands were subject to energy minimization and were converted to PDBQT format. Grid were created with binding site of nevirapine as centre for 1S1X and binding site of indanavir as centre for 3WJS and with grid box dimensions in Angstroms x-25, y-25, z-25. Docking of the ligands were done using Auto dock Vina with exhaustiveness of 8. The output PDBQT files were opened in PYRX and the individual ligand poses were separately saved in pdb format. The protein molecule and output ligand poses were loaded in PYMOL and their hydrogen bond interactions of ligands with Amino Acid residues were studied.

Results and Discussion

1. 6KDJ- Reverse transcriptase with Nucleoside reverse-transcriptase inhibitor Lamivudine

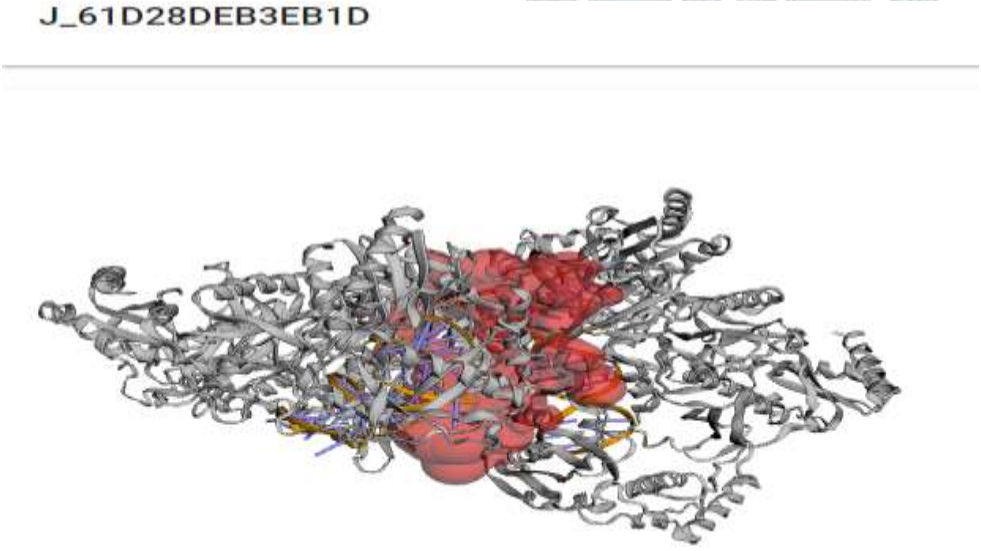

Figure 1: Active site of 6KDJ protein identified in CAST-p

Serial number	Ligand	DOCKING SCORE	INTERACTION
Control ligand	Lamivudine	-8.4	GLN336, GLN278,GLN334, LYS275, GLU305,GLN278
1.	Amentoflavone	-12.1	LYS512,ASP364
2.	Cordifolide	-10.7	LYS281, ARG277,LYS527
3.	Neoandrographolide	-10.1	GLN334,ARG277
4.	Queretaroic acid	-12.1	LYS512,LYS281
5.	Amritoside	-9.4	LYS527,GLU516,LYS512

Table 1: Phytochemical showing significant docking scores to 6KDJ protein

From the table 1, we can see that phytochemicals show comparable docking scores to the native ligand Lamivudine. Neoandrographolide binds to GLN334 amino acid which also forms bonds with Lamivudine. Apart from above chemicals isovitexin-8.8, 8-piperamide -7.7, berberine-7.9, tembatarine-7.9, bicalein-8.4, dihydrpipericide -8.2, isochavicine-7.7 also show high docking scores

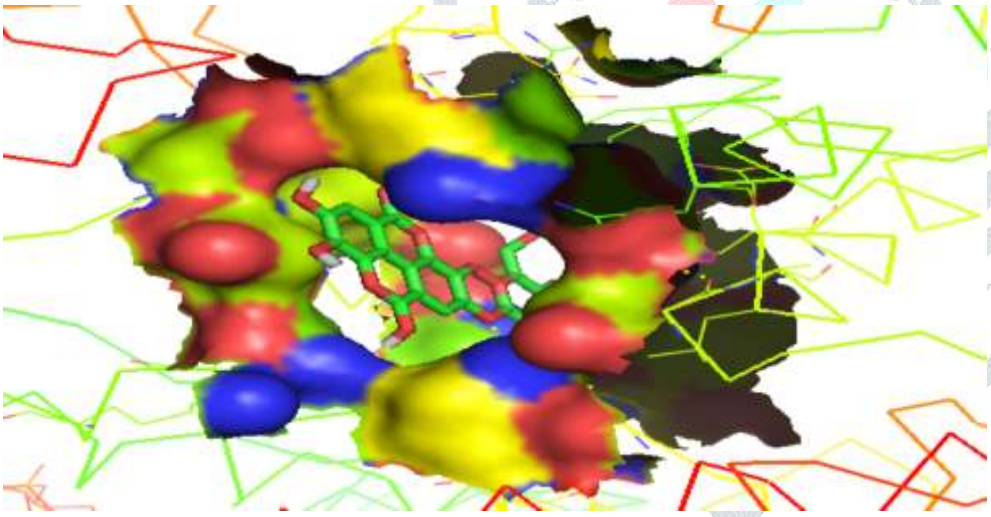

Figure 2: 6KDJ Amritoside in docking pocket

2.1S1X- Reverse transcriptase with Non-nucleoside reverse-transcriptase inhibitor Nevirapine

Serial number	Ligand	DOCKING SCORE	INTERACTION
Control ligand	Nevirapine	-5.4	LYS101,GLU138
1.	Tinosporide	-6.6	TYR188
2.	Piperlonguminine	-5.8	MET230
3.	Serratin	-5.7	THR139,ARG172
4.	Tricostachine	-5.5	TYR188
5.	Tinosporaside	-5.1	LYS103,LYS101,THR139

Table 2: Phytochemicals showing significant docking scores to 1S1X protein

From the table 2 we can see that phytochemicals show comparable docking scores to the native ligand Nevirapine .Tinosporaside binds to LYS101 amino acid which also forms bonds with Nevirapine. Apart from above chemicals Bicalein-5.4, Bisibaloene-5.1, coumaperine-4.6, Dihydropipernonaline -4.8, isochavicine-4.8 also show good docking scores.

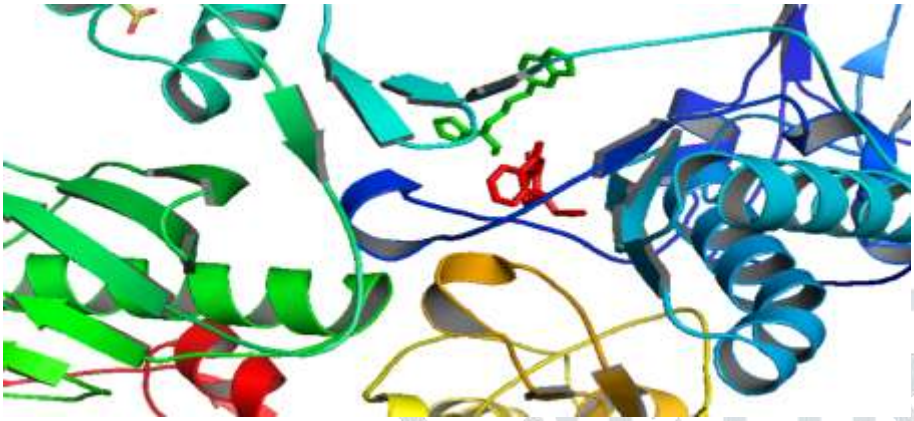

Figure 3: 1S1X reverse transcriptase showing Nevirapine and Tricostachine in same binding pocket

3. 3WJS-Protease with inhibitor Indanavir

Serial number	Ligand	DOCKING SCORE	INTERACTION
Control ligand	Indanavir	-11.5	ASP36, ARG10
1	Palmatoside	-11.4	ASP36, MET37, GLY102
2	Cordifolide	-11.2	ASP36, MET37, ARG10
3	Neoandrographolide	-10.9	ASP32,ASP36,ASN97
4	Tinasporaside	-10.6	ALA99,LEU57
5	Amritoside	-10.2	ARG10,TRP98,GLY34

Table 3: Phytochemicals showing significant docking scores to 3WSJ protein

From the table 3 we can see that phytochemicals show comparable docking scores to the native ligand Idanavir. Palmatoside and Cordifolide form hydrogen bond with ASP36 aminoacid similar to Indanavir. Cordifolide and Amritoside form hydrogen bond with ARG10 aminoacid similar to Indanavir. Apart from above chemicals Berberine-9.3, biclein-7.8, dihydropipericide-8.1, magnoflorin-8.8, tricostachine-7.9 also show good docking scores to the protein.

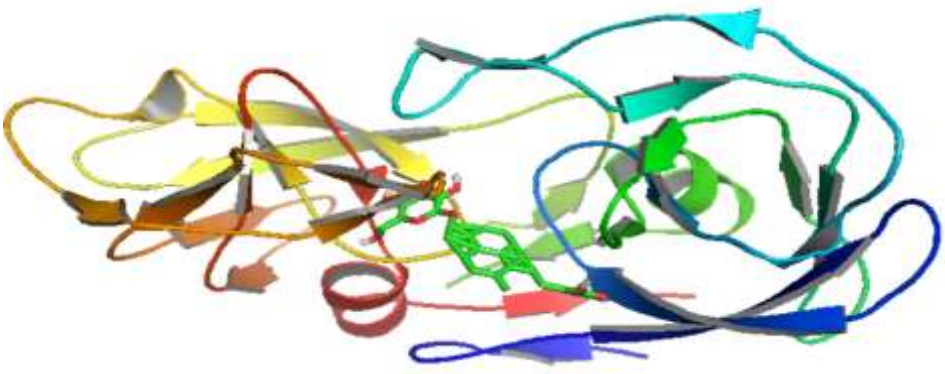

Figure 4: Neoandrographolide docked in 3WSJ protease

Conclusion

In this study we have shown that many phytochemicals which are present in herbs of MV Kashayam have good binding activity to various drug target proteins of HIV virus and hence there is a possibility that the Kashayam may show antiviral properties against HIV.

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