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AN IN-SILICO METHOD TO IDENTIFY AND ANALYZE PHYTOCOMPOUNDS FROM MEDICINAL PLANTS AS HIV-1 PROTEASE INHIBITORS

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ABSTRACT

The most severe stage of Human Immunodeficiency Virus (HIV) infection is Acquired Immune Deficiency Syndrome. There has been a push in recent research to extract phytochemicals from plants to suppress HIV, but few studies have focused on the impact of these phytochemicals on the activity of enzymes/transporters involved in the virus. One of the goals of this work is to assess the antiviral efficacy of these chemicals against the HIV-1 protease enzyme using computational techniques. The ADMET Lab 2 and pkCSM servers were used to determine the Physicochemical properties and toxicity prediction of the chosen Phytocompounds. Using Computational tools, potential structural inhibitory activities of these phytochemicals were explored. Free binding energy analysis for antiviral activities identified two phytocompounds with lower binding energy than Standard drug against HIV-1 protease enzyme. Among all Phytocompounds Antheraxanthin has similar binding energy to Dolutegravir (Standard Drug) Amentoflavone, Nimocinol and Nicotiflorin exhibited pronounced structural evidence as potential HIV-1 protease enzyme inhibitors. On the Basis of ADMET analysis, Nimocinol is highly recommended Phytocompound for further study.

Keywords: HIV-1, Protease enzyme, Protease inhibitors, ADMET, Drug likeliness, Anti-viral Activities

INTRODUCTION

According to the World Health Organisation (WHO), around 72 million persons were infected with the Human Immunodeficiency Virus (HIV) in 2017 (WHO, 2018). Among these are Sub-Saharan Africa was the most severely afflicted region, accounting for more than 69% of all infected cases. According to the Joint United Nations (UNAIDS) report, while there has been a steady decline in Acquired Immune Deficiency Syndrome (AIDS) related illnesses over the last decade, the global rate of new HIV infections is not falling fast enough to meet the milestones set by 2020 **[1]**.

The HIV protease enzyme is one of the most important enzymes necessary to create mature and infectious HIV virions in the HIV replication cycle in human immune cells. As a result, the enzyme has become the most prominent target for anti-HIV drugs **[2]**. The protease enzyme is a C2-symmetric active homodimer composed of a non-covalently linked dimer of 99 amino acid residues apiece. The two monomeric chains combine to create an enclosed tunnel covered by two flaps that "open and close" in response to substrate binding **[3]**. HIV protease activity is critical for the development of infectious HIV virions during the viral cycle. As a result, there is no doubt that inhibition or inactivation of the enzyme will result to the production of less viable and non-infectious virions and will eventually lead to a reduction in the spread of the infection to vulnerable hosts or cells **[4]**.

Protease inhibitor medicines (PIs) suppress HIV viral replication by binding to the HIV proteases and subsequently blocking the protease. Proteolytic cleavage of protein precursors required for the production of mature HIV virions **[5, 6]**. PIs are intended to resemble the natural substrate of the viral protease. They inhibit the HIV-1 protease from cleaving the precursor proteins by specifically binding the active site of the virus protease, resulting in the generation of immature non-infectious viral particles **[7]**. The use of traditional herbal medicine is becoming more popular in the treatment of illnesses such as HIV in many countries, despite the likelihood of herbal-drug interactions and toxicity as a result of co-administration of herbs and antiretroviral medicines. Nonetheless, there have been significant increases in the use of herbal medicine not only in developing countries but also in developed countries, causing great public health concern among scientists and physicians who are sometimes unsure about the safety of herbal preparations, particularly when used concurrently with regular orthodox medications such as ARV. Many patients on antiretroviral treatment in

South Africa also use traditional herbal medicine **[8]**.

In the present study, we have selected more than 50 Phytocompounds for docking and among all total 8 Phytocompounds for ADME analysis and then best four Phytocompounds are selected for toxicity analysis to identify potent Protease inhibitors from various medicinal Plants. In light of the foregoing, this article contains an in-silico investigation of more than 50 Phytocompounds as HIV-1 Protease inhibitors.

MATERIAL AND METHOD

Protein Retrieval

The 3D structures of HIV-1 protease served as the foundation for this investigation. The protein data bank (http://www.rcsb.org/ utilised to retrieve the 3D structure of HIV-1 Protease (PDB:1HPV), which were used as a receptor the experimental object (Resolutions for the HIV-1 protease enzyme structures with the PDB codes 1HPV was 1.90Å is shown in **Figure 1**). All water molecules were eliminated prior to analysis, and ADT software was used to create the necessary files for Auto Dock by assigning hydrogen polarities, figuring Gasteiger charges for protein structures, and converting protein structures from the PDB file format into PDBQT format.

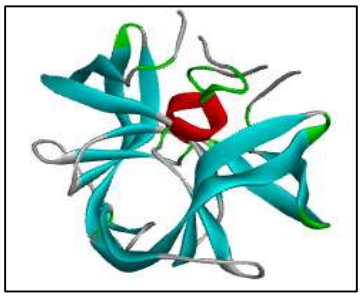


Figure 1: HIV-1 Protease (PDB: 1HPV)

Preparation of ligands

The National Center for Biotechnology Information's PubChem Compound Database was used to retrieve the structure of Phytocompounds in the Spatial Data File (SDF) file format. Structure of phytocompounds in the Spatial Data File (SDF) file format from the PubChem Compound Database (National Center for Biotechnology Information;).

The SDF format of chemical structures was converted utilising Discovery Studio visualizer to the PDB format. The investigation of ligand structures using non-polar hydrogen combinations, Gasteiger charge additions, and rotatable bonds was done next using ADT components of the ligand. Then, the ligand converted from PDB format to ADT PDBQT format allows for usage with AutoDock4 (AD4) **[9]**.

Molecular Docking study

The ready proteins and ligand structures were saved in the PDBQT file format for molecular docking experiments. As a tool for molecular graphical visualisation, Auto Dock Tools (ADT) was employed. Both the Auto Grid and algorithms, the initial

population people, the number of energy function evaluations was set to 2.5 maximum number of generations was set at 27,000. The phytochemical compounds were then docked into the binding pocket of Protease (by defining the grid box with a spacing of 1 Å and size of 26.27*20.52*25.0 pointing in x, y and z directions respectively.

ADME Prediction

ADME (adsorption, distribution, metabolism, and excretion) is significant to search the pharmacodynamics of the designed compounds which could be a target agent in drug design and discovery studies. ADMET lab 2 server is a web-based platform that lets the user upload or draw their hit compounds with structure or SMILES code. This tool supplies many parameters such as lipophilicity (LOGP,), water solubility- Log S, drug-likeness rules (Lipinski, Ghose, Veber, Egan, and Muegge) methods **[10]**. The HIV-1 IN inhibitors were uploaded with SMILES codes and analyzed.

Toxicity Prediction

Toxicology prediction of bioorganic compounds is substantial to estimate the amount of tolerability of the hit compounds before in vitro, in vivo, and clinical studies. pkCSM is also a web-based platform for analyzing physicochemical properties of small compounds, and this online website supplies many toxicology results such as hERG inhibitor, AMES Toxicity, cardiotoxicity, human maximum tolerated dose, Skin Toxicity, *T. pyriformis* toxicity, Hepatotoxicity, and Minnow toxicity. The designed compounds' SMILES codes were uploaded pkCSM website to analyze their target prediction **[11]**.

RESULTS AND DISCUSSION:

It has been difficult to find an HIV cure. Many years have passed. Several compounds have been tested against certain viral components in order to Stop the virus from spreading in the host. It's thrilling to think of using a well-known phytochemical to develop a disease. The virus encodes the viral protease, reverse transcriptase, and integrase enzymes.

Molecular Docking Analysis

Molecular docking is one of the most successful and extensively used structure-based in silico tools for predicting interactions between molecules and their biological targets. Typically, it entails first predicting a ligand's molecular orientation inside a receptor and then calculating complementarity using a scoring function **[12, 13, 14]**. It enables the drug researcher to proceed with the evaluation of the most promising drug.

In order to molecularly dock with the HIV-1 protease enzyme, Phytocompounds with their binding energies are shown in **Table 1**, and their values show that they have good affinity for the target enzyme, and compares the binding energy value of standard drug

used to treat HIV-1 with different Phytocompounds.

Figure 2 displays the 2D interaction of Phytocompounds with amino acid residues of HIV-1 Protease enzymes. Amino acid residues of protein bind with the Phytocompounds with various bonds such as hydrogen bond, Vander Waal bond, alkyl bond, hydrogen conventional bond (**Figure 2**).

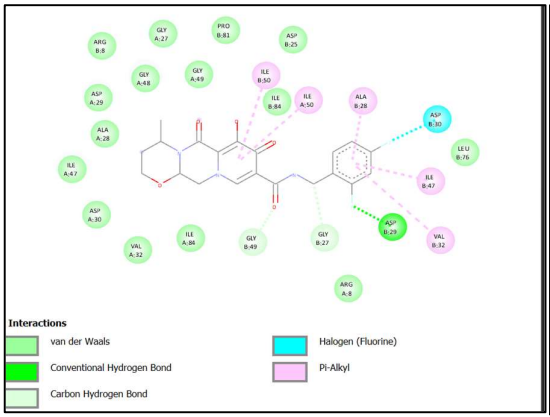
Active site of HIV-1 Protease enzyme with key amino acids ILE 50, ILE 47, VAL 32, ALA 28 forms Hydrophobic bond with ring in Standard drug Dolutegravir and Phytocompounds **Amentoflavone, Antheraxanthin, Cynaroside, Schottenol, Quercimeritrin, Nicotiflorin and ALA 28 in Licoflavone B. Amino acid residue ASP 29 involved as hydrogen bond in both Standard drug and selected Phytocompounds.**

The docking score of the standard compound and Phytocompounds are given in **Table 1**.

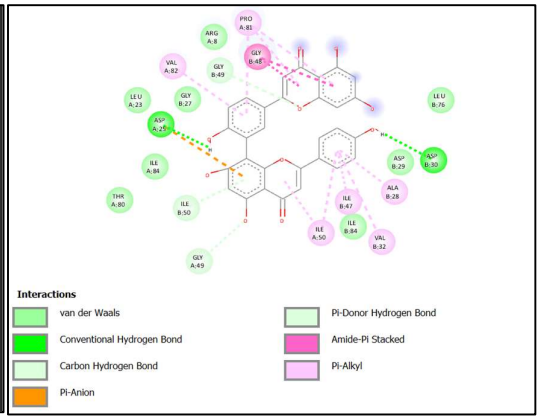
Table 1: Binding energies of Selected Phytocompounds

S. No.	Phytocompounds	Binding Energy (kcal/mol)
1	(-)-Isoshyobunone	-6.8
2	5, 7-Dihydroxy-4'-methoxy-8, 3 di-C prenylflavanones	-8.9
3	alpha-Amyrin	-8.2
4	alpha-Spinasterol	-8.4
5	Amentoflavone	-10.4
6	Antheraxanthin	-9.8
7	Apigenin_5-O-beta-D-glucopyranoside	-9.4
8	Aromadendrin	-8.2
9	Auranetin	-8.4
10	Bilobetin	-8.7
11	Capsanthin	-8.7
12	Capsorubin	-8.7
13	Chrysoeriol	-8.2
14	Citroxanthin	-8.7
15	Citrusin A	-8.3
16	Cryptoflavin	-8.5
17	Cynaroside	-9.5
18	Diosgenin	-9.0
19	Diosmetin_7-O-beta-D-glucopyranoside	-8.2
20	Ellagic acid	-8.4
21	Ginkgetin	-8.5
22	Glabrocoumarin	-9.4
23	Glabroisoflavanone	-9.3
24	Glycyrin	-8
25	Gossypetin	-8.2
26	Gossypitrin	-8.9
27	Herbacetin	-6.9
28	Herbacitrin	-8.4
29	Hesperidin	-9.1
30	Hispaglabridin	-9.7
31	Isoaloesin	-8.4
32	Isoquercetin	-9.3
33	Isoquercitrin	-8.5
34	Isorhamnetin	-8.1
35	Isovitexin	-9.2
36	Licoflavone B	-9.5
37	Linderoflavone	-8.3
38	Liquiritigenin	-8.7

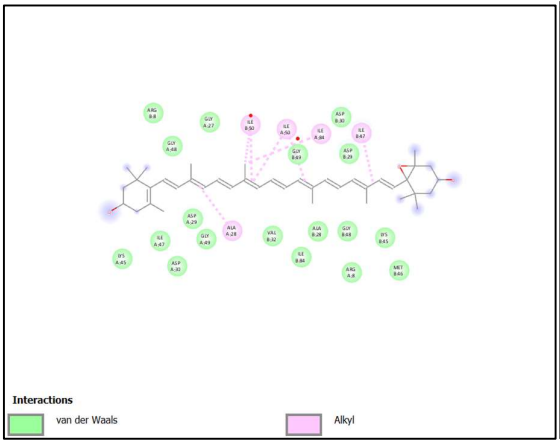
39	**Morindin**	**-9.4**
40	**Morindone**	**-8.5**
41	**Myricetin**	**-8.4**
42	**Narcissin**	**-8.2**
43	**Naringetol**	**-8.1**
44	**Narirutin**	**-9.1**
45	**Nicotiflorin**	**-9.9**
46	**Nimocinol**	**-10.4**
47	**Primeveroside**	**-8.5**
48	**Quercetin_3,4 diglucoside**	**-8.2**
49	**Quercetin-3-glucoside**	**-8.5**
50	**Quercimeritrin**	**-9.7**
51	**Rhababerone**	**-8**
52	**Rubiadin**	**-8.2**
53	**Rutin**	**-8**
54	**Schottenol**	**-9.7**
55	**Sciadopitysin**	**-8.1**
56	**Vincarodine**	**-8.6**
57*	**Dolutegravir (Standard Drug)**	**-9.8**



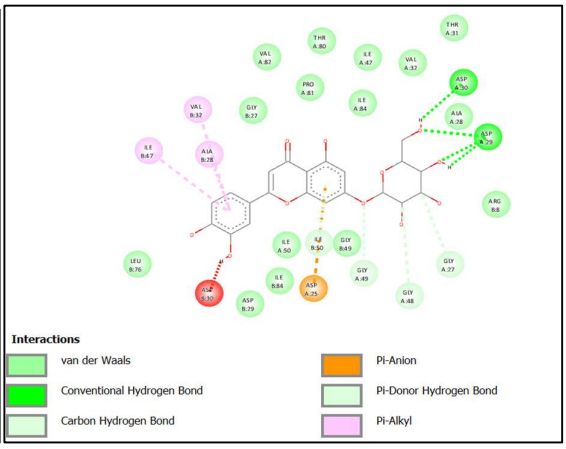
A: Standard: Dolutegravir



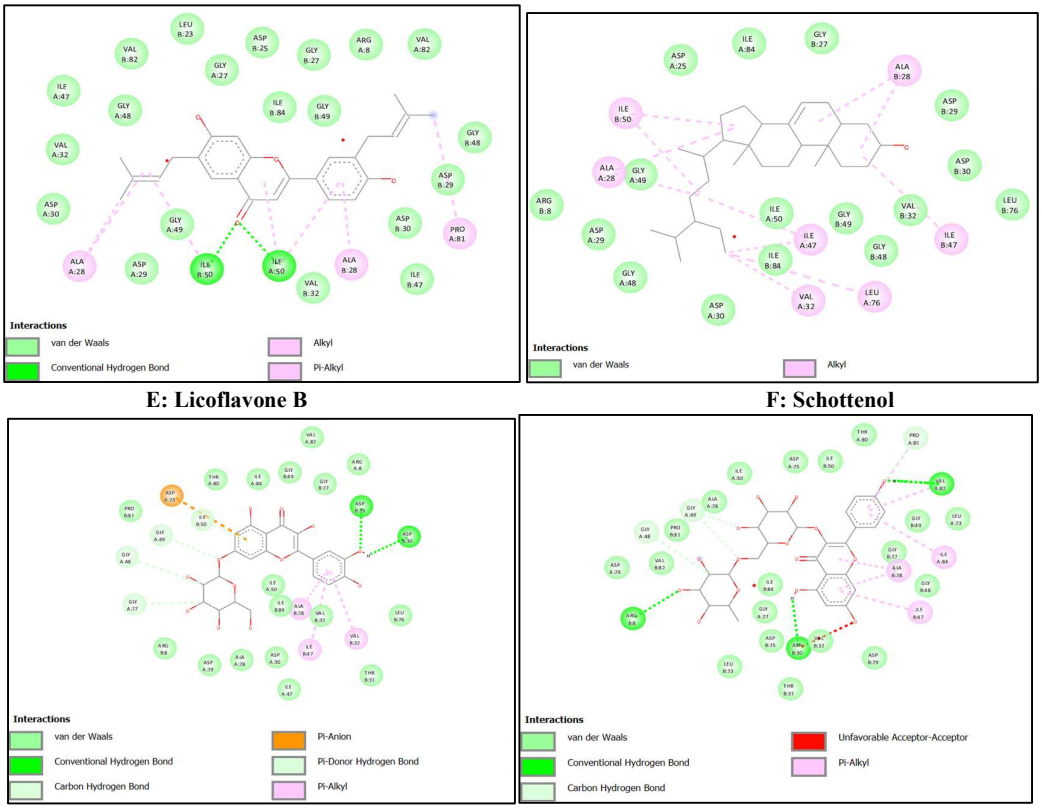
B: Amentoflavone



C: Antheraxanthin



D: Cynaroside



E: Licoflavone B **F: Schottenol**

G: Quercimeritrin **H: Nicotiflorin**

Figure 2: 2D Interaction of Amino acid residues with standard compound and Selected Phytocompounds

ADME Prediction:

The ADME predictions of the designed compounds were performed by ADMET lab 2 server, Besides, lipophilicity, water-solubility properties and drug-likeness factors (Lipinski's Rules, Ghose, Veber, Egan, Muegge) were also calculated. All selected phytocompounds showed high topological polar surface area (TPSA) ranging from 20.23 Å2 to 206.6Å2. nHD of the compounds was found between 1 to 9, whereas nHA of compounds was between 0 and 11. The calculated lipophilicity properties are given in the **Table 2**. Pharmacokinetic properties such as GI absorption, BBB permeant, P-gp substrate, and skin permeation (Log Kp) were also calculated, and selected compounds exhibited good Gastrointestinal absorption (GI). SAscore is 4.68 for Nicotiflorin (Synthetic accessibility score is designed to estimate ease of synthesis of drug-like molecules.) shows that it is easy to synthesize drug like molecules. SAscore is 5.018 for Nimocinol describes its proficiency for synthesis.

In **Figure 3**, the light orange area describes the upper limit range for each property (lipophilicity: XLOGP3 between -0.7 and +5.0, size: MW between 150 and 500 g/mol, polarity: TPSA between 20 and 130 Å2, solubility: log S not higher than 6,

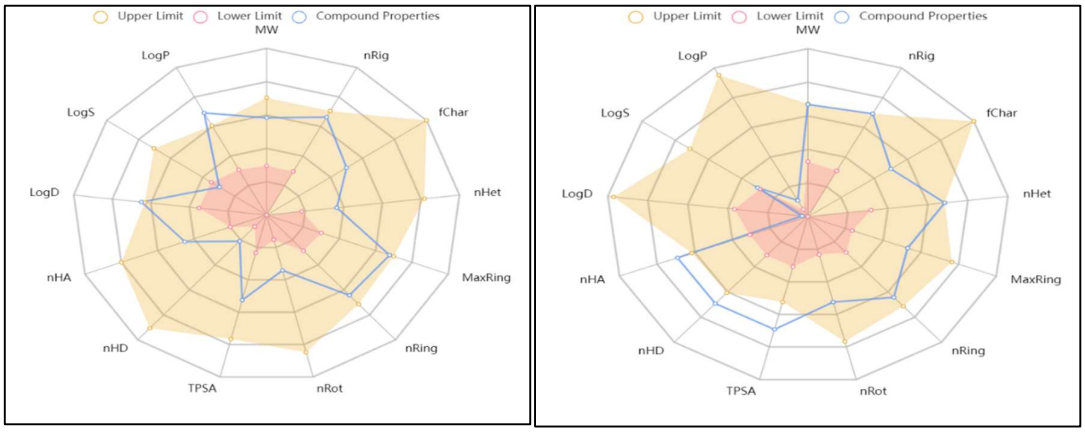
saturation: fraction of carbons in the sp3 hybridization not less than 0.25, and flexibility: no more than 9 rotatable bonds Blue colour line describes the properties for selected Phytocompounds (Nimocinol and Nicotiflorin) while small pink area shows lower limit range of all Physicochemical Properties.

Toxicity Prediction

Toxicity predictions are screened for selected Phytocompounds after ADME analysis. Among all compounds, four best compounds are found to have better Physicochemical Properties. Moreover, all compounds were not found to be cause skin sensitivity. Although they are also predicted as hERG inhibitors, none of them showed any hERG inhibition results. Nimocinol is found to be better Phytocompound among all for further study **(Table 3)**.

Table 2: Physicochemical Properties of Phytocompounds

S. No.	Title	Mol. Wt.	log P	Log S	Log D	HBA	HBD	TPSA	nRB	RC	nRigid	nHB
1	Antheraxanthin	584.42	5.2	-5.05	4.014	3	2	52.99	10	3	35	5
2	Nicotiflorin	594.16	0.2	-3.8	-0.119	11	9	245.29	6	5	40	20
3	Nimocinol	452.26	3.693	-4.6	3.12	5	1	76.74	3	5	34	5
4	Schottenol	414.39	8.0	-7.05	6.5	1	1	20.23	6	4	27	2
5	Quercimeritrin	464.1	0.373	-3.8	-0.06	7	8	206.6	4	4	32	15
6	Amentoflavone	538.09	6.002	-4.5	2.3	10	6	181.98	3	6	42	8
7	Cynaroside	448	0.907	-3.8	0.15	11	7	186.37	4	4	31	13
8	Hispaglabridin A	392.2	6.677	-3.2	4.7	4	2	58.92	3	4	29	2
9	Licoflavone B	390	6.6	-3.4	3.8	4	2	70	3	-	-	-



A: Nimocinol **B: Nicotiflorin**

Figure 3: Diagrammatic representation of Physicochemical Properties of Best Phytocompounds.

Table 3: Toxicity Assessment of Selected Phytocompounds

S. No.	Phytocompounds	hERG Blockers	AEMS toxicity	Carcinogenicity	Skin sensitization
1	Nicotiflorin	No	No	No	-
2	Nimocinol	No	No	No	No
3	Quercimeritrin	No	No	No	-
4	Cynaroside	No	-	No	-

CONCLUSION

Amentoflavone, Nicotiflorin and Nimocinol have lower binding free energy on HIV-1 Protease enzyme than Standard Compound Dolutegravir. Antheraxanthin showed similar binding energy with Dolutegravir. Among Eight selected compounds Nicotiflorin Nimocinol, Quercimeritrin and Cynaroside have shown good drug likeness properties. Nimocinol is found to be best Phytocompound from all selected Phytocompounds from different medicinal Plants. This result desires to be explored further by *in vitro* and/or *in vivo* analysis.

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