Acquired Immune Deficiency Syndrome is an incurable and terminal disease of the human immune system caused by the Human Immunodeficiency Virus (HIV). It is associated with Envelope Glycoprotein (EGP) and Glycoprotein 120 (GP 120). 3D structures of these two proteins were generated using Homology Modeling. Active compounds of medicinal herbs Castanospermum australe which may inhibit HIV replication and syncytium formation induced by EGP and of Ancistrocladus korupensis which may act on HIV life cycle by inhibiting reverse transcriptase, cellular fusion and syncytium formation were selected. Chemical structures of the active component of these herbs were drawn using chemsketch, combined & converted to .pdb. Both the proteins were successfully docked with the Castanospermum australe - Ancistrocladus korupensis active component combination. RNAi treatment was later employed to block the translation pathway. Our study concluded that the combination of plant alkaloids Michellamine B and Castanospermin could be used as the herbal remedy for HIV infection.

Keywords: AIDS, Homology modeling, Castanospermum australe, Ancistrocladus korupensis, plant alkaloids.

INTRODUCTION

Acquired immunodeficiency syndrome (AIDS) is a clinical syndrome that is the result of infection with human immunodeficiency virus (HIV), which causes profound immunosuppression. It has been a serious, life-threatening health problem since the first case was identified in 1981 and is the most quickly spreading disease of the century. Since the epidemic began, more than 60 million people have been infected with the virus. HIV/AIDS is now the leading cause of death in Sub-Saharan Africa. Worldwide, it is the fourth biggest killer. According to recent reports of WHO and UNAIDS an estimated 40 million people (37.2 million adults and 2.2 million children) globally were living with HIV, out of which about 22 million had died. The most affected is Sub-Saharan Africa, where 3.1 million adults and children became infected with HIV and 2.3 million died. South Africa is reported to have the largest population living with the disease, at well over 5 million people infected, followed by Nigeria in second place and India being the third largest population of HIV infected with more than 2 million people reported. Around 1.2 million people in Asia acquired HIV in 2009, bringing the number of people living with HIV to an estimated 8.2 million. The spread of HIV in India has been diverse, with much of India having a low rate of infection and the epidemic being most extreme in the southern states. As of now, 92% of all nationally reported AIDS cases have been found in 10 of the 28 states and 7 union territories. The greatest numbers were in Maharashtra and Gujarat in the west; Tamil Nadu and Andhra Pradesh in the south; and Manipur in the Northeast. In the southern states, the infections are mostly due to heterosexual contact, while infections are mainly found amongst injecting drug users in Manipur and Nagaland. A very high proportion of men and women infected with HIV virus are in their active reproductive ages and around half of the people who acquire HIV become infected before they turn 25. Of greater concern is the possibility of infected mothers transferring the disease to their babies. Two major types of HIV have been identified so far, HIV-1 and HIV-2. HIV-1 is the cause of the worldwide epidemic and is most commonly referred to as HIV. It is a highly variable virus, which mutates readily. There are many different strains of HIV-1, which can be classified according to groups and subtypes; there are two groups, M and O. Within group M, there are currently known to be at least ten genetically distinct subtypes of HIV-1. These are subtypes A to J. In addition, Group O contains another distinct group of heterogeneous viruses. HIV-2 is much less pathogenic and occurs rarely; it is found mostly in West Africa HIV begins its infection of a susceptible host cell by binding to the CD4 receptor on the host cell. CD4 is present on the surface of many lymphocytes, which are a critical part of the body’s immune system. It is now known that a co-receptor is needed for HIV to enter the cell. Following fusion of the virus with the host cell, HIV enters the cell. The genetic material of the virus, RNA, is released and undergoes reverse transcription into DNA. An enzyme in HIV called reverse transcriptase is necessary to catalyze this conversion of viral RNA into DNA. Once the genetic material of HIV has been changed into DNA, this viral DNA enters the host cell nucleus where it can be integrated into the genetic material of the cell. The enzyme integrate catalyses this process. Once the viral DNA is integrated into the genetic material of the host, it is possible that HIV may persist in a latent state for many years. This ability of HIV to persist in certain latently
infected cells is the major barrier to eradication or cure of HIV. For this reason, based on current knowledge, patients must remain on anti-viral therapy for life. Several reviews on the natural products for chemotherapy of HIV infection have been reported. Matthee et al.\textsuperscript{11} reported naturally occurring HIV reverse transcriptase inhibitors. Jung et al.\textsuperscript{12} discussed anti-HIV agents according to their chemical classes. Yang et al.\textsuperscript{13} reviewed natural products-based anti-HIV drug discovery and development facilitated by NCI development programme. Cos et al.\textsuperscript{14} reviewed different plant substances as anti-HIV agents according to their mechanism of action.

### MATERIALS AND METHODS

The softwares or web servers used for this study includes Modeller9v7, RASMOL, ACD/ChemSketch version 11.00, RAMPAGE, ArgusLab 4.0.1, Genamics, Si RNA Design Tool and PATCHDOCK.

**In silico** modeling and docking simulations

The key protein responsible for HIV infection, envelope glycoprotein with accession number ABH02539.1\textsuperscript{15} was taken for this study. Human receptor protein to which HIV protein binds, gp120 with accession number AAF69492.1\textsuperscript{16} was taken from NCBI GenBank database. Homology modeling was carried out using Modeller 9v7\textsuperscript{17}, for predicting 3 D structures for the above mentioned proteins. The best templates used for homology modeling were identified by similarity searching tool BLAST\textsuperscript{18} and the best homologous protein structures from RCSB PDB were retrieved. The following templates were used:

**Envelope glycoprotein:** 3JWD, Chain A (Identity=100%, Source: Human immunodeficiency virus 1), 3JWO, Chain A (Identity=100%, Source: Homo sapiens), 2BF1, Chain A (Identity=37%, Source: Simian immunodeficiency virus).

**Gp120**: 2BF1, Chain A (Identity=37%, Source: Simian immunodeficiency virus), 1YYM, Chain G (Identity=75%, Source: Homo sapiens), 1G9M, Chain G (Identity=74% Source: Homo sapiens).

Five models of each of the above proteins were retrieved. The models were analyzed by Rampage Ramachandran plot server\textsuperscript{19} and the best model of each was selected. Chemical structures of active component of Ancistrocladus korupensis\textsuperscript{20}, Michellamine B (Fig-1) and active component of Castanospermum austral\textsuperscript{21}, Castanospermine (Fig-2) were drawn and combined using ACD chemsketch\textsuperscript{22} software (Fig-4). The combined structure was saved as .mol file and was later converted to .pdb file using Argus lab\textsuperscript{23} software (Fig-4).

Each of the proteins used for this work, namely Envelope glycoprotein and gp120 was successfully docked with active components of Ancistrocladus korupensis and Castanospermum austral i.e. Michellamine B and Castanospermine combination. The docking study was conducted by PatchDock\textsuperscript{24}. It is an automatic server for molecular docking and it uses molecular docking algorithm based on shape complementarity principles.
RNAi treatment

RNA sequences of the proteins (i.e. envelope glycoprotein and gp120) were generated using the software GENAMICS and the fragments of the RNA sequence (Si RNA fragments) are generated using online Si RNA design tool from Microsynth.

RESULTS AND DISCUSSION

In silico homology modeling of target proteins

The first target protein identified in this study was the envelope glycoprotein of human immunodeficiency virus 1 with the Gene index number GI: 110893758. The protein consists of 455 amino acid residues. The next sequence used in our study was gp120 of Human immunodeficiency virus 1 with GI: 7769644. The sequence also consists of 455 amino acids. The 3D structure of envelope glycoprotein obtained by homology modeling was analyzed by RAMPAGE Ramchandran plot server. The results obtained for best model is good quality because almost all residues of the protein are located in to the allowed region of Ramchandran plot. (Fig-5) The stereochemical validation of the structure is follows:

- Number of residues in favored region (~98.0% expected): 411 (90.7%)
- Number of residues in allowed region (~2.0% expected): 37 (8.2%)
- Number of residues in outlier region: 5 (1.1%)

Similarly the 3D structure of gp120 obtained by homology modeling was also analyzed by RAMPAGE Ramchandran plot server. This model also qualifies the quality as per the Ramchandran rule (Fig-6). The stereochemical validation of alpha carbon atoms of the model are given below:

- Number of residues in favored region (~98.0% expected): 394 (87.0%)
- Number of residues in allowed region (~2.0% expected): 47 (10.4%)
- Number of residues in outlier region: 12 (2.6%)

Docking simulations and in silico drug discovery

The docking studies were conducted with the modeled proteins (Envelope protein and gp 120) and the combinations of Michellamine B and Castanospermine. Michellamine B is a plant alkaloid isolated from Ancistrocladus korupensis. Michellamine B has exhibited significant in vitro activity against HIV-1 and HIV-2. Castanospermine is an indolizine alkaloid isolated from the seeds of Castanospermum australe. It is a potent inhibitor of some glucosidase enzymes and has antiviral activity.

The docking simulations were performed with PatchDock server. The docking mechanism in PatchDock relies on three concepts. First, molecular shape representation, the programme computes the molecular surface of the molecule followed by a segmentation algorithm for detection of geometric patches (concave, convex and flat surface pieces). The patches are filtered, so that only patches with ‘hot spot’ residues are retained. The second step is the surface patch matching, here we applied a hybrid of the geometric hashing and pose-clustering matching techniques to match the patches detected in the previous step. Concave patches are matched with convex and a flat patch with any type of patches. The final step is filtering and scoring. In this case the candidate complexes from the previous step are examined. The programme discards all complexes with unacceptable penetrations of the atoms of the receptor to the atoms of the ligand. Finally, the remaining candidates are ranked according to a geometric shape complementarity scores.

The PatchDock Server generated many best poses and the conformations which were stable with minimum energy. The envelop glycoprotein is bind to the inhibitors and produced stable protein-ligand complex (Fig-7). Similarly the combinations of inhibitors successfully docked with gp-120 protein with minimum binding energy and visualized by RASMOL (Fig-8). Docking scores for envelope glycoprotein was -8768 and gp120 was -9124. Our study
revealed that Michellamine B–Castanospermine complex could be used as a potential inhibitor against HIV infection as these ligands perfectly interacting with both the proteins.

RNAi treatment
The Si RNA fragment of envelop glycoprotein and gp120 were generated by the tools mentioned in materials and methods (Fig- 9 & 10). The translation pathways of envelop glycoprotein and gp120 proteins were blocked by the formation of Si RNA, thus inhibiting the disease. If RNAi treatment proves efficient in wet lab, the virus can be killed.

CONCLUSION
The in silico herbal work makes use of ayurvedic herbs in computer aided drug designing. The principle outlined in homology modeling is used to model the 3D structure of the proteins envelop glycoprotein and gp120. The docking studies of active components of the herbs Ancistrocladus korupensis and Castanospermum australe with the envelop glycoprotein and gp120 proteins provide a new insight to drug discovery process against HIV infection. Our study concluded that the successful docking of envelop glycoprotein and gp120 with Michellamine B and Castanospermine combination proves that the combination can be effective in the treatment of AIDS disease. Again, since the work is done in in silico platform, the combination (Michellamine B and Castanospermine) needs to go to clinical testing to establish its efficacy.

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