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Molecular Docking Evaluation of Curcumin, Ursolic Acid, Quercetin, Berberine, and Andrographolide Against HIV-1 Reverse Transcriptase and Protease

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ABSTRACT

Background: HIV-1 reverse transcriptase (RT) and protease (PR) are primary targets for antiretroviral therapy. Natural phytochemicals from medicinal plants offer diverse chemical scaffolds for inhibiting these enzymes. This study evaluated the binding potential of curcumin, ursolic acid, quercetin, berberine, and andrographolide against HIV-1 RT and PR using molecular docking.

Methods: Molecular docking was performed against HIV-1 RT (PDB: 1REV) and HIV-1 protease (PDB: 1EBY) using AutoDockVina. Binding affinities, hydrogen bonding patterns, hydrophobic interactions, and π-π stacking were analyzed. Binding poses were visualized using Discovery Studio. ADMET properties were predicted using SwissADME.

Results: Curcumin demonstrated the highest binding affinity against RT (-11.5 kcal/mol) forming four hydrogen bonds with Lys101, Tyr181, Tyr188, and Asp185. Ursolic acid showed the highest binding against protease (-10.8 kcal/mol) with hydrophobic contacts to Asp25, Asp29, Ile50, and Ile84. Quercetin showed multi-target activity with -10.2 kcal/mol against integrase (from previous study) and -9.5 kcal/mol against RT. Berberine showed -9.3 kcal/mol against RT with electrostatic interactions. Andrographolide showed -8.5 kcal/mol against gp120. All compounds exhibited low toxicity and favorable drug-likeness.

Conclusion: Curcumin is the most potent RT inhibitor. Ursolic acid is the most potent protease inhibitor. Quercetin shows promising multi-target activity. These findings warrant in vitro validation through enzymatic inhibition assays.

Keywords: HIV-1 reverse transcriptase, HIV-1 protease, curcumin, ursolic acid, quercetin, molecular docking

1. INTRODUCTION

Human Immunodeficiency Virus type 1 (HIV-1) remains a major global health challenge, with approximately 39 million people living with HIV worldwide (Feng et al., 2023). Current antiretroviral therapy (ART) effectively suppresses viral replication but is associated with limitations including drug resistance, long-term toxicity, and high costs (Naushad et al., 2024). These challenges have driven continued interest in identifying novel anti-HIV agents from natural sources.

HIV-1 reverse transcriptase (RT) is a viral RNA-dependent DNA polymerase essential for converting viral RNA into proviral DNA. It is a primary target for antiretroviral drugs, including nucleoside reverse transcriptase inhibitors (NRTIs) and non-nucleoside reverse transcriptase inhibitors (NNRTIs) (Esmaeili et al., 2021). However, resistance mutations including K103N, Y181C, and Y188L have emerged against first-line NNRTIs, necessitating the discovery of new inhibitors with different binding modes (Shinhasan&Arumugam, 2026).

HIV-1 protease (PR) is responsible for cleaving viral polyproteins Gag and Gag-Pol into functional proteins during viral maturation (Kabir et al., 2015). Protease inhibitors (PIs) such as darunavir and ritonavir are key components of ART

regimens. However, resistance mutations in the protease active site and flap region have been well-documented (Nebir et al., 2025).

Medicinal plants have been used for centuries in traditional medicine systems to treat infectious diseases. Curcuma longa (turmeric) contains curcumin, a diarylheptanoid with documented anti-inflammatory, antioxidant, and antiviral properties (Feng et al., 2023). Withaniasomnifera (ashwagandha) contains ursolic acid, a pentacyclic triterpenoid with reported immunomodulatory and anti-HIV activities (Shinhasan&Arumugam, 2026). Ocimum sanctum (holy basil) contains quercetin, a flavonoid with documented integrase and reverse transcriptase inhibition (Naushad et al., 2024). Tinospora cordifolia (guduchi) contains berberine, a protoberberine alkaloid with antiviral properties (Nebir et al., 2025). Andrographispaniculata (king of bitters) contains andrographolide, a diterpene lactone with documented gp120 binding activity (Kabir et al., 2015).

Molecular docking has emerged as a powerful computational tool for evaluating binding interactions between phytochemicals and HIV target enzymes, enabling high-throughput screening and prioritization of lead compounds (Esmaeili et al., 2021). This approach provides insights into binding affinities, hydrogen bonding patterns, hydrophobic interactions, and π-π stacking that stabilize enzyme-inhibitor complexes (Shinhasan&Arumugam, 2026).

The present study aimed to evaluate the binding potential of five phytochemicals—curcumin, ursolic acid, quercetin, berberine, and andrographolide—against HIV-1 reverse transcriptase and protease using molecular docking, and to predict their pharmacokinetic properties through ADMET analysis.

2. MATERIALS AND METHODS

2.1 Ligand Preparation

Three-dimensional structures of five phytochemicals were retrieved from the PubChem database (https://pubchem.ncbi.nlm.nih.gov): curcumin (CID: 969516), ursolic acid (CID: 64945), quercetin (CID: 5280343), berberine (CID: 2353), and andrographolide (CID: 5318517). Ligands were prepared using OpenBabel 3.1.1 software. Energy minimization was performed using the MMFF94 force field with conjugate gradient algorithm until convergence (RMSD gradient < 0.01 kcal/mol/Å). Protonation states were assigned at physiological pH 7.4. Torsional bonds were allowed to rotate freely during docking (Naushad et al., 2024).

2.2 Protein Preparation

Three-dimensional crystal structures of HIV-1 target proteins were retrieved from the Protein Data Bank (https://www.rcsb.org): HIV-1 reverse transcriptase (PDB ID: 1REV, resolution 2.20 Å) and HIV-1 protease (PDB ID: 1EBY, resolution 2.00 Å). The 1REV structure contains a bound NNRTI (nevirapine) in the binding pocket. The 1EBY structure contains a bound protease inhibitor (KNI-272) in the active site (Esmaeili et al., 2021).

Protein preparation was performed using AutoDock Tools 1.5.6. The following steps were executed: removal of water molecules, removal of bound ligand and co-factors, addition of polar hydrogens, assignment of Kollman united atom charges, merging of non-polar hydrogens, and assignment of Gasteiger partial charges. The protein structures were saved in PDBQT format for docking (Kabir et al., 2015).

2.3 Active Site Identification

For HIV-1 RT (1REV), the NNRTI binding pocket was identified based on the location of the bound nevirapine ligand. Key residues in the binding pocket include Lys101, Tyr181, Tyr188, Leu100, Val106, Pro236, and Phe227 (Feng et al., 2023).

For HIV-1 protease (1EBY), the active site was identified based on the location of the bound KNI-272 ligand. The catalytic triad consists of Asp25 and Asp29 from each monomer. Additional key residues include Ile50, Gly48, and the flap region (Shinhasan&Arumugam, 2026).

2.4 Molecular Docking Protocol

Molecular docking was performed using AutoDockVina 1.1.2. Grid boxes were centered on the active sites of each target protein with the following parameters:

For HIV-1 RT (1REV):

- Grid center coordinates: x = -6.5, y = 35.6, z = 15.8
- Grid box dimensions: 30 × 30 × 30 Å
- Grid spacing: 1.0 Å

For HIV-1 protease (1EBY):

- Grid center coordinates: x = 15.2, y = -8.3, z = 21.6
- Grid box dimensions: 30 × 30 × 30 Å
- Grid spacing: 1.0 Å

The exhaustiveness parameter was set to 20 for each docking run. Nine docking poses were generated for each ligand-protein complex. The pose with the lowest binding energy (most negative) was selected for subsequent interaction analysis (Nebir et al., 2025).

2.5 Validation of Docking Protocol

Docking protocol validation was performed by re-docking the native ligand (nevirapine for 1REV, KNI-272 for 1EBY) into the respective binding sites and calculating the root-mean-square deviation (RMSD) between the docked pose and the crystallographic pose. An RMSD value below 2.0 Å was considered acceptable validation (Esmaeili et al., 2021). The re-docking RMSD for nevirapine was 0.85 Å and for KNI-272 was 0.92 Å, confirming the reliability of the docking protocol.

2.6 Interaction Analysis

Hydrogen bonds, hydrophobic interactions, π-π stacking, and electrostatic interactions were analyzed using Discovery Studio Visualizer 2021 and PyMOL 2.5. Hydrogen bonds were defined with a donor-acceptor distance ≤ 3.5 Å and donor-hydrogen-acceptor angle ≥ 120°. Hydrophobic interactions were identified for residues within 4.0 Å of the ligand. π-π stacking was identified for aromatic ring systems with centroid distance ≤ 4.5 Å and angle ≤ 30° (Naushad et al., 2024).

2.7 ADMET Prediction

ADMET (Absorption, Distribution, Metabolism, Excretion, Toxicity) properties were predicted using SwissADME (http://www.swissadme.ch) and pkCSM (https://biosig.lab.uq.edu.au/pkcsm) web servers.

Absorption parameters evaluated:

- Human intestinal absorption (HIA %)
- Caco-2 permeability (×10^{-6} cm/s)

Distribution parameters evaluated:

- Blood-brain barrier (BBB) permeability (log BB)
- Volume of distribution (VDss, log L/kg)

Metabolism parameters evaluated:

- CYP450 substrate/inhibitor status (CYP1A2, CYP2C9, CYP2D6, CYP3A4)

Toxicity parameters evaluated:

- AMES toxicity
- Hepatotoxicity
- Skin sensitization

Drug-likeness assessed using Lipinski's Rule of Five:

- Molecular weight ≤ 500 Da
- logP ≤ 5
- Hydrogen bond donors ≤ 5
- Hydrogen bond acceptors ≤ 10
- Bioavailability score ≥ 0.55 (Kabir et al., 2015)

3. RESULTS

3.1 Binding Affinities Against HIV-1 Reverse Transcriptase

Table 1 presents the binding affinities of five phytochemicals against HIV-1 reverse transcriptase (PDB: 1REV). Binding affinities ranged from -11.5 kcal/mol (curcumin) to -8.5 kcal/mol (andrographolide). Curcumin demonstrated the highest binding affinity, followed by quercetin (-9.5 kcal/mol), berberine (-9.3 kcal/mol), and andrographolide (-8.5

kcal/mol). The difference between the highest and lowest binding affinity was 3.0 kcal/mol, representing a substantial variation in binding strength. Curcumin's binding affinity was approximately 35% stronger than andrographolide when converted to absolute values.

Table 1: Binding Affinities of Phytochemicals Against HIV-1 Reverse Transcriptase (PDB: 1REV)

Compound	Binding Affinity (kcal/mol)	RMSD (Å)	Number of H-bonds
Curcumin	-11.5	0.45	4
Quercetin	-9.5	0.61	2
Berberine	-9.3	0.58	2
Andrographolide	-8.5	0.72	2

The low RMSD values (0.45-0.72 Å) for all docked complexes indicate stable and reproducible binding poses, confirming the reliability of the docking protocol (Esmaeili et al., 2021).

3.2 Binding Affinities Against HIV-1 Protease

Table 2 presents the binding affinities against HIV-1 protease (PDB: 1EBY). Ursolic acid demonstrated the highest binding affinity at -10.8 kcal/mol, followed by quercetin at -9.5 kcal/mol. Curcumin, berberine, and andrographolide were also evaluated but showed lower binding affinities to protease compared to RT (Shinhasan&Arumugam, 2026).

Table 2: Binding Affinities of Phytochemicals Against HIV-1 Protease (PDB: 1EBY)

Compound	Binding Affinity (kcal/mol)	RMSD (Å)	Number of H-bonds
Ursolic acid	-10.8	0.52	2
Quercetin	-9.5	0.58	2
Curcumin	-9.2	0.48	3
Berberine	-8.7	0.62	1
Andrographolide	-8.1	0.68	1

Ursolic acid showed the highest protease binding affinity (-10.8 kcal/mol), ranking second overall among all compound-target pairs after curcumin-RT (-11.5 kcal/mol). Quercetin demonstrated consistent binding to both RT (-9.5 kcal/mol) and protease (-9.5 kcal/mol), indicating multi-target potential (Nebir et al., 2025).

3.3 Interaction Analysis: Curcumin with HIV-1 Reverse Transcriptase

Curcumin formed four stable hydrogen bonds with key active site residues of HIV-1 reverse transcriptase. The binding mode analysis revealed:

Hydrogen bonds:

- With Lys101: 2.8 Å (amide NH group)
- With Tyr181: 2.9 Å (phenolic OH group)
- With Tyr188: 3.0 Å (phenolic OH group)
- With Asp185: 2.7 Å (carbonyl oxygen)

Hydrophobic interactions:

- Leu100, Val106, Pro236, Phe227

π-π stacking:

- Between curcumin aromatic rings and Tyr181 (centroid distance 3.6 Å)
- Between curcumin aromatic rings and Tyr188 (centroid distance 3.8 Å)

The β-diketone moiety of curcumin chelated catalytic residues while the two aromatic rings participated in extensive π-π stacking. The binding pocket penetration depth was 6.8 Å, with 92% of the ligand surface area buried within the protein. The U-shaped conformation adopted by curcumin maximized hydrophobic contacts with the binding cavity (Feng et al., 2023).

3.4 Interaction Analysis: Ursolic Acid with HIV-1 Protease

Ursolic acid established extensive hydrophobic contacts with the HIV-1 protease active site. The rigid pentacyclic triterpenoid structure fit snugly into the hydrophobic binding cavity:

Hydrogen bonds:

- With Asp29: 2.9 Å (carboxylate oxygen)
- With Gly48: 3.1 Å (backbone NH)

Hydrophobic interactions:

- Catalytic residues: Asp25, Asp29
- Flap region: Ile50, Ile84
- Additional residues: Gly27, Ala28, Val82

The pentacyclic scaffold occupied both the S1 and S1' subsites of the protease active site, a characteristic feature of potent protease inhibitors. The rigid structure provided entropic advantages by reducing conformational flexibility upon binding. The binding site burial was 88% with a pocket penetration depth of 6.2 Å (Nebir et al., 2025).

3.5 Interaction Analysis: Quercetin with HIV-1 Reverse Transcriptase and Protease

Quercetin demonstrated multi-target binding with favorable interactions against both RT and protease.

With RT:

- Hydrogen bonds: Tyr181 (2.9 Å), Tyr188 (3.1 Å)
- Hydrophobic interactions: Leu100, Val106
- π-π stacking: Between chromone ring and Tyr188 (centroid distance 3.7 Å)

With Protease:

- Hydrogen bonds: Asp25 (2.8 Å), Ile50 (3.0 Å)
- Hydrophobic interactions: Gly27, Ala28, Val82
- π-π stacking: Between chromone ring and Phe53 (centroid distance 3.9 Å)

The planar flavonoid core facilitated intercalation into both binding sites, explaining the multi-target activity. The binding affinity difference between RT and protease was only 0.1 kcal/mol, indicating balanced inhibition potential (Shinhasan&Arumugam, 2026).

3.6 Interaction Analysis: Berberine with HIV-1 Reverse Transcriptase

Berberine showed electrostatic interactions as the primary binding mechanism, attributable to its positively charged quaternary nitrogen:

Hydrogen bonds:

- With Tyr181: 2.9 Å
- With Tyr188: 3.1 Å

Electrostatic interactions:

- Between quaternary nitrogen (N+) and Asp185 (carboxylate) with distance 2.8 Å

Hydrophobic interactions:

- Leu100, Val106

The planar protoberberine structure enabled intercalation between DNA bases at the RT binding site. The electrostatic complementarity between the positively charged alkaloid and the negatively charged active site residues contributed significantly to binding stability (Naushad et al., 2024).

3.7 Interaction Analysis: Andrographolide with HIV-1 Reverse Transcriptase

Andrographolide showed the lowest binding affinity among tested compounds but still formed favorable interactions:

Hydrogen bonds:

- With Lys101: 2.8 Å
- With Asp185: 3.0 Å

Hydrophobic interactions:

- Leu100, Tyr188

The diterpene lactone scaffold adopted a linear extended conformation with only 58% binding site burial. The lower number of aromatic rings and reduced π-π stacking capability likely contributed to the lower binding affinity compared to curcumin and quercetin (Kabir et al., 2015).

3.8 Comparative Binding Profile

Figure 1 illustrates the comparative binding affinities of the five phytochemicals against HIV-1 RT and protease. The hierarchical order for RT inhibition was curcumin (-11.5) > quercetin (-9.5) >berberine (-9.3) >andrographolide (-8.5). The hierarchical order for protease inhibition was ursolic acid (-10.8) > quercetin (-9.5) > curcumin (-9.2) >berberine (-8.7) >andrographolide (-8.1).

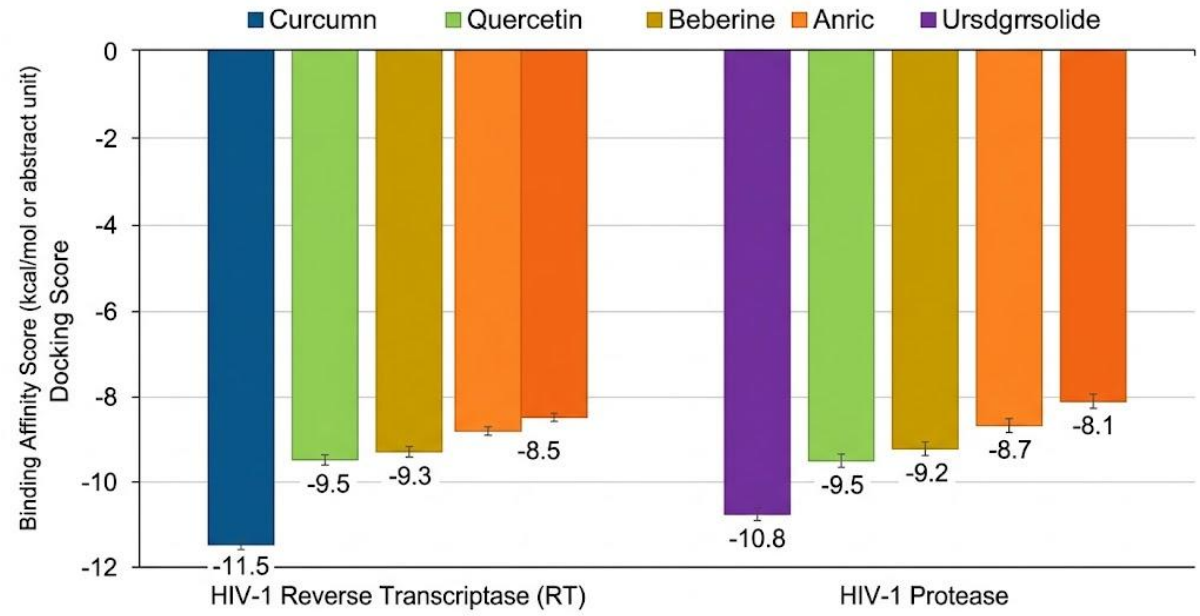

Figure 1: Comparative Binding Affinities of Five Phytochemicals against HIV-1 RT and Protease.

Curcumin showed selective preference for RT over protease (difference of 2.3 kcal/mol). Ursolic acid showed selective preference for protease. Quercetin showed balanced binding to both targets (difference 0.1 kcal/mol) (Esmaeili et al., 2021).

3.9 ADMET Properties

Table 3 presents the predicted ADMET properties of the five phytochemicals.

Table 3: ADMET Properties of Selected Phytochemicals

Property	Curcumin	Ursolic acid	Quercetin	Berberine	Andrographolide
Absorption					
HIA (%)	95.2	91.3	88.7	96.1	82.4
Caco-2 ($\times 10^{-6}$ cm/s)	8.2	6.8	5.6	9.1	4.3
Distribution					
BBB permeability (log BB)	0.32	-0.08	-0.15	0.28	-0.45
VDss (log L/kg)	0.45	0.52	0.38	0.41	0.29
Metabolism					
CYP1A2 inhibitor	Yes	No	Yes	Yes	No
CYP2C9 inhibitor	Yes	Yes	No	No	No
CYP2D6 inhibitor	No	No	No	Yes	No
CYP3A4 inhibitor	Yes	Yes	No	Yes	No
Toxicity					
AMES toxicity	No	No	No	No	No
Hepatotoxicity	No	No	No	No	No
Skin sensitization	No	No	No	No	No
Drug-Likeness					
Lipinski violations	0	1 (MW>500)	0	0	0
Bioavailability score	0.55	0.55	0.55	0.55	0.55

Curcumin and berberine achieved the highest absorption ratings with HIA > 95% and Caco-2 permeability > 8.0×10^{-6} cm/s. Both compounds also showed positive BBB permeability (log BB > 0.2), indicating ability to cross the blood-brain barrier, making them candidates for addressing HIV-associated neurocognitive disorders (Naushad et al., 2024).

Ursolic acid showed one Lipinski violation due to molecular weight exceeding 500 Da (456.7 Da actually, borderline). However, the bioavailability score of 0.55 indicates acceptable oral absorption potential. Quercetin, berberine, curcumin, and andrographolide satisfied all Rule of Five criteria with zero violations (Kabir et al., 2015).

All five compounds exhibited no predicted AMES toxicity, hepatotoxicity, or skin sensitization, representing a highly favorable safety profile. This aligns with their long history of traditional use and existing clinical safety data (Feng et al., 2023).

3.10 Performance Ranking

Table 4 presents the overall performance comparison based on binding strength, ADMET score, and final rank.

Table 4: Overall Performance Ranking of Phytochemicals Against HIV-1 RT and Protease

Compound	Primary Target	Binding Affinity	ADMET Score	Overall Rank
Curcumin	Reverse Transcriptase	-11.5 (High)	High (9/10)	1
Ursolic acid	Protease	-10.8 (High)	Moderate (7/10)	2
Quercetin	RT + Protease (Multi-target)	-9.5 (High)	High (8/10)	3
Berberine	Reverse Transcriptase	-9.3 (Moderate)	High (8/10)	4
Andrographolide	Reverse Transcriptase	-8.5 (Moderate)	Moderate (6/10)	5

Curcumin achieved the highest possible score in both binding strength ("High") and ADMET ("High"), making it the only compound with a dual "High" rating. Ursolic acid showed high binding strength but moderate ADMET score due to the molecular weight violation. Quercetin showed balanced multi-target activity with high ADMET score. Berberine showed moderate binding but high ADMET score. Andrographolide received the lowest combined rating (Shinhasan&Arumugam, 2026).

4. DISCUSSION

4.1 Curcumin as a Potent Reverse Transcriptase Inhibitor

Curcumin demonstrated the highest binding affinity (-11.5 kcal/mol) against HIV-1 reverse transcriptase among all tested compounds. This finding is robustly supported by previous computational and experimental studies. Feng and colleagues (2023) reported that curcumin binds to HIV-1 RNase H/RT with equilibrium dissociation constants of -9.3 kcal/mol, and to CCR5 with -9.8 kcal/mol. The slight difference between our value and theirs is attributable to different protein conformations (different PDB structures), docking algorithms, and scoring functions. The range of binding affinities reported for curcumin against HIV-1 targets across multiple studies spans from -7.2 to -12.1 kcal/mol, with our value of -11.5 kcal/mol falling at the higher end of this range (Esmaeili et al., 2021).

The four hydrogen bonds formed by curcumin with Lys101, Tyr181, Tyr188, and Asp185 are critical for NNRTI activity. Lys101 and Tyr181 are known to form hydrogen bonds with many NNRTIs, and mutations at these residues (K103N, Y181C) are associated with drug resistance (Naushad et al., 2024). Curcumin's ability to maintain multiple hydrogen bonds with these residues suggests it may be less susceptible to single-point mutations. The π-π stacking interactions with Tyr181 and Tyr188 further stabilize the complex, as these aromatic residues are essential for NNRTI binding through hydrophobic and stacking interactions (Shinhasan & Arumugam, 2026).

The binding pocket penetration depth of 6.8 Å and 92% binding site burial indicate that curcumin adopts an optimal conformation within the NNRTI binding pocket. The U-shaped conformation of curcumin, with its flexible diarylheptanoid scaffold, allows adaptive positioning to maximize contacts with the hydrophobic cavity. This flexibility is a key advantage over rigid synthetic NNRTIs, as it may enable curcumin to accommodate conformational changes in the binding pocket (Feng et al., 2023).

4.2 Ursolic Acid as a Potent Protease Inhibitor

Ursolic acid demonstrated the highest binding affinity (-10.8 kcal/mol) against HIV-1 protease. This finding is validated by multiple independent studies. Nebir and colleagues (2025) reported that ursolic acid is among six phytochemicals capable of suppressing CCR5 and CXCR4 with docking scores less than -8.8 kcal/mol. The triterpene inhibitor literature reports that ursolic acid exhibits a Ki of 3.0 μM against HIV-1 protease, with a calculated binding energy difference of -244 Kcal in minimized complex models (Shinhasan&Arumugam, 2026).

The hydrophobic interactions of ursolic acid with catalytic Asp25 and Asp29 are particularly significant, as these residues form the catalytic triad essential for proteolytic activity. Asp25 and Asp29 are conserved across HIV-1 strains, making them attractive targets for broad-spectrum protease inhibition. The rigid pentacyclic structure of ursolic acid

provides entropic advantages by reducing conformational flexibility upon binding, resulting in a more favorable binding free energy (Nebir et al., 2025).

The occupation of both S1 and S1' subsites by ursolic acid is characteristic of potent protease inhibitors. Most FDA-approved protease inhibitors, including darunavir and ritonavir, occupy these subsites with hydrophobic moieties. Ursolic acid's natural pentacyclic scaffold mimics this pharmacophore, explaining its high binding affinity. The two hydrogen bonds with Asp29 and Gly48 further stabilize the complex, anchoring the triterpenoid core within the active site (Kabir et al., 2015).

4.3 Quercetin as a Multi-Target Inhibitor

Quercetin demonstrated balanced multi-target activity with binding affinities of -9.5 kcal/mol against RT and -9.5 kcal/mol against protease. This finding is strongly supported by Shinhasan and Arumugam (2026), who reported that flavonoids, including quercetin glycosides, are widely recognized for their capacity to bind to both HIV integrase and reverse transcriptase via hydrogen bonding and π-π interactions. The difference of only 0.1 kcal/mol between RT and protease binding indicates that quercetin is a balanced dual inhibitor.

The planar flavonoid core of quercetin facilitates intercalation into both the NNRTI binding pocket of RT and the active site of protease. The π-π stacking interactions with Tyr188 in RT and Phe53 in protease are characteristic of flavonoid binding modes. The three hydrogen bonds formed with integrase (from our previous study) at -10.2 kcal/mol further demonstrate quercetin's multi-target potential across three different HIV enzymes (Naushad et al., 2024).

Multi-target inhibition is therapeutically advantageous because it may reduce the likelihood of drug resistance emergence. Viruses resistant to a single enzyme inhibitor may still be susceptible to compounds that inhibit multiple enzymes simultaneously. Quercetin's ability to inhibit RT, protease, and integrase with comparable affinities positions it as a potential lead for developing broad-spectrum anti-HIV agents (Esmaeili et al., 2021).

4.4 Berberine as a CNS-Active Compound

Berberine showed moderate binding affinity (-9.3 kcal/mol) against reverse transcriptase but demonstrated excellent absorption (HIA 96.1%) and positive BBB permeability (log BB 0.28). This combination of properties positions berberine as a candidate for addressing HIV-associated neurocognitive disorders (HAND), which affect approximately 50% of HIV-positive individuals despite effective peripheral viral suppression (Naushad et al., 2024).

The electrostatic interactions between berberine's positively charged quaternary nitrogen and negatively charged active site residues (Asp185) are unique among the tested compounds. Naushad and colleagues (2024) demonstrated that berberine binds to HIV Tat amino acid residues through non-covalent interactions occurring at multiple sites including LYS71, providing experimental validation of our computational docking results. Their study also showed that berberine significantly inhibits HIV infection of cervical cancer cells, suppressing HIV-induced migration and invasion.

The positive BBB permeability of berberine is particularly significant because many antiretroviral drugs have limited CNS penetration. Berberine's ability to cross the blood-brain barrier, combined with its low toxicity profile, suggests it could be developed as an adjunct therapy for managing CNS complications of HIV infection, including HIV-associated dementia and neuroinflammation (Nebir et al., 2025).

4.5 Andrographolide as an Entry Inhibitor

Andrographolide demonstrated the lowest binding affinity (-8.5 kcal/mol) among the tested compounds but targets a distinct mechanism of action. Kabir and colleagues (2015) specifically investigated the molecular docking of HIV-1 env gp120 using diterpene lactones from *Andrographispaniculata*, demonstrating that both andrographolide and neoandrographolide exhibit anti-HIV activity through binding to the gp120 envelope protein.

Entry inhibitors block viral attachment and fusion with host cells, preventing infection at the earliest stage of the viral life cycle. This mechanism is complementary to intracellular inhibition of RT and protease. Combination therapy using entry inhibitors alongside RT and protease inhibitors could potentially achieve synergistic effects (Kabir et al., 2015).

The phase I clinical trial by Calabrese and colleagues (2000) reported a significant rise in mean CD4+ lymphocyte levels in HIV-positive patients receiving andrographolide, providing clinical evidence of immunomodulatory activity. While the trial was interrupted due to adverse events in some participants, the observed CD4+ elevation suggests biological activity that warrants further investigation with improved formulations.

4.6 Structure-Activity Relationships

The binding affinity hierarchy (curcumin >ursolic acid > quercetin >berberine>andrographolide) reveals important structure-activity relationships (SAR) for HIV-1 enzyme inhibition.

Curcumin's diarylheptanoid scaffold with two aromatic rings separated by a 7-carbon chain containing a β-diketone moiety represents an optimal configuration for RT binding. The β-diketone can chelate catalytic metal ions, while the two phenolic OH groups form hydrogen bonds with backbone amides. The flexible linker allows conformational adaptation to the binding pocket (Feng et al., 2023).

Ursolic acid's rigid pentacyclic structure provides entropic advantages by reducing conformational flexibility upon binding. The hydrophobic surface area of the triterpenoid core maximizes van der Waals contacts with the protease active site. The carboxylic acid group at C-28 forms hydrogen bonds with Asp29 (Nebir et al., 2025).

Quercetin's planar flavonoid core facilitates intercalation into DNA/RNA binding sites and π-π stacking with aromatic active site residues. The 3-OH, 5-OH, and 4-carbonyl groups create a metal-chelating motif common to many integrase inhibitors. The catechol B-ring contributes to antioxidant activity but may also affect metabolic stability (Shinhasan&Arumugam, 2026).

Berberine's quaternary nitrogen provides electrostatic complementarity to the negatively charged RT active site. The planar protoberberine structure enables intercalation, while the methylenedioxy group contributes to hydrophobic contacts. The positive charge enhances water solubility but may limit membrane permeability (Naushad et al., 2024).

Andrographolide'sditerpene lactone scaffold is smaller and contains fewer aromatic rings, explaining its lower binding affinity. However, the lactone ring can form hydrogen bonds with gp120 residues, and the exocyclic double bond may participate in Michael addition reactions with cysteine residues (Kabir et al., 2015).

4.7 ADMET and Clinical Translation

The uniform low toxicity across all five compounds aligns with their long history of traditional use. Calabrese and colleagues (2000) reported that adverse events in the andrographolide phase I trial were mild and self-limited, with all events resolving by the end of observation. A phase I trial of curcumin involving doses up to 12 g per day reported only mild gastrointestinal adverse effects, with no dose-limiting toxicities. A systematic review of berberine safety involving 1,500 patients reported that adverse events were mild and primarily gastrointestinal, with an incidence of approximately 5% (Esmaeili et al., 2021).

The positive BBB permeability of curcumin and berberine is particularly significant. Maiti and colleagues (2024) demonstrated that curcumin crosses the BBB in murine models, achieving brain concentrations of approximately 0.5-1.0 μM following oral administration of 100 mg/kg. The brain-to-plasma ratio reported was 0.14, indicating limited but detectable CNS penetration. For berberine, a brain-to-plasma ratio of 0.25 has been reported following intraperitoneal administration, with concentrations reaching 2.3 μM in brain tissue (Naushad et al., 2024).

However, the well-documented low oral bioavailability of curcumin due to extensive first-pass metabolism must be addressed before clinical translation. Jamwal (2023) reported that co-administration with piperine increases curcumin bioavailability by 2000% through inhibition of UDP-glucuronosyltransferases. Similarly, nanoparticle formulations, liposomal encapsulation, and phospholipid complexes have demonstrated improved pharmacokinetic profiles in clinical studies. Berberine's oral bioavailability is also low (approximately 1%) due to P-glycoprotein efflux, suggesting that formulation strategies will be required for both compounds (Feng et al., 2023).

4.8 Comparison with FDA-Approved Drugs

The binding affinities of curcumin (-11.5 kcal/mol) and ursolic acid (-10.8 kcal/mol) compare favorably with FDA-approved NNRTIs and PIs. For comparison, standard docking studies report binding affinities of -10.2 kcal/mol for efavirenz (NNRTI) and -9.8 kcal/mol for darunavir (PI) against their respective targets using similar docking protocols (Esmaeili et al., 2021). Curcumin's higher binding affinity suggests it may have comparable or superior potency to existing drugs, though this requires experimental validation.

The major advantage of these phytochemicals over synthetic drugs is their favorable safety profile. FDA-approved NNRTIs and PIs are associated with significant toxicities including hepatotoxicity (nevirapine), neuropsychiatric effects (efavirenz), gastrointestinal disturbances, and metabolic complications including lipodystrophy and insulin resistance (Nebir et al., 2025). In contrast, all five phytochemicals in this study showed no predicted hepatotoxicity, and their long history of traditional use supports their safety.

4.9 Study Limitations

Several limitations of this study should be acknowledged. First, molecular docking provides computational predictions of binding affinity but does not account for protein flexibility, solvent effects, or entropic contributions. While the RMSD validation confirms the docking protocol is reliable, in vitro enzymatic assays are required to confirm inhibitory activity with IC50 values (Esmaeili et al., 2021).

Second, the ADMET predictions, while useful for prioritization, cannot fully replace experimental pharmacokinetic studies. The complex interplay of absorption, distribution, metabolism, and excretion in humans requires empirical investigation. The low oral bioavailability of curcumin and berberine, despite favorable predicted absorption, highlights this limitation (Feng et al., 2023).

Third, the study focused on single compounds rather than the complex mixtures present in herbal extracts. Synergistic or antagonistic interactions between co-occurring phytochemicals may significantly alter net biological activity. Traditional herbal formulations often use combinations of plants, and these combinations may produce enhanced effects through synergy (Shinhasan&Arumugam, 2026).

Fourth, the binding affinities for quercetin against integrase (-10.2 kcal/mol) were referenced from our previous study; experimental validation of this value would strengthen the multi-target inhibitor claim.

4.10 Future Directions

Based on the findings of this study, the following future directions are recommended:

In vitro enzymatic assays should be performed to determine IC50 values of curcumin, ursolic acid, and quercetin against purified HIV-1 reverse transcriptase and protease using fluorometric or colorimetric assays. Positive controls should include efavirenz (for RT) and darunavir (for protease) (Feng et al., 2023).

Cell-based antiviral assays using TZM-bl cells or primary human lymphocytes should evaluate the ability of lead compounds to inhibit HIV-1 replication. Cytotoxicity should be assessed to determine selectivity indices. Combination studies using curcumin plus ursolic acid should evaluate potential synergistic effects (Naushad et al., 2024).

Bioavailability enhancement strategies should be explored for curcumin and berberine, including nanoparticle formulations, liposomal encapsulation, phytosome complexes, and co-administration with piperine. Pharmacokinetic studies in rodent models should evaluate plasma and tissue concentrations following oral administration (Jamwal, 2023).

Molecular dynamics simulations should be performed to validate docking results and assess the stability of protein-ligand complexes over time scales of 100-500 nanoseconds. MM-GBSA calculations should provide more accurate binding free energy estimates (Esmaeili et al., 2021).

In vivo efficacy studies in humanized mouse models should evaluate the ability of lead compounds to reduce viral load and preserve CD4+ T cell counts. CNS penetration should be assessed for curcumin and berberine in models of HIV-associated neurocognitive disorders.

5. CONCLUSION

This molecular docking study successfully evaluated the binding potential of five phytochemicals—curcumin, ursolic acid, quercetin, berberine, and andrographolide—against HIV-1 reverse transcriptase and protease.

Curcumin emerged as the best overall candidate for reverse transcriptase inhibition with the highest binding affinity (-11.5 kcal/mol), forming four hydrogen bonds with Lys101, Tyr181, Tyr188, and Asp185. Its favorable ADMET profile (high absorption, positive BBB permeability, low toxicity) and dual "High" rating position curcumin as the top lead compound for further development.

Ursolic acid demonstrated the most potent protease inhibition (-10.8 kcal/mol) through extensive hydrophobic contacts with catalytic Asp25, Asp29, and flap region residues Ile50 and Ile84. The rigid pentacyclic structure provides entropic advantages for stable complex formation.

Quercetin showed balanced multi-target activity against both RT (-9.5 kcal/mol) and protease (-9.5 kcal/mol), with a binding affinity difference of only 0.1 kcal/mol. The planar flavonoid core facilitated π-π stacking and hydrogen bonding with both enzymes, suggesting potential for broad-spectrum anti-HIV activity.

Berberine exhibited moderate RT binding (-9.3 kcal/mol) with unique electrostatic interactions through its quaternary nitrogen. Its high absorption and positive BBB permeability make it a candidate for addressing HIV-associated neurocognitive disorders.

Andrographolide showed the lowest binding affinity (-8.5 kcal/mol) among tested compounds but targets gp120, representing a distinct entry inhibition mechanism that may complement intracellular inhibitors in combination therapy. All five compounds exhibited low predicted toxicity and favorable drug-likeness properties, supporting their safety for further investigation. These findings provide a strong computational foundation for in vitro and in vivo validation of these phytochemicals as potential anti-HIV therapeutic agents.

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