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RESEARCH ARTICLE

In Silico approach to inhibit Synthetic HIV-TAT activity using Phytoconstituents of *Moringa oleifera* leaves extract

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ABSTRACT:
TAT (Trans-activator-Transcription Protein), a viral protein is encoded by the TAT gene in HIV-1-which is a lethal subtype of HIV (Human immunodeficiency Virus). It is vital for the transcription of the viral genome. Previous studies show that in Human TAT is a toxin-producing protein allowing cell death in normal T-cells. Thereafter allows for progression towards AIDS (Acquired immunodeficiency syndrome). Traditionally herbal medicines have played a vital role in the treatment of many diseases and ailments. Although studies have been conducted to find anti-HIV activities against other HIV-1 proteins, there are no traces of studies against HIV Trans-activator-Transcription protein (PDB: 1JFW). The main objective of this study is to find an efficacious inhibitor against a synthetic HIV-TAT protein (PDB: IJFW). After a thorough literature survey, the molecular and biological activity random compounds of *Moringa oleifera* (drum-stick plant) have been recorded for molecular and biological activities to evaluate drug-likeness of the compounds. Thereafter which the highest binding affinity compound was identified by performing protein-ligand docking analysis. Finally, the compound with the highest binding affinity along with its measurement has been visualized and recorded using the Pymol software. This study can further be confirmed using molecular dynamics to identify the lead inhibitor against HIV-1 TAT protein

KEYWORDS: HIV Trans-activator-transcription protein, *Moringa oleifera*, Drug-likeness, Molecular Docking.

INTRODUCTION:
Moringa oleifera, Lam (M. oleifera) is a member of the Moringaceae family belongs to the sub-Himalayan tracts of India, Pakistan, Bangladesh and Afghanistan and is consumed well as a dietary source[1,2]. Phytochemical studies have revealed that *M. oleifera* leaves are rich in essential amino acids and sources such as potassium, calcium, phosphorous, iron, vitamins A and D. They are also rich in antioxidants such as β-carotene, vitamin C and flavonoids[3,4]. In many regions of Africa, from traditional days M. oleifera is widely consumed for self-medication by patients affected HIV/ AIDS[5,6]. However, there is room to exploit the potential M. oleifera in the battle against HIV.

In the present study, an attempt has been made to answer the role of M. oleifera as an inhibitor against as a target receptor, Trans-activator-Transcription protein.

HIV-I TAT (Trans-activator-Transcription Protein) is a viral protein encoded by the TAT gene which plays a pivotal role in reproducing the virus but it also plays a pivotal role in HIV-based immunodeficiency. TAT is a short protein encoded by two exons and its size varies from 86 to 106 residues[7]. TAT contains six different regions with distinct functions. Region I (residues 1–21) is a proline-rich region and has the conserved Trp 11.

Region II (residues 22–31) has seven conserved cysteines at positions 22, 25, 27, 30, 31, 34 and 37. No other cysteines are found in the sequence and there is no evidence of disulphide bridges required for TAT function. Region III (residues 38–48) has the Phe 38 and the conserved sequence LGISYG from residues 43–48. Region IV (residues 49–59) is rich in basic residues and has the conserved sequence RKKRRQRRRPP. Region V (60–72) is a glutamine-rich region. Region VI constitutes

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the C-terminus of TAT encoded by the second exon but its size can be variable depending on the HIV isolate. The C-terminus shows similarities with the N-terminus in a high percentage of prolines. Resolution of this structure was a determining factor in drug designing. The chemical synthesis of the drugs allowed the specific binding and the inhibition of TAT to be verified[8].

Although previous studies have shown the efficiency of the compounds to be effective against lethal diseases, there are no such studies to show the efficiency of compounds against HIV-1 TAT proteins. Therefore the current studies were performed to predict the binding affinities of the active sites of the synthetic HIV-1 TAT (PDB: IJFW) protein to identify the best candidates against the activities of synthetic HIV-1 TAT protein using *in silico* molecular docking studies.

MATERIALS AND METHODS:

Compounds Collection:

Phytochemical compounds from *Moringa oleifera* were obtained from previous literature studies[9] (Table1).

Ligand and Protein Preparation:

The 2-Dimensional (2-D) structures of compounds were obtained from Pubchem. PubChem is a database of chemical molecules and their activities against biological assays. The system is maintained by the National Center for Biotechnology Information (NCBI), a component of the National Library of Medicine, which is part of the United States National Institutes of Health (NIH). PubChem can be accessed for free through a web user interface. Millions of compound structures and descriptive datasets can be freely downloaded. The 2-D structure of compounds was downloaded in SDF format and converted to PDB format by using Pymol molecular graphics system, version 1.5.0.3 (www.pymol.org). A Three Dimensional structure of synthesized TAT protein (PDB ID: 1JFW) was identified and obtained from Protein Data Bank (PDB) The Protein Data Bank (PDB) is a database that contains three-dimensional structural data of large biological molecules, such as proteins and nucleic acids (https:// www. Rcsb.org)[8].

Calculation of ADME properties:

Computational methods were employed to obtain the molecular properties by processing the Molinspiration Cheminformatics server (http://www.molinspiration. com)[10]. Drug-likeness is a qualitative concept used for drug-like property which is described as a complex balance of various molecular properties and structural features which determine whether a particular molecule is similar to the known drugs. These molecular properties are mainly hydrophobicity, electronic distribution, Hydrogen bonding characteristics, molecule size, and flexibility. Drug-likeness evaluated by the Lipinski rule of five that deals four simple physicochemical parameter ranges (MWT $\leq$ 500, log P $\leq$ 5, H-bond donors $\leq$ 5, H-bond acceptors $\leq$ 10) associated with 90% of orally active drugs that have passed phase II clinical status Protein and ligand preparation[11]. The ADME properties for ten *Moringa oleifera* compounds were performed

Active site prediction:

A small region or cleft where the ligand molecule can bind to the receptor protein and produce the preferred outcome is termed as an active site/catalytic site. Identification of this active site residue in the target protein structure has a great range of applications in molecular docking. The binding pockets of synthesized TAT protein were predicted out using Metapocket server[12,13].

Molecular Docking:

Molecular docking is a key tool in structural molecular biology and computer-assisted drug design. The goal of ligand-protein docking is to predict the predominant binding mode (s) of a ligand with a protein of known three-dimensional structure. Successful docking methods search high-dimensional spaces effectively and use a scoring function that correctly ranks candidate dockings[14]. In this study, docking has been carried out using Argus lab docking tool. Drug-like phytochemical compounds from *Moringa oleifera* were used to perform molecular docking studies using Argus Lab[15]. The docking interaction of synthesized TAT protein with the drug-like compound obtained from Moringa Oleifera was carried out using Argus lab software[16]. Docking was performed using "Genetic Algorithm (GA) Dock" exhaustive search with grid resolution of 0.40 Å whereas the 'Docking precision' was set to "Regular precision" and "Flexible" ligand docking mode was employed for each docking run. The stability of each docked pose was evaluated using energy calculations and the number of hydrogen bonds formed[17,18].

RESULTS AND DISCUSSION:

The following *Moringa oleifera* phytochemical compounds were obtained from previous literature studies. The PubChem ID, molecular formulae and canonical SMILES for the same were obtained from PubChem database.

Table1.Showing the compounds obtained from *Moringa oleifera* along with its Pubchem ID, Molecular formula and Canonical SMILES

S. No	Name of Compounds	Pubchem ID	Molecular Formula	Canonical Smiles
1.	6,6-dimethyl-5,6- dihydroimidazo[2,1- b] [1,3] thiazol-3- yl) methyl N,N'-dicyclohexylimidothiocarbamate	46926547	$C_{21}H_{35}N_4S_2$	CC1(C[N+]2=C(N1)SC=C2CSC(=NC3CCCCC3)NC4CCCCC4)C
2.	2-Pyrrolidinone	12025	C_4H_7NO	C1CC(=O)NC1
3.	Linalool oxide	22310	$C_{10}H_{18}O_2$	CC1(CCC(O1)C(C)(C)O)C=C
4.	Upiol	2447	$C_6H_{11}BrN_2O_2$	CC(C)C(C(=O)NC(=O)N)Br
5.	Ellagic acid	5281855	$C_{14}H_6O_8$	C1=C2C3=C(C(=C1O)O)OC(=O)C4=CC(=C(C(=C43)OC2=O)O)O
6.	Gallic acid	370	$C_7H_6O_5$	C1=C(C=C(C(=C1O)O)O)C(=O)O
7.	Ferulic acid	445858	$C_{10}H_{10}O_4$	COC1=C(C=CC(=C1)C=CC(=O)O)O
8.	Vanillin	1183	$C_8H_8O_3$	COC1=C(C=CC(=C1)C=O)O
9.	Aurantiamide acetate	10026486	$C_{27}H_{28}N_2O_4$	CC(=O)OCC(CC1=CC=CC=C1)NC(=O)C(CC2=CC=CC=C2)NC(=O)C3=CC=CC=C3
10.	Kaempferol	5280863	$C_{15}H_{10}O_6$	C1=CC(=CC=C1C2=C(C(=O)C3=C(C=C(C=C3O2)O)O)O)O

Table 2: The drug-likeness of the phytochemical compounds of *Moringa oleifera* were identified using Molinspiration server where the properties

S. No	Name of Compounds	mi LogP	TPSA	N atm	MW	nON	nOHNH	nviol	Nrtb	Vol	Drug-likeness (Yes/Nno)
1.	6,6-dimethyl-5,6-dihydroimidazo[2,1- b] [1,3] thiazol-3- yl) methyl N,N'-dicyclohexylimidothiocarbamate	2.09	40.30	27	407	4	2	0	6	392	Yes
2.	2-Pyrrolidinone	-0.18,	29.10	6	85	2	1	0	0	83	Yes
3.	Linalool oxide	1.94	29.46	12	170	2	1	0	2	179	Yes
4.	Upiol	0.54	72.19	11	223	4	3	0	2	158	Yes
5.	Ellagic acid	0.94	141.33	22	302	8	4	0	0	221	Yes
6.	Gallic acid	0.59	97.98	12	170	5	4	0	1	135	Yes
7.	Ferulic acid	1.25	66.76	14	194	4	2	0	3	172	Yes
8.	Vanillin	1.07	46.53	11	152	3	1	0	2	136	Yes
9.	Aurantiamide acetate	3.89	84.50	33	444	6	2	0	11	418	Yes
10.	Kaempferol	2.71	111.12	21	286	6	4	0	1	232.	Yes
[Note: TPSA- Total Polar surface area, natm- number of atoms, MW- Molecular weight, nON- No. of H- bond acceptors, nOHNH- No. of H-bond donors, nviol- number of violation, nrtb –number of rotatable bonds and Vol- Volume].											

Such as miLogP, TPSA, Natm, Molecular weight, nON, nOHNH, nviol, Nrtb, vol were analyzed.

The drug-likeness of the phytochemical compounds of Moringaoleifera were identified using Molinspirationserver where the properties such as miLogP, TPSA, Natm, Molecular weight, nON, nOHNH, nviol, Nrtb, vol were analyzed.

Figure1. The above illustrates the sorted 3D image of synthetic HIV-1(Human Immunodeficiency Virus) TAT protein, obtained from Protein Databank (PDB ID: 1JFW) and Visualized in Pymol

Active Sites and Docking Results:
The active site of the protein were as follows: ALA (A) 42, THR(A) 40, GLN(A)35, CYS(A) 37, PHE(A) 38, LYS(A) 41, VAL(A) 36.

Docking Result:
Table3.Shows the binding affinities of Phytochemical compounds of Moringa Oleifera against HIV-TAT protein

S. No	Compounds	Interacting sites	Binding energy (Kcal/mol)	Distance (Å)
1	6,6-dimethyl-5,6- dihydroimidazo[2,1- b] [1,3] thiazol-3- yl) methyl N,N'- dicyclohexylimidothiocarbamate	GLN-35..O….H PHE-38...O….H	-9.34	2.4 1.9
2.	2-Pyrrolidinone	LYS-41…O…H PHE-38…O…H	-5.25	2.8 2.2
3.	Ellagic acid	LYS-41…H…O ALA-42...H…O PHE-38…H…O	-8.83	2.2 2.3 2.0
4.	Upiol	GLN-35..O…H CYS-37..H…O CYS-37..H…H LYS-41..H…O	-7.90	2.9 1.9 2.7 2.2
5.	Linalool oxide	NO INTERACTIONS	-7.03	N/A
6.	Gallic acid	CYS-37..H…O PHE-38…O…H LYS-41..H…O	-6.36	2.6 2.4 2.5
7.	Ferulic acid	GLN-35..O…H ALA-42...H…O LYS-41..H…O CYS-37..H…O	-8.12	2.8 2.2 2.4 1.9
8.	Vanillin	LYS-41.. H..O CYS-37.. H..O CYS-37.. H..O CYS-37.. H..O PHE-38.. H..O	-6.49	1.8 2.2 2.7 2.7 2.2
9.	Aurantiamide acetate	LYS41..H..O PHE-38…H..O CYS37…H…O	-9.99	2.2 2.2 2.7
10.	Kaempferol	ALA-42..H..O PHE-38..H..O CYS-37..H..H	-9.82	2.2 2.4 2.2

DISCUSSION:

The present study investigates the efficiency of phytochemical compounds extracted from *Moringa Oleifera* leaves against the HIV TAT protein (PDB ID-IJFW). TAT is a regulatory protein that drastically enhances the efficiency of viral transcription. This protein is a synthetic protein which was designed for studies against HIV[8] and the protein was also previously processed for molecular dynamics and molecular docking studies. Wherein the quassinoid compounds holacanthone and oricinolide were found to be more efficacious in terms of binding energy as well as bonding distance[15]. In the current studies, the compounds from *Moringa Oleifera* were subjected to ADME studies. ADME studies on molinspiration tool was performed to find out the drug-likeness of the herbal compound. As mentioned earlier on drug-likeness is a concept that is used to find an oral medicinal property within the herbal compounds. It complies of various molecular properties and structural features which help to identify and compare the properties of known drugs to the compound. These molecular properties are mainly hydrophobicity, electronic distribution, and hydrogen bonding characteristics, molecule size, and flexibility. Drug-likeness evaluated by the Lipinski rule of five that deals four simple physicochemical parameter ranges (MWT ≤ 500, log P ≤ 5, H-bond donors ≤ 5, H-bond acceptors ≤ 10)[10,19]. The ten compounds that matched the drug-like properties: 6,6-dimethyl-5,6- dihydroimidazo[2,1- b] [1,3] thiazol-3- yl) methyl N, N'-dicyclohexylimidothiocarbamate, 2-Pyrrolidinone, Ellagic acid, Upiol, Linalool oxide, Gallic acid, Ferulic acid, Vanillin, Aurantiamide acetate, and Kaempferol. These aforementioned compounds were further processed for molecular docking studies.

Docking studies where Aurantiamide acetate and Kaempferol were found to be the best compounds amongst the ten drug-like compounds. This result was found to be more reasonable as these compounds bound to the synthetic HIV TAT protein(PDB-IJFW) with the lowest binding energy scores: Aurantiamide acetate -9.99kcal/mol (LYS41… H-O, PHE- 38…H..O. CYS37…H…O) and Kaempferol -9.82 kcal/mol (ALA-42...H...O, PHE-38...H...O, CYS-37...H...H) (Please refer to Table 3)[20, 21]. All Tat proteins contain a cluster of

seven cysteine residues (Region 22–37), which are important for trans-activation. The Hydrogen bonding formed between two different molecules is called intermolecular H bonding. Alpha helices and beta sheets are the two important secondary structures in a protein.

Literature survey has shown that kaempferol and Aurantiamide acetate contribute to diverse pharmacological properties. Whereas, Aurantiamide acetate has demonstrated significant anti-inflammatory, antiarthritic and analgesic activity mediated via inhibition of TNF-alpha, IL-2 and other cytokines. *In vitro* and *in vivo* studies demonstrate that Aurantiamide acetate may suppress the growth of human malignant gliomas via inhibiting intracellular autophagic flux[22]. From the above references and our research findings, we suggest the possibility of Aurantiamide acetate and Kaempferol to develop as an HIV-TAT protein (PDB ID: IJFW) antagonist. However, further in vitro and *in vivo* studies needed to validate their biological potential.

CONCLUSION:

In the present study, the molecular docking is performed to deduce the possible binding affinity of synthesized TAT protein with the best predicted flavonoid compound. The best ligand conformation is chosen based on binding free energy value, hydrogen bonding, and hydrophobic interaction. The conclusion drawn from the docking analysis is that Aurantiamide acetate and Kaempferol interact well with the synthesized TAT protein (PDB: IJFW).

ABBREVIATIONS:

2-D:2- Dimensional
Å: Ångström
ADME: Absorption, Distribution, Metabolism, Excretion
AIDS: Acquired immunodeficiency syndrome
Arg: Arginine
GA: Genetic Algorithm
GLN-Glutamine
H-Bond: Hydrogen Bond
HIV Human immunodeficiency Virus
Kcal/Mol: Kilo Calories Per Mol
MilogP: Molecular hydrophobicity
MLR: Multiple Linear Regressions
MW- Molecular weight
nOHNH- No. of H-bond donors
nON- No. of H-bond acceptors,
nrotb – no of rotatable bond
PDB: Protein Data Bank
Ser: Serine
TAT: Trans-activator-Transcription Protein
TPSA- Total Polar surface area

CONFLICT OF INTEREST:

The authors declare they have no competing interests.

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