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RESEARCH PAPER

MOLECULAR DOCKING STUDIES OF PHYTOCONSTITUENTS IDENTIFIED FROM TRADITIONAL MEDICINAL PLANTS FOR PROTEASE AND REVERSE TRANSCRIPTASE TARGETED ANTI-HIV ACTIVITY

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The aim of the present study was to identify specific phytoconstituents by *in silico* molecular docking studies on selected medicinal plants for AIDS which is an autoimmune disorder caused by immunodeficiency virus (HIV). The replication of HIV virus inhibition was studied by the ligand docking result of phytoconstituents such as Calanolide C, Calanolide B, Calanolide A, Buchenavianine, Agastenol, O-Demethyl buchenavianine and Artemisinin on the medicinal plants *Callophyllum lanigerum*, *Buchenavia capitata* and *Artemisia annua* respectively with that of HIV Integrase and HIV Reverse transcriptase. The molecular docking study was carried by using Molegro Virtual Docker and the results were expressed based on the ability of the chemical constituents to bind with the targets given in terms of MolDock score, Rerank score and Hydrogen bond binding energy. Calanolide C and Calanolide E_2 have least MolDock score than Buchenavianine, as compared with that of standard anti-HIV drugs such as Indinavir and Saquinavir. According to the docking studies, least MolDock score have high anti-HIV activity and phytoconstituents from *Callophyllum lanigerum* predicted for better anti-HIV activity through molecular docking studies.

Key words: Phytoconstituents, Anti-HIV activity, MolDock score, *Callophyllum lanigerum*.

INTRODUCTION

AIDS is caused by infectious virus which is known as human immunodeficiency virus (HIV). It is an immunosuppressant disease which results into life threatening action. According to United Nation, 36 million people were infected by AIDS globally every year. AIDS has worldwide approach and most commonly in South Africa and Swaziland [1]. The replication of HIV virus in a host cell is majorly done by three important enzymes namely HIV-protease, HIV-reverse transcriptase and HIV-protease with two glycoproteins [2].

The pathogenesis of human immunodeficiency virus is to attack on immune system and central nervous system and hence suppress the immunity. The first step is entry of HIV into the cell required certain receptor and the cell surface CD4 receptor and co-receptor such as

CCR5 or CXCR4. The receptor interacts with protein complexes which are embedded in viral envelope. These complexes are composed of two glycoprotein gp120 and glycoprotein gp41. Gp120 binds with CD4 receptor called attachment, co-receptor binding results in conformational changes of gp120. This allows gp41 to unfold and insert hydrophobic terminus into the cell membrane. Gp41 then folds back itself which draws the virus towards the cell and facilitates the fusions of membrane [3].

The viral nuclear capsid enters the host cell and breaks open release two viral RNA strain and three essential enzymes such as protease, integrase and reverse transcriptase. Then the reverse transcriptase begins the reverse transcription of viral RNA. It has two catalytic domains, the ribonuclease active site and pilminarase active site. Here single standard viral RNA transcribes into RNA-DNA double helix. Ribonuclease each breaks down the RNA. The pilminarase then completes the remaining DNA structure to form DNA double helix. After this stage, integrase cleaves a dinucleotide from each three-prime end of DNA creating two sticky end. Integrase then transfers the DNA into the cell nuclease and facilitates each integration into the host cell genome [4].

The host cell genome now contains genetic information of HIV. Activation of the cell induces transcription of proviral DNA anti-messenger RNA (mRNA). The viral messenger RNA into the cytoplasm becomes building blocks for new virus synthesis. Some of them are processed by viral protease. Protease cleaves longer protein into smaller core protein. This step is crucial to create an infectious virus. Two viral RNA strains and the replication enzymes then, comes together and core protein assembles around them forming the particle leaves the cell accruing a new envelop host and viral protein. The virus matures and becomes Redding to infect others. HIV replicate billions of times per day destroying the host immune cell and eventually causing disease progression. The number of people living with HIV on antiretroviral therapy has increased by about a third fold reaching 17.0 million people – 2 million more than 15 million by 2015 target. In year 2015, there were 2.1 million new HIV infections worldwide, adding up to a total of 36.7 million people living with HIV [5].

Medical science turned towards natural plants, marine organisms, and other organisms. These phytoconstituents show very potent action against the HIV replication. Based upon a through literature survey [6], the work was designed to focus on traditional medicinal plants like *Callophyllum lanigerum*, *Buchenavia capitata*, *Agastache rugose* and *Artemisia annua* having anti-HIV activities. Calanolide A is a compound isolated from the latex of the tree, *Calophyllum lanigerum* var. *austrocoriaceum* which grows in the rain forest of the Malaysian state of Sarawak on the island of Borneo. There are about 200 species in the genus 'Calophyllum'. Plants from the genus 'Calophyllum' have been shown to contain xanthones, steroids, triterpenes, Coumarins, and benzopyrans. Calanolide A falls into the category of a dipyranocoumarin [7]. It is classified as a non-nucleoside HIV-1 specific reverse transcriptase (RT) inhibitor. A piperidine flavones related to alkaloid isolated from *Buchenavia capitata* (family *Combretaceae*), showed activity in both anti-HIV and anticancer cell-based screening [8].

There have been reports in Africa that people infected with HIV have also benefitted from the use of the plant *Artemisia annua*. This is of obvious benefit, due additionally to the problem that HIV poses in the region and the potential low cost and high accessibility of the plant. Therefore, *Artemisia annua* tested against HIV, has shown that it does indeed have very strong activity [9]. Keeping in view the significance of molecular docking in drug discovery [10, 11], screening these large numbers of secondary metabolites of plant origin using computational tools will help in drug design in a very short time as compared to the conventional methods.

MATERIALS AND METHODS

Molecular docking studies

Preparation of ligand

The 3D structures of the phytoconstituents (Calanolide C, Calanolide E_2, Calanolide B, Calanolide A, Buchenavianine, Agastenol, *O*-Demethyl buchenavianine & Artemisinin) and standard drugs (Indinavir & Saquinavir) were obtained from PubChem compound [9, 12-24] and saved in Mol format. The ligands are imported to the workspace and preparation was done. The docking scores of active constituents are compared against standard drugs.

Preparation of protein

The target for docking studies is selected as HIV-1 protease enzyme (PDB 1HXW) and HIV-1 reverse transcriptase (PDB ID: 1RT2). Docking analysis was done by initially selecting the target

for the disease and followed by obtaining the 3D structure of HIV-1 protease & HIV-1 reverse transcriptase from protein data bank in pdb format [25-30]. It is well known that PDB files often have poor or missing assignments of explicit hydrogens, and the PDB file format cannot accommodate bond order information. Therefore, proper bonds, bond orders, hybridization and charges were assigned using the MVD. The potential binding sites of both the targets were calculated using the built-in cavity detection algorithm implemented in MVD. The search space of the simulation exploited in the docking studies was studied as a subset region of 25.0 Angstroms around the active side cleft. The water molecules are also taken into consideration and the replaceable water molecules were given a score of 0.50.

Parameters for scoring functions

MolDock score

The ignore-distant-atoms option was used to ignore atoms far away from the binding site. Additionally, hydrogen bond directionality was said to check whether hydrogen bonding between potential donors and acceptors can occur. The binding site on the protein was defined as extending in X, Y and Z directions around the selected cavity with a radius of 25 Å.

Rerank Score

The re ranking scoring functions are used to create and predict models for estimation of chemical properties. The re-ranking score function is computationally more expensive than the scoring function used during the docking simulation, but it is generally better than the docking score function at determining the best pose among several poses originating from the same ligand. While the Rerank-score in MVD provides an estimate of the strength of the interaction, it is not calibrated in chemical units, and it does not take complex contributions (such as entropy) into account.

RESULTS AND DISCUSSION

The possibility of the natural phytoconstituents to bind with the targets is given in terms of MolDock score. The important parameters for evaluating a docking study are MolDock score and Rerank Score. The phytoconstituents are ranked according to MolDock score. The ligand possessing the least MolDock and Rerank score exhibit a powerful affinity towards its target. Analysis of the molecular docking of the phytoconstituents *C. lanigerum*, *B. capitata* and *A. annua* on HIV-I protease (PDB: IHXW) based on MolDock score is represented in **Table 1** and based on H Bond in **Table 2**.

Table 1. Molecular docking analysis of phytoconstituents obtained from medicinal plants on HIV-1 protease (PDB ID : IHXW) ranking based on MolDock score

Name	Ligand	MolDock score	Rerank score	H Bond
Indinavir	Indinavir	-149.797	-108.412	-2.6882
Calanolide C	Calanolide C	-128.706	-56.0842	0
Calanolide E_2	Calanolide E_2	-124.558	-56.5087	-2.84033
Calanolide B	Calanolide B	-123.957	-75.7958	-3.63525
Buchenavianine	Buchenavianine	-123.87	-101.248	-4.06235
Agastenol	Agastenol	-116.098	-17.6589	-1.82337
Calanolide A	Calanolide A	-112.997	-90.9635	0
O-Demethyl buchenavianine	*O*-Demethyl buchenavianine	-103.988	-37.3842	-1.7955
Saquinavir	Saquinavir	-99.183	190.016	-1.28327
Artemisinin	Artemisinin	-63.3541	-63.8414	-4.89917

Analysis of the molecular docking of the phytoconstituents *C. lanigerum*, *B. capitata* and *A. annua* on HIV-1 reverse transcriptase (PDB ID : 1RT2) based on MolDock Score is represented in **Table 3** and based on H Bond in **Table 4**.

The residues in the 1HXW chain which are involved in the binding to the standard drug Indinavir and Saquinavir are Ala 28, Arg 8, Asp 25, Asp 29, Asp 30, Gly 27, Gly 48, Ile 32, Ile 47, Ile 50, Ile 84, Leu 10, Leu 23, Pro 53, Pro 81, Thr 31, Thr 80, Val 32, Val 84. The docked view of Indinavir and Calanolide C are represented in the **Figure 1A** and **Figure 1B** respectively. The residues in the 1RT2 enzyme which are involved in the binding to the standard drug Abacavir are Ser 134, Thr 139, Pro 140, Gly 141, Met 184, Pro 157, Thr 115, Gln 161, Gly 93, Gln 91. Docked view of Abacavir and Buchenavianine on HIV-1 reverse transcriptase (PDB ID : 1RT2) is represented in **Figure 1C** and **Figure 1D**.

Table 2. Molecular docking analysis of phytoconstituents obtained from medicinal plants on HIV-1 protease (PDB ID : 1HXW) ranking based on H Bond

Name	Ligand	MolDock score	Rerank score	H Bond
Artemisinin	Artemisinin	-63.3541	-63.8414	-4.89917
Buchenavianine	Buchenavianine	-123.87	-101.248	-4.06235
Calanolide B	Calanolide B	-123.957	-75.7958	-3.63525
Calanolide E_2	Calanolide E_2	-124.558	-56.5087	-2.84033
Indinavir	Indinavir	-149.797	-108.412	-2.6882
Agastenol	Agastenol	-116.098	-17.6589	-1.82337
O-Demethyl buchenavianine	*O*-Demethyl buchenavianine	-103.988	-37.3842	-1.7955
Saquinavir	Saquinavir	-99.183	190.016	-1.28327
Calanolide C	Calanolide C	-128.706	-56.0842	0
Calanolide A	Calanolide A	-112.997	-90.9635	0

Table 3. Molecular docking analysis of phytoconstituents obtained from medicinal plants on HIV-1 reverse transcriptase (PDB ID : 1RT2) ranking based on MolDock score

Name	Ligand	MolDock score	Rerank score	H Bond
Abacavir	Abacavir	-131.483	-106.605	-6.27362
Zidovudine	Zidovudine	-128.716	-106.149	-7.78922
Buchenavianine	Buchenavianine	-119.879	-90.8804	0
Calanolide A	Calanolide A	-113.097	-4.50677	-6.78752
Agastenol	Agastenol	-106.97	-73.374	-5.4959
Efavirenz	Efavirenz	-106.795	-70.738	-0.978297
Stavudine	Stavudine	-106.578	-87.854	-4.23572
Calanolide E_2	Calanolide E_2	-105.913	-63.9523	-0.723649
Calanolide C	Calanolide C	-97.1397	-48.9806	0.726116
Calanolide B	Calanolide B	-92.702	-46.8593	-2.42709
O-Demethyl buchenavianine	*O*-Demethyl buchenavianine	-71.6317	-61.7347	-6.45446
Nevirapine	Nevirapine	-65.9948	-57.8608	0
Artemisinin	Artemisinin	-65.5836	-42.1011	-0.76748
Nelfinavir	Nelfinavir	11218.8	451.23	-4.84985

Table 4. Molecular docking analysis of phytoconstituents obtained from medicinal plants on HIV-1 reverse transcriptase (PDB ID : 1RT2) ranking based on H Bond

Name	Ligand	MolDock score	Rerank score	H Bond
Zidovudine	Zidovudine	-128.716	-106.149	-7.78922
Calanolide A	Calanolide A	-113.097	-4.50677	-6.78752
O-Demethyl buchenavianine	*O*-Demethyl buchenavianine	-71.6317	-61.7347	-6.45446
Abacavir	Abacavir	-131.483	-106.605	-6.27362
Agastenol	Agastenol	-106.97	-73.374	-5.4959
Nelfinavir	Nelfinavir	11218.8	451.23	-4.84985
Stavudine	Stavudine	-106.578	-87.854	-4.23572
Calanolide B	Calanolide B	-92.702	-46.8593	-2.42709
Efavirenz	Efavirenz	-106.795	-70.738	-0.978297
Artemisinin	Artemisinin	-65.5836	-42.1011	-0.76748
Calanolide E2	Calanolide E2	-105.913	-63.9523	-0.723649
Buchenavianine	Buchenavianine	-119.879	-90.8804	0
Nevirapine	Nevirapine	-65.9948	-57.8608	0
Calanolide C	Calanolide C	-97.1397	-48.9806	0.726116

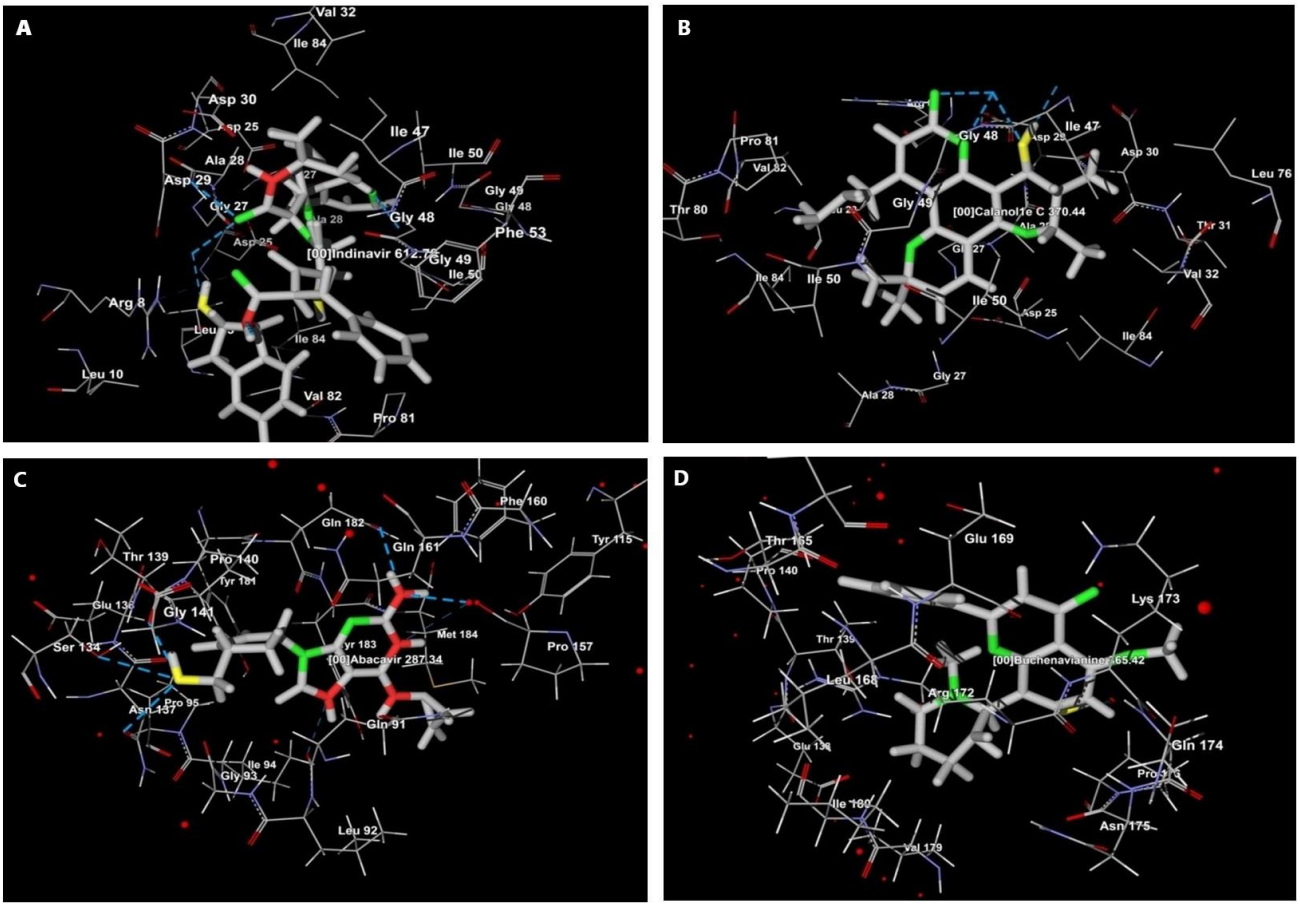

Fig. 1A. Docked view of Indinavir on HIV-1 protease (PDB ID : IHXW), **Fig. 1B.** Docked view of Calanolide C on HIV-1 protease (PDB ID : IHXW), **Fig. 1C.** Docked view of Abacaviron on HIV-1 reverse transcriptase (PDB ID : IRT2), **Fig. 1D.** Docked view of Buchenavianine on HIV-1 reverse transcriptase (PDB ID : IRT2)

CONCLUSION

The molecular docking analysis helps to predict the novel drugs with the phytoconstituent and the target protein on protease and reverse transcriptase for targeted anti-HIV activity by Molegro Virtual Docker. From thorough literature studies, eight compounds were obtained, such as Calanolide A, Calanolide B, Calanolide C, Calanolide E_1 from plant *Callophyllum lanigerum*, Buchenavianine and *O*-Demethyl buchenavianine from plant *Buchenavia capitata*, Agastenol from plant *Agastache rugose* and Artemisinin from *Artemisia annua*, the phytoconstituents which are known as ligand in molecular study. The 3D structures of ligands were obtained from PubChem compound. HIV-protease (PDB ID: 1HXW) and HIV-1 reverse transcriptase (PDB ID: 1RT2) were selected for docking studies. The ability of chemical constituents to bind with the targets was given in term of MolDock score, Rerank score and hydrogen bond binding energy. Calanolide C and Calanolide E_2 have least MolDock score than Buchenavianine with that of standard anti-HIV drugs such as Indinavir and Saquinavir. The least MolDock score have high anti-HIV activity. Therefore, phytoconstituents from *Callophyllum lanigerum* predicted better anti-HIV activity through molecular docking studies, and this plant can be use in future for *in-vitro* and *in-vivo* anti-HIV studies.

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