Exploring the HIV-1 Reverse Transcriptase Inhibition and Anti-HIV Potential of Garcinia Species Compounds through Computational Analysis

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Abstract

Human immunodeficiency virus (HIV) infection remains a global health threat, and a significant number of individuals are unaware of this disease. Gender-related testing disparities persist, particularly among men, citing stigma as a major barrier. Sub-Saharan Africa shoulders a disproportionate burden, emphasizing the need for global epidemic management. While antiretroviral therapy has extended the lifespan of those with HIV, challenges in treatment adherence persist. This study focused on HIV-1 reverse transcriptase, a pivotal enzyme involved in inhibiting HIV replication. In parallel, the potential anti-HIV effects of the bioactive compounds from five Garcinia species were explored through computational analysis. Molecular docking revealed 124 phytocompounds from five different species of Garcinia, five compounds with notable binding affinity, including moreollin, surpassing the FDA-approved drug dolutegravir. Physicochemical assessments demonstrated favorable drug likeness attributes, emphasizing optimal lipophilicity and absorption. Pharmacokinetic predictions suggest efficient distribution across barriers. Toxicity analysis indicated that the selected compounds had few adverse effects. These findings underscore the potential of Garcinia-derived compounds as anti-HIV agents, warranting further experimental validation. This study contributes to the ongoing battle against HIV/AIDS by addressing disparities, combating stigma and highlighting potential treatment avenues.

Keywords*: Human immunodeficiency virus; HIV-1 reverse transcriptase; Garcinia; Moreollin.*

Introduction

Human immunodeficiency virus (HIV) continues to pose a significant and persistent threat to global public health (Stockdale et al., 2019). In this era of medical advancements, notably, a substantial portion of individuals living with HIV remain unaware of their infection, with approximately 40% of HIV-positive individuals remaining undiagnosed (Staveteig et al., 2017). Despite considerable progress in HIV testing and treatment, disparities in testing rates persist, particularly among specific demographic groups. A notable gender-related disparity emerges, as men tend to undergo HIV testing less frequently than women do, often citing concerns about anticipated stigma as a significant barrier (Treves-Kaga et al., 2017). Sub-Saharan Africa bears a disproportionate burden, hosting approximately 70% of the world's 25.5 million HIV-positive individuals, emphasizing the urgent need to address testing gaps and optimize epidemic management on a global scale (Stockdale et al., 2019). HIV, a virus that ultimately leads to acquired immunodeficiency syndrome (AIDS), operates by compromising the body's immune system, primarily through the progressive reduction of CD4+ cells. Although a cure for AIDS has not been identified, antiretroviral medications effectively slow the disease's progression and enhance the overall health and longevity of individuals living with HIV (Coffin, 1999). The intricacies of the HIV-1 life cycle are marked by a sequence of critical events influenced by the type of host cell and its activation status (Coffin, 1997). These events include viral entry, mediated by the envelope glycoproteins gp120 and gp41; viral fusion with host cells; reverse transcription of the viral RNA genome into DNA; integration of this viral DNA into the host genome; and the subsequent assembly and release of new viral particles (Ray and Doms, 2006; Eckert et al., 2001; Platt et al., 2005). Central to this process is the vital role of the reverse transcriptase enzyme, which catalyzes the conversion of viral RNA into DNA, facilitating further replication. Reverse transcription and integration are hallmark features of the Retroviridae family, which includes HIV-1, as these viruses employ reverse transcriptase (RT) to convert their RNA genomes into DNA (Hu and Hughes, 2012). Upon infection, the virus enters a susceptible target cell, undergoes reverse transcription, and integrates its genetic material into the host cell's genome, a pivotal step for viral replication (Wilen et al, 2012). While a considerable proportion of individuals infected with HIV exhibit no discernible symptoms, a subset of individuals experiences flu-like manifestations within weeks of infection (Kapila et al., 2016). HIV infection can

lead to various health complications, including herpes zoster virus (shingles), pneumonia, and neurological disturbances (Downs et al., 1996). HIV-1 transmission occurs through the exchange of infected bodily fluids, such as blood, semen, vaginal secretions, or breast milk, into a recipient's body (Hübner et al., 2009; Sourisseau et al., 2007). Importantly, the risk of transmission is closely linked to the level of cell-associated viral load (Rousseau et al., 2004). The advent of antiretroviral therapy (ART) marked a pivotal moment in the battle against HIV, commencing with the approval of zidovudine in 1987. Research has demonstrated the benefits of combining multiple antiretroviral medications, collectively referred to as highly active antiretroviral therapy (HAART) (Kemnic and Gulick, 2023). Health authorities such as the Department of Health and Human Services (DHHS) and the World Health Organization (WHO) endorsed HAART, which significantly extended the life spans of individuals living with HIV while also reducing the risk of viral transmission. However, the pathogenesis of HIV infection is highly intricate and patient specific, presenting unique challenges in treatment and management (Brass et al., 2008). Adherence to ART can be hindered by adverse effects, social stigma, and economic disparities (Bhatti et al., 2016). In summary, HIV-1 reverse transcriptase plays a central role in inhibiting HIV replication, but comprehensive strategies are essential for addressing these disparities, combating stigma, and overcoming treatment challenges in the ongoing battle against HIV/AIDS.

Garcinia species are known to harbor bioactive constituents such as flavonoids, xanthones, triterpenoids, and benzophenones, which possess antibacterial, antifungal, anti-inflammatory, and antioxidant properties. Moreover, several of these compounds exhibit intriguing biological attributes, including potential effects on human immunodeficiency virus (Adnan et al., 2019). On the basis of these findings, our research has focused mainly on five different species of Garcinia, namely, *Garcinia morella*, *Garcinia brasiliensis*, *Garcinia cambogia*, *Garcinia gardneriana* and *Garcinia pedunculata,* to determine the reported phytocompounds against human immunodeficiency virus through computational analysis.

Materials and Methods

3D protein structure

The 3D structural framework of HIV-1 reverse transcriptase (RT) was retrieved from the Protein Data Bank (PDB). Based on its resolution, the X-ray crystallographic structure of the target protein was chosen. The AutoDock tool was subsequently used to perform further optimization, which included removing all water molecules, heteroatoms and inhibitors before adding a polar H atom, computing the Kollman charge and calculating the Gasteiger charge. Furthermore, the 3D coordinates of the optimized protein structures bearing partial charges were recorded in the PDBQT format (Trott and Olson, 2009).

Preparation of ligands

The reported phytocompounds from *Garcinia morella*, *Garcinia brasiliensis*, *Garcinia cambogia*, *Garcinia gardneriana* and *Garcinia pedunculata* were selected for the study. The SDF files of these phytocompounds were obtained from the PubChem database. The SDF files were converted into PDB files using Open Babel software.

Molecular docking analysis

Docking analysis was performed utilizing AutoDock 5.6, employing the Lamarckian genetic algorithm and default procedures. The objective was to dock a flexible ligand with a rigid protein. The binding and catalytic sites of the protein targets were the focal points for these docking calculations. Once potential binding sites were identified, compound docking at these sites was executed to determine the most likely and energetically favorable binding conformations. To ensure robust docking simulations, AutoDock Vina 1.1.2 was utilized, which included a grid box positioned at the previously identified binding site. For each protein–compound pair, the grid dimensions were set at 32 A3 with a grid spacing of 0.375A, encompassing the active site. Affinity scores were computed based on the free energy binding theory provided by AutoDock Vina and quantified in kcal/mol, with more negative values indicating stronger binding affinity. Subsequently, the resulting structures and binding docking poses were visually inspected and analyzed using software tools such as DS Visualizer 2.5 (http://3dsbiovia.com/products/) and PyMOL Molecular Graphics Framework 2.0 (Piret and Boivin, 2011) to assess the relationships and interactions.

Lead-likeliness properties

The physicochemical, medicinal, and drug likeness properties of the selected compounds were determined using SwissADME, a freely accessible web tool (Lipinski et al., 2001). In this analysis, Lipinski's rule, also known as the rule of five (RO5) (Lipinski, 2004), was applied to evaluate drug likeness and ascertain whether the chemical compounds in question, with their particular pharmacological or biological activity, possess attributes conducive to oral administration.

Toxicity analysis

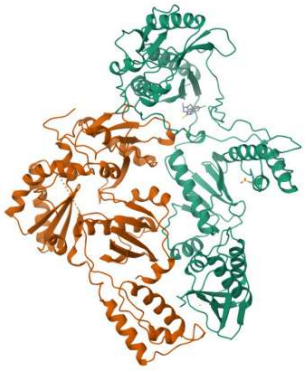
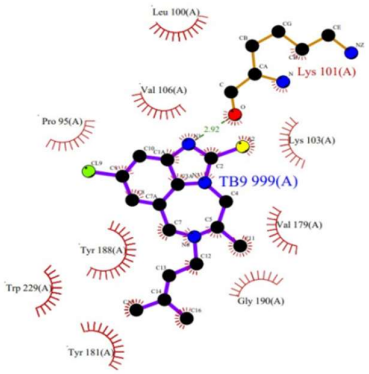
Preclinical toxicity testing of many biological systems, with specificity to species, organs, and doses, is used to determine the deleterious effects of an investigational substance (Parasuraman, 2011). ProTox-II is a publicly accessible webserver for *in silico* toxicity prediction for toxicologists, regulatory agencies, and computational and medicinal chemists with the canonical SMILES of selected compounds (Banerjee et al., 2018).

Results and Discussion

3D structure of the proteins and binding site residues

The 3D structure of the HIV-1 reverse transcriptase protein was retrieved from the Protein Data Bank. The PDB ID, sequence length of the amino acid, chain selected for docking analysis and binding site residues obtained from online servers are tabulated (**Table 1**).

Table 1. PDB ID, amino acid length and active site residues of the target

Protein Name and Structure	PDB ID	Amino Acid length	Chain	Active site residues	Server used
 HIV-1 reverse transcriptase	1REV	560	A		PDBSum

Molecular docking

The reported 124 phytocompounds from *Garcinia Morella*-34, *Garcinia brasiliensis*-45, *Garcinia cambogia*-15, *Garcinia gardneriana*-13 and *Garcinia pedunculata*-17 were screened against the selected HIV-1 reverse transcriptase protein. An *in silico* docking study revealed that out of 124 phytocompounds from five different species of Garcinia, five major compounds had greater binding efficiencies, ranging from -12.4 to -10.3 kcal/mol, which is similar to that of the positive control drug Dolutegravir (Table 2). Among all the compounds, moreollin had a better binding affinity (-12.4 kcal/mol) than did the FDA-approved drug dolutegravir (-11 kcal/mol).

Table 2. Binding affinity, interacting residues and hydrogen bonds of phytocompounds against HIV-1 reverse transcriptase

Compound name	Binding affinity (Kcal/mol)	Interacting residues	No. of hydrogen bonds
Standard (Dolutegravir)	-11.0	PRO95, VAL106, VAL179, TYR181, TYR188 and TRP229	3
Moreollin	-12.4	PRO420, LEU422, LYS424 and TRP426	2
Khusinol	-10.9	PRO392, THR397, THR419 and LEU425	10
Oleanolic acid	-10.6	PRO420 and LEU422	1
Stigmasterol	-10.3	TYR232, LYS65 and GLU370	1
Salvial-4(14)-en-1-one	-10.3	ILE94, HIS96, PRO133, ASN136 and GLN23	6

Physicochemical properties of the best compounds from *Garcinia* species

Chemical distribution, metabolism, excretion, and toxicity (ADMET) are important factors in the development of new drugs. A high-quality drug candidate should have suitable ADMET characteristics at a therapeutic dose in addition to sufficient activity against the therapeutic target (Abdolmaleki, 2017). A high-quality drug candidate should have suitable ADMET characteristics at a

therapeutic dose in addition to sufficient activity against the therapeutic target (Abdolmaleki, 2017). The physicochemical properties included molecular weight, heavy atoms, aromatic heavy atoms, rotatable bonds, hydrogen bond donors, acceptors and molar refractivity (Table 3). Based on the majority of criteria (rule of 5) for drug likeness, a lipophilic range of 0 to 5 is widely regarded as excellent for pharmaceutical design (Banerjee et al., 2018).

Table 3. Basic physicochemical properties of the best phytocompounds in *Garcinia* species

Basic physicochemical properties	Moreollin	Khusinol	Oleanolic acid	Stigmasterol	Salvial-4(14)-en-1-one
Molecular Weight(g/mol)	590.70	220.35	456.70	412.69	220.35
Heavy atoms	43	16	33	30	16
Aromatic heavy atoms	6	0	0	0	0
Rotatable bonds	7	1	1	5	1
H-bond acceptors	8	1	3	1	1
H-bond donors	1	1	2	1	0
Molar Refractivity	163.66	70.20	136.65	132.75	69.46

The 5 compounds with the best efficacy against the 5 different Garcinia species were demonstrated to have optimum lipophilicity, which suggests that these compounds have high bioavailability due to their efficient absorption across membranes and into the systemic circulation (Table 4). The ability of a chemical compound to dissolve in oil, lipids, or nonpolar solvents is known as its lipophilicity (hydrophobicity), which is a metric of lipid solubility. It is critical to comprehend how the overall quality of prospective medicinal substances is impacted by lipophilicity. In addition to establishing preclinical ADMET (absorption, distribution, metabolism, elimination, and toxicity) properties, recent research has revealed that compounds with adequate lipophilicity may be more likely to be successfully created (Raies and Bajic, 2016).

Table 4. Lipophilic properties of phytocompounds on *Garcinia* species

Lipophicility	Moreollin	Khusinol	Oleanolic acid	Stigmasterol	Salvial-4(14)-en-1-one
TPSA	108.36 Å²	20.23 Å²	57.53 Å²	20.23 Å²	17.07 Å²
iLOGP	4.51	3.17	3.89	5.01	2.87
XLOGP3	5.29	3.16	7.49	8.56	3.58
WLOGP	5.61	3.55	7.23	7.80	3.98
MLOGP	2.07	3.56	5.82	6.62	3.56
Silicos-IT Log P	6.92	3.10	5.85	6.86	4.09
Consensus Log P	4.88	3.31	6.06	6.97	3.62

This approach predicts the drug likeness of a chemical compound that has biological activity and is designed for oral administration. To have the best pharmacokinetics, oral medications must be distributed uniformly throughout the GIT. According to a study performed on *Garcinia* compounds, the BBB and GI tract can effectively absorb these substances into the bloodstream. P-gp normally serves as an efflux transporter, returning drugs or foreign substances to the GIT lumen to reduce the levels of drugs in the blood and tissues (Straehla and Warren, 2020).

To be effective as a drug, a potent molecule must reach its target in the body at a sufficient concentration and remain in a bioactive form long enough for the expected biological events to occur. Having a soluble molecule greatly facilitates the development of many drugs, primarily because of the ease of handling and formulation (Ononamadu and Ibrahim, 2021). The compound showed high GI absorption and exhibited blood–brain barrier permeability (Table 5).

Table 5. Predicted pharmacokinetic parameters

Predicted pharmacokinetics (ADME) parameters	Moreollin	Khusinol	Oleanolic acid	Stigmasterol	Salvial-4(14)-en-1-one
GI absorption	Low	High	Low	Low	High
BBB permeant	No	Yes	No	No	Yes
Pgp substrate	Yes	No	No	No	No
CYP1A2 inhibitor	No	No	No	No	No
CYP2C19 inhibitor	No	Yes	No	No	Yes
CYP2C9 inhibitor	No	Yes	No	Yes	Yes
CYP2D6 inhibitor	No	No	No	No	No
CYP3A4 inhibitor	Yes	No	No	No	No
log Kp (cm/s)	-6.15 cm/s	-5.40 cm/s	-3.77 cm/s	-2.74 cm/s	-5.10 cm/s

Drug-blocked isoforms of this network of enzymes may be inhibited by drugs due to poor excretion, which can lead to drug-induced toxicity. Therefore, it is vital that a proposed drug has only a negligible inhibitory effect on some enzyme isoforms. These findings suggested that these substances are rapidly and efficiently metabolized in the liver and removed from the body.

Drug-likeness and medicinal chemistry parameters

The whole drug likeness attribute measures how closely the physicochemical and structural characteristics of chemicals match or are comparable to those of a sizable number of well-known drugs. The Ghose (Amgen), Veber (GSK), Egan (Pharmacia) and Muegge (Bayer) methods were used (Ghose, 1999). Multiple estimations allow consensus views or selection of methods best fitting the end-user's specific needs in terms of chemical space or project-related demands. Any violation of any rule described here appears explicitly in the output panel. The compound moreollin had four violations in the Ghose rule, one violation in the Muegge rule and one violation in the Lipinski rule, whereas Veber and Egan rules showed that the compound had better drug-like characteristics (Table 6).

Table 6. Predicted pharmacokinetic parameters

Drug-likeness and medicinal chemistry parameters	Moreollin	Khusinol	Oleanolic acid	Stigmasterol	Salvial-4(14)-en-1-one
Lipinski #violations	Yes; 1 violation: MW>500	Yes; 0 violation	Yes; 1 violation: MLOGP>4.15	Yes; 1 violation: MLOGP>4.15	Yes; 0 violation
Ghose #violations	No; 4 violations	Yes	No; 3 violations: WLOGP>5.6	No; 3 violations: WLOGP>5.6	Yes
Veber #violations	Yes	Yes	Yes	Yes	Yes
Egan #violations	Yes	Yes	No; 1 violation: WLOGP>5.88	No; 1 violation: WLOGP>5.88	Yes
Muegge #violations	No; 1 violation	No; 1 violation: Heteroatoms<2	No; 1 violation: XLOGP3>5	No; 2 violations: XLOGP3>5	No; 1 violation
Bioavailability score	0.55	0.55	0.85	0.55	0.55
PAINS #alerts	0 alert	0 alert	0 alert	0 alert	0 alert
Brenk #alerts	3 alerts	1 alert	1 alert	1 alert	1 alert
Synthetic accessibility	7.51	4.39	6.08	6.21	3.56

Drugs that are now in use have been demonstrated to prevent or treat HIV, with some side effects. Docking studies are crucial tools for comprehending the inhibitory or binding mechanisms of pairing ligands with appropriate proteins. Structure-based ligand discovery was employed in conjunction with bioinformatics database analysis for drug repurposing (Zhang and Tang, 2018). One of the most commonly used computer-modeling approaches for identifying ligands and producing pharmaceutical drugs for biological protein–ligand interactions is molecular docking (Abdolmaleki, 2017).

Toxicity Prediction

The *in silico* toxicity of the compounds from *Garcinia* sp. was evaluated using two online tools. The toxicity class, carcinogenicity, and mutagenicity were determined using ProTox-II online software. The properties are tabulated in Table 7. Chemical exposure increases with the amount of chemicals and the exponential growth of chemical mixes (Raies and Bajic, 2016). Thus, *in silico*

toxicity prediction is highly evolving as an integral platform for predicting the toxicity of chemicals that could be harmful to humans, animals, plants, and the environment (Banerjee et al., 2018).

Table 7. Predicted toxicity of the phytocompounds

Compounds	Hepatotoxicity	Carcinogenicity	Immunotoxicity	Mutagenecity	Cytotoxicity
Moreollin	0.81	0.58	0.99	0.64	0.64
Khusinol	0.83	0.69	0.76	0.77	0.92
Oleanolic acid	0.52	0.57	0.79	0.85	0.99
Stigmasterol	0.87	0.6	0.99	0.98	0.94
Salvial-4(14)-en-1-one	0.71	0.63	0.94	0.89	0.87

Conclusion

This *in silico* investigation investigated phytocompounds from various Garcinia species to evaluate their inhibitory potential against HIV-1 reverse transcriptase. This study identified five compounds, including moreollin, that exhibited significant binding affinity, surpassing that of the FDA-approved drug dolutegravir. Physicochemical assessments indicated favorable drug likeness attributes, such as optimal lipophilicity and absorption. Pharmacokinetic predictions suggested efficient distribution across the gastrointestinal tract and blood–brain barrier. Toxicity analysis revealed low hepatotoxicity, carcinogenicity, immunotoxicity, mutagenicity, and cytotoxicity. While acknowledging the computational nature of this study, these findings underscore the potential of Garcinia-derived compounds as anti-HIV agents, warranting further experimental validation and clinical investigations to advance their development in the context of comprehensive HIV/AIDS management.

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