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Original Article

# EXPLORATION OF THE ACTIVE COMPOUNDS OF *MORINGA OLEIFERA* LAM AS HIV-1 REVERSE TRANSCRIPTASE INHIBITOR: A NETWORK PHARMACOLOGY AND MOLECULAR DOCKING APPROACH

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**ABSTRACT**

**Objective:** This study aims to predict the active compound of *Moringa oleifera* for the treatment of *Human Immunodeficiency Virus* (HIV), specifically targeting the HIV-1 reverse transcriptase (HIV-1 RT) enzyme using network pharmacology and molecular docking approach.

**Methods:** The active ingredients of *M. oleifera*, were screened from the Knapsack database. Subsequently, HIV-1 RT and its related target compounds were retrieved from the Genecard database. The analysis of common targets involved protein-protein interactions (PPI) analysis using string databases and constructing interaction IDs using Cytoscape software. Gene Ontology (GO) functional and the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses were performed. Molecular docking studies were conducted using AutoDock Vina software to validate the results of the network pharmacological analysis.

**Results**: A total of 63 active ingredients and 8601 targets related to HIV-1 RT were identified. The network analysis, encompassing GO and KEGG enrichment, revealed strong associations of common targets with key signaling pathways such as Tumor Necrosis Factor (TNF), Toll-Like Receptor (TLR), and apoptosis. Additionally, 11 compounds of *M. oleifera,* including apigenin, benzyl isothiocyanate, benzylamine, caffeic acid, ferulic acid, epicatechin, kaempferol, gallic acid, luteolin, syringic acid and vanillin were identified as potential vital compounds. Molecular docking analysis highlighted apigenin and kaempferol as the most promising compounds, exhibiting the lowest binding affinity to the HIV-1 RT enzyme. These compounds correlated with caspase-3(CASP3), caspase-9 (CASP9), and BCL2 Apoptosis Regulator (BAX) protein, stimulating cell apoptosis through multiple pathways.

**Conclusion:** The study highlighted that apigenin and kaempferol are potential compound of *M. oleifera* in HIV-1 treatment through inhibition activity at HIV-1 RT Enzyme.

**Keywords:** HIV-1 reverse transcriptase, *Moringa oleifera*, Network pharmacology, Molecular docking


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## INTRODUCTION

*Human Immunodeficiency Virus* (HIV) remains a global health issue and causes the deaths of 630,000 people by the end of 2022 [1]. The objectives of HIV treatment are to impede the advancement of HIV infection to Acquired Immune Deficiency Syndrome (AIDS) and extend the lifespan of individuals with AIDS while maintaining a high standard of living. Antiretroviral medications (ARV) can impede viral replication by suppressing the activity of enzymes implicated in viral replication, specifically reverse transcriptase (RT), protease (PR), and integrase (IN). One of the most efficient interventions occurs at the stage of the reverse transcription process [2]. HIV-1 RT is crucial in facilitating the creation of double-stranded DNA from single-stranded HIV RNA [3]. A downside of ARV therapy is the presence of numerous side effects, leading to reduced patient adherence and stimulating the emergence of resistance [4]. Resistance to antiretroviral drugs are the essential development of research on compounds as anti-HIV agents, including those from a natural sources [5].

*M. oleifera* is traditionally used in HIV treatment in South Africa [6]. This plant is native and grows widely in northern India and spread to the continents of Africa and Asia [6]. The plant is widely used for treatment due to its high nutritional content and has various activities such as an antioxidant [7], antimicrobial, anti-inflammatory, antihypertensive, immunomodulator, and as hypoglycemic agent [6, 8] and hepatoprotector [9]. Based on the analysis carried out by Kerdsomboon [10], the leaf extract contains flavonoids and phenolics including apigenin, protocatechuic acid, gallic acid, chlorogenic acid, vanillic acid, caffeic acid, catechin, rutin, *para*-coumaric acid, ferulic acid, quercetin, rosmarinic acid, cinnamic acid, and kaempferol. The major compounds are quercetin, ellagic acid, chlorogenic acid, niazimicin, gallic acid, rutin, ferulic acid, and moringin [11]. Furthermore, this leaf also contains glycoside compounds such as apigenin glucoside, kaempferol 3-O-glucoside, kaempferol diglucoside, and kaempferol-di-acetyl-rhamnoside [12].

A previous study revealed that water and 50% methanol extracts of *M. oleifera* have inhibitory activity on the HIV-1 RT with $IC_{50}$ values of 9 µg/ml and 79 µg/ml, respectively [3]. However, the active compound that has HIV-1 RT inhibition activity remains unclear. The present study aims to determine the active compound in the *M. oleifera* leaves that is responsible for the inhibitory activity of the HIV-1 RT. This active compound could be predicted through network pharmacology and molecular docking. By constructing an interactive network connecting drug, component, target, pathway, and disease and using GO and KEGG pathway enrichment analysis along with molecular docking technology, the potential molecular mechanism of *M. oleifera* as an HIV-1 RT inhibitor was explored. Our research findings guide future basic experimental research.

## MATERIALS AND METHODS

### Data mining of active compounds in *M. oleifera*

Bioactive compounds from *M. oleifera* were retreived from the Knapsack database (http://www.knapsackfamily.com/KNApSAcK/, NAIST Comparative Genomics Laboratory, Japan), accessed on July 23, 2023. The Knapsack family database integrates information about plant species and their secondary metabolite content, supporting various plant-related research area, including metabolomics and plant taxonomy studies [13]. The

physicochemical and absorption, distribution, metabolism, excretion and toxicity (ADMET) properties of the compounds were obtained from PKCSM Pharmacokinetics (https://biosig.lab.uq.edu.au/pkcsm/, American Chemical Society) [14] and Tox Prediction (https://tox-new.charite.de/protox_II/index.php) [15], accessed on July 23, 2023 using the canonical name. These pharmacokinetics (ADMET) studies and drug-likeness were predicted by adopting Lipinski's rule of five parameters: molecular weight ≤ 500 Da, Log p ≤ 5, Hydrogen bond acceptor (HBA) ≤ 10, Hydrogen bond donor (HBD) ≤ 5 [16, 17]. A Log P value greater than 5 indicates that the compound has poor permeation and absorption. Therefore, a molecule will have good bioavailability if it meets the criteria of Lipinski's Rule of 5 [17]. Human intestine absorption (HIA) should be ≥ 30% and not act as Cytochrome P450 2D6 (CYP2D6) inhibitors with class toxicity>3.

**Target of HIV disease**

The target proteins corresponding to the active compounds of *M. oleifera* were sourced from GeneCard (www. genecards. org) [18], accessed on July 23, 2023, using the compound name as a keyword. The top 30 targets listed were collected based on their codes and target names. The target of HIV disease was collected from hsa05170 HIV 1 infection on KEGG (https://www.genome.jp/entry/hsa05170, Keisha Laboratories). Specifically, HIV-1 RT, was extracted from GeneCard (www.genecards.org), accessed on July 23, 2023, using the keyword "HIV-1 Reverse Transcriptase". The entire set of targets listed was taken based on data collection of codes and target names.

**Active compounds-target interaction**

The relationship between target compounds and disease was visualized using a Venn diagram (https://bioinfogp.cnb.csic.es/tools/venny/) by inputting data related to the target protein for HIV disease and the target protein of the active compounds from *M. oleifera*.

**Analysis of protein-protein interaction (PPI)**

PPI analysis was conducted using the Search Tool for the Retrieval of Interacting Genes (STRING, https://string-db.org/) by inputting the target protein set obtained from Venn diagram intersection. *Homo Sapiens* species restrictions and confidence score>0.4 were applied to ensure the reliability and confidence of the results [19, 20]. Cytoscape 3.9.1 was utilized to create networks and quantify the data obtained from STRING by calculating closeness, degree, and between centrality as degree values. Degree, a vital parameter within the topological structure, is employed to evaluate the importance of each node in the PPI network. It represents the number of connections to a node and is commonly used to illustrate the topological significance of a protein within the PPI network. Furthermore, hub target genes are identified as nodes with a 'Degree' exceeding double the median degree of the network [20]. Subsequently, the top 10 targets with the highest values were selected as the main targets [21].

**Functional enrichment analysis**

Enrichment analysis, covering GO functions (molecular function–MF, biological process–BP, and cellular component–CC) and KEGG pathways, was conducted using ShinyGO 0.77 (http://bioinformatics.sdstate.edu/go/, South Dakota State University) [22]. The target protein of HIV-1 RT and the active compound of *M. oleifera* served as input, with the species "*Human*", False Discovery Rate (FDR) cutoff at 0.05, and display of 30 pathways [23]. ShinyGO is an intuitive, visual application specifically designed for enrichment analysis. This tool offers more comprehensive gene sets associated with TF and miRNA target genes for human, mouse, and Arabidopsis. By using ShinyGO, it becomes *possible* to gain a summary of gene associations through GO enrichment and explore KEGG pathways [22]. The GO functions were visualized using a histogram bar plot and the resulting pathways were correlated with HIV disease.

**Molecular docking**

The active compounds of *M. oleifera* were drawn using the Marvin application (Chemaxon https://www.chemaxon.com, trial version) with the canonical smile and were saved in PDB format. The protein target was obtained through the RCSB PDB website (https://www.rcsb.org/) using "HIV-1 Reverse Transcriptase" as a keyword. The X-Ray crystal 3MEE was considered due to the lower resolution (<2.5 A) [24] and its complex with TMC278 (Rilpivirine), which is a second-generation HIV-1 RT. When creating the macromolecule 3MEE, the mutated amino acids from the first-generation were considered [25]. Proteins were downloaded in PDB format, visualized, and corrected for missing residues using the Chimera 1.17.1 application (UCSF Resource for Biocomputing, University of California, San Francisco) [26]. The process of preparing protein molecules as both receptors and ligands involved the utilizing Discovery Studio 2021 (BIOVIA, Dassault Systèmes, Biovia Discovery Studio, License, San Diego: Dassault Systèmes, 2023) [27]. This is included the removal of water molecules and heteroatoms.

Molecular docking is conducted to validate the relationship between compounds and the target. Molecular docking was performed using the PyRx software (version 0.8) [28] to investigate a range of ligand orientations, conformations, and binding affinities at the active sites of HIV-1 and ligands. To ensure compatibility with AutoDock Vina docking, all ligands were converted into the PDBQT format using Open Babel software[29]. Subsequently, a grid box was established to encompass the entire protein for a blind docking strategy. Validation of native ligands and receptors was carried out using the AutoDock Vina application, utilizing grid box settings derived from optimization. The grid box settings include a center at X: 10.9796, Y: 12.5195, Z: 16.2002, and dimensions at X: 12.4367, Y: 11.7488, Z: 14.0701. Gridbox validation was conducted using the Yasara application [30] with a Root-Mean-Square Deviation (RSMD) acceptable value of<2 Å (Angstroms). Following this, the results of molecular docking were visualized, and the nonbonding interactions between protein-ligand complexes and docking poses were assessed using BIOVIA Discovery Studio Client 2021 [27]. The conformation with the most negative binding affinity (in kcal/mol) and RSMD value closest to 0 was selected as the lead compound [31].

**RESULTS**

**Active compounds of *Moringa oleifera***

A total of 63 *M. oleifera* active compounds were obtained from the Knapsack database, accessed on July 23, 2023. Screening for physicochemical properties was carried out using Lipinski's rules of five, which include criteria of molecular weight ≤ 500 Da, Log p ≤ 5, HBA ≤ 10, (HBD ≤ 5 [16, 17] and 35 active compounds (table 1) met these criteria and were used in further analysis.

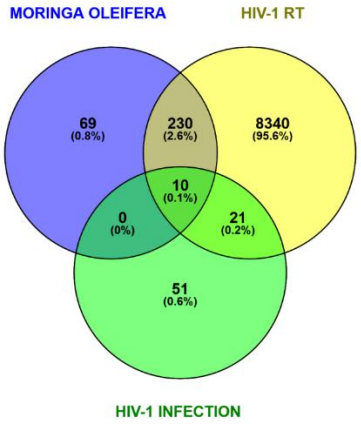



**Fig. 1: Venn diagram of *M. oleifera* active compounds, HIV-1 RT Enzyme and HIV-1 Infection**

**Active compounds-target interaction**

The outcomes derived from the Genecard search, accessed on July 23, 2023, revealed 309 targets associated with the active components found in *M. oleifera*, along with 8,601 targets related to

HIV-1 RT. Furthermore, in the KEGG pathway 'HIV-1 infection,' 82 target proteins were identified. Subsequently, all the targets will be analyzed using a Venn diagram to illustrate the overlapping proteins. The Venn diagram (fig. 1) shows that there were 10 intersections between the target active compounds of *M. oleifera*, HIV-1 RT, and HIV-1 Infection. It was shown that 77% of *M. oleifera* target compounds were associated with HIV-1 RT. Additionally, 10 proteins named Caspase 3 (CASP3), BCL2 Apoptosis Regulator (BCL2), Caspase 8 (CASP8), CD4 Molecule (CD4), C-X-C Motif Chemokine Receptor 4 (CXCR4), Toll Like Receptor 2 (TLR2), Toll Like Receptor 4 (TLR4), Caspase 9 (CASP9), C-C Motif Chemokine Receptor 5 (CCR5),BCL2 Associated X, Apoptosis Regulator (BAX) were identified as potential targets correlated with *M. oleifera* active compounds and HIV-1 disease. These targets will be analyzed for protein-protein interactions using the STRING database and visualized using Cytoscape 3.9.1.

**Table 1: Information on the 35 active compounds of *M. oleifera* that meet Lipinski's rules of five and ADMET parameters**

| No | Compound | MW | LogP | HBA | HBD | HIA | CYP2D6 inhibitor | Toxicity class |
|---|---|---|---|---|---|---|---|---|
| 1 | Epicatechin | 290,271 | 1.5461 | 6 | 5 | 65,425 | No | 6 |
| 2 | alpha-Phellandrene | 136,238 | 3.1648 | 0 | 0 | 96,548 | No | 6 |
| 3 | Niazimin A | 383,397 | 0.7098 | 8 | 3 | 44,845 | No | 5 |
| 4 | Niazicin A | 385,438 | 0.4846 | 8 | 3 | 45,796 | No | 5 |
| 5 | Niazimicin A | 357,428 | 0.3039 | 7 | 4 | 50,995 | No | 5 |
| 6 | Niazinin B | 343,401 | 0.3251 | 7 | 4 | 51,997 | No | 5 |
| 7 | Niazicinin A | 369,370 | 0.3197 | 8 | 3 | 56,015 | No | 5 |
| 8 | Niazidin | 369,370 | 0.3197 | 8 | 3 | 56,015 | No | 5 |
| 9 | Niazinin A | 343,401 | 0.3251 | 7 | 4 | 56,775 | No | 5 |
| 10 | Niaziminin B | 399,465 | 1,286 | 8 | 3 | 58,560 | No | 5 |
| 11 | Niazirine | 279,292 | -0.04102 | 6 | 3 | 59,546 | No | 5 |
| 12 | Niazirinine | 321,329 | 0.52978 | 7 | 2 | 60,000 | No | 5 |
| 13 | Niaziminin A | 399,465 | 1,286 | 8 | 3 | 68,647 | No | 5 |
| 14 | Caffeic acid | 180,159 | 1,1956 | 3 | 3 | 69,407 | No | 5 |
| 15 | Isorhamnetin | 316,265 | 2.2910 | 7 | 4 | 69,585 | No | 5 |
| 16 | Rhamnetin | 316,265 | 2.2910 | 7 | 4 | 72,598 | No | 5 |
| 17 | Kaempferol | 286,239 | 2.2824 | 6 | 4 | 74,290 | No | 5 |
| 18 | Luteolin | 286,239 | 2.2824 | 6 | 4 | 75,170 | No | 5 |
| 19 | Gentisic acid | 154,121 | 0.796 | 3 | 3 | 80,078 | No | 5 |
| 20 | Apigenin | 270,240 | 2.5768 | 5 | 3 | 90,555 | No | 5 |
| 21 | *p*-Coumaric acid | 164,160 | 1.4900 | 2 | 2 | 93,494 | No | 5 |
| 22 | *o*-Coumaric acid | 164.16 | 1.49 | 2 | 2 | 93.4941 | No | 5 |
| 23 | Genistein | 270.24 | 2.5768 | 5 | 3 | 93,831 | No | 5 |
| 24 | Daidzein | 254,241 | 2.8712 | 4 | 3 | 94,772 | No | 5 |
| 25 | Gallic acid | 170,120 | 0.5016 | 4 | 4 | 43,374 | No | 4 |
| 26 | Moringyne | 312,318 | -0.73986 | 7 | 4 | 54,312 | No | 4 |
| 27 | Syringic acid | 198,174 | 1.1076 | 4 | 2 | 74,963 | No | 4 |
| 28 | Vanillin | 152,149 | 1.2131 | 3 | 1 | 78,206 | No | 4 |
| 29 | Ellagic acid | 302,194 | 1.3128 | 8 | 4 | 83,817 | No | 4 |
| 30 | Benzylamine | 107,156 | 1.1453 | 1 | 1 | 87,626 | No | 4 |
| 31 | Salicylic acid | 138,122 | 1.0904 | 2 | 2 | 89,961 | No | 4 |
| 32 | Eugenol | 164,204 | 2.1293 | 2 | 1 | 91,651 | No | 4 |
| 33 | Sinapic acid | 224,212 | 1.5072 | 4 | 2 | 91,779 | No | 4 |
| 34 | Ferulic acid | 194,186 | 1.4986 | 3 | 2 | 93,490 | No | 4 |
| 35 | Benzyl isothiocyanate | 149,218 | 2.2894 | 2 | 0 | 94,988 | No | 4 |

MW: Molecular Weight, Log p: Log of octanol/water partition coefficient, HBA*:* Hydrogen Bond Acceptor, HBD*:* Hydrogen Bond Donor, HIA: Human intestinal absorption

**PPI network**

The establishment of the PPI network involved entering 10 protein targets into the STRING database. In this network, there were 10 nodes and 32 edges that correlated with *M. oleifera* and HIV disease. Finally, an analysis of these 10 proteins using Cytoscape software was performed to determine their total degree, closeness, and betweenness centrality values, illustrating the proteins with the greatest impact within this network. These targets are essential in HIV therapy, underscoring their significance in potential treatment strategies the information on the total degree value of this network is shown in table 2 and fig. 2.

**Table 2: Information of total degree value on PPI Network**

| No | Protein code | Protein name | Betweenness | Closeness | Degree | Total |
|---|---|---|---|---|---|---|
| 1 | CASP3 | Caspase 3 | 0,15 | 1,00 | 9 | 10,15 |
| 2 | BCL2 | BCL2 Apoptosis Regulator | 0,15 | 1,00 | 9 | 10,15 |
| 3 | CASP8 | Caspase 8 | 0,11 | 1,00 | 8 | 9,11 |
| 4 | CD4 | CD4 Molecule | 0,06 | 1,00 | 8 | 9,06 |
| 5 | CXCR4 | C-X-C Motif Chemokine Receptor 4 | 0,06 | 1,00 | 8 | 9,06 |
| 6 | TLR2 | Toll Like Receptor 2 | 0,00 | 1,00 | 7 | 8,00 |
| 7 | TLR4 | Toll Like Receptor 4 | 0,00 | 0,00 | 8 | 8,00 |
| 8 | CASP9 | Caspase 9 | 0,07 | 0,80 | 7 | 7,87 |
| 9 | CCR5 | C-C Motif Chemokine Receptor 5 | 0,00 | 1,00 | 6 | 7,00 |
| 10 | BAX | BCL2 Associated X, Apoptosis Regulator | 0,00 | 0,64 | 4 | 4,64 |

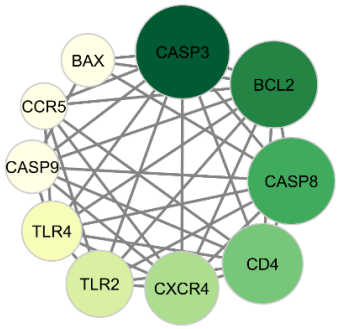
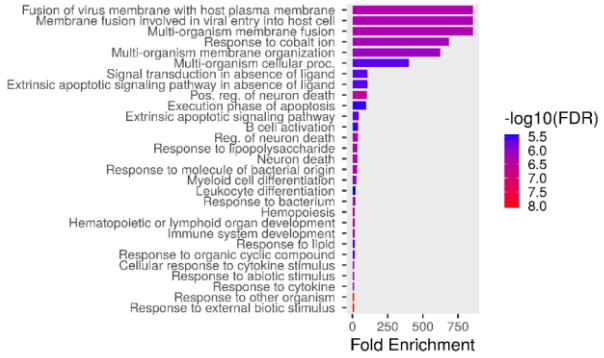


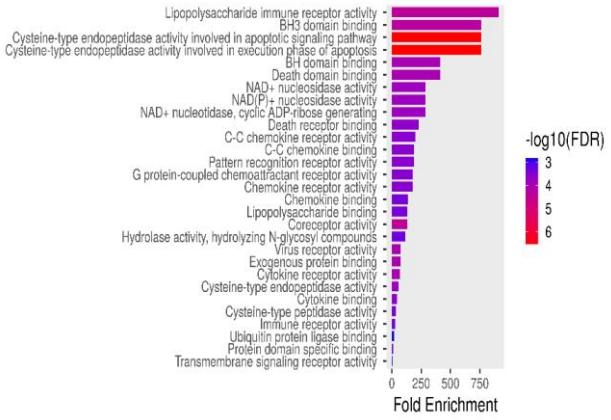

**Fig. 2: PPI network of the targets in *M. oleifera*, HIV-1 RT and HIV-1 Infection the darker color and node size indicated to the impact of the network**

**Results of gene ontology and KEGG pathway analysis**

Intersection target proteins obtained from the Venny platform were analyzed using ShinyGo 0.77 by inputting 10 target proteins, specifying the species as "human", and setting FDR cut off 0.05. GO enrichment was performed, including GO BP, GO MF, and GO CC. The GO enrichment results were in 1000 BP pathway, 56 CC pathway, and 107 MF pathway. The top 30 of each GO pathway are shown in fig. 3. The KEGG enrichment analysis indicated that apoptosis was the primary pathway associated with those 10 targets, as shown by fold enrichment (fig. 4a). This demonstrates the extent to which proteins/genes from a specific pathway are present in the experimental data compared to random data. However, when evaluated through FDR enrichment (fig. 4b), the HIV-1 infection exhibited the most minimal FDR enrichment value (FDR<0.05). This suggests that this pathway significantly contributes to the conducted networking analysis.




**(a)**

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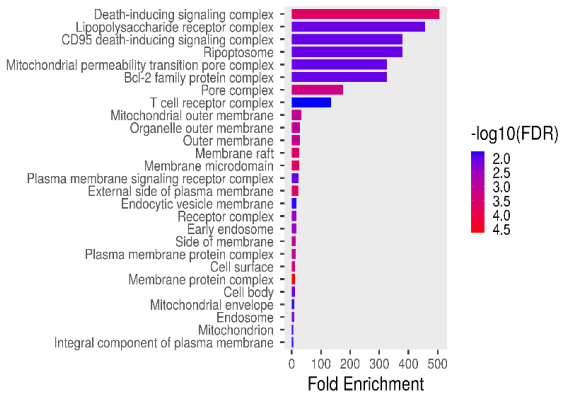
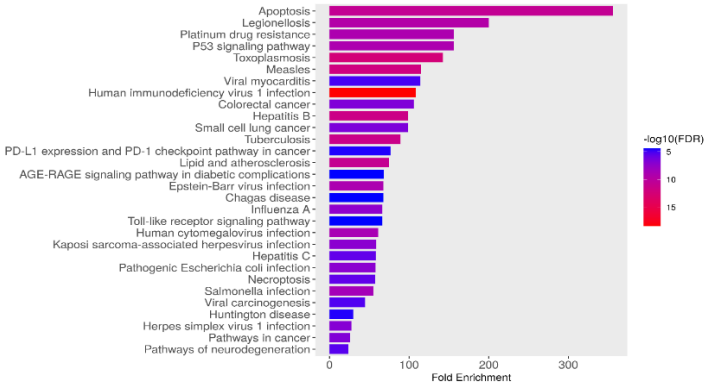
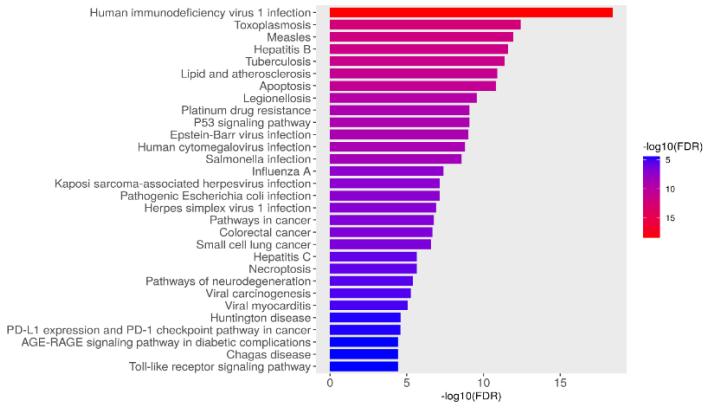



**(c)**

**Fig. 3: Top 30 gene ontology pathways, a. GO Biological process (BP), b. GO Cellular component (CC), c. GO molecular function (MF)**




**(a)**




**(b)**

**Fig. 4: KEGG pathway enrichment, the analyzed results based on fold enrichment (a) and FDR enrichment (b)**

Moreover, these 10 shared proteins were linked to 11 bioactive compounds found in *M. oleifera* (fig. 5), specifically apigenin, benzyl isothiocyanate, benzylamine, caffeic acid, epicatechin, ferulic acid, gallic acid, kaempferol, luteolin, syringic acid, and vanillin. Thereafter, these compounds were subsequently employed as ligands for the molecular docking analysis.

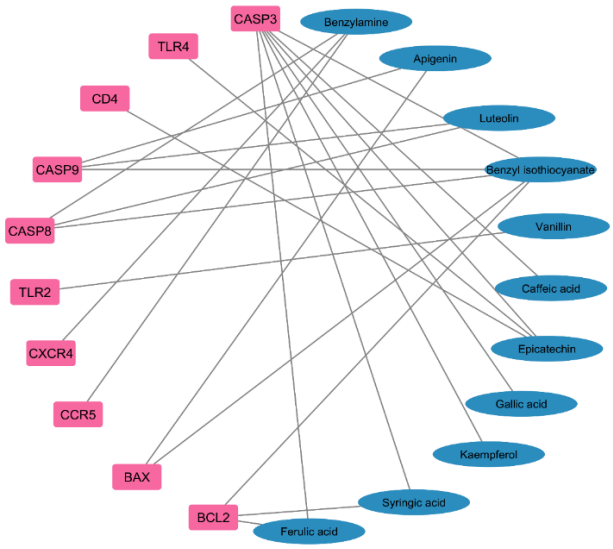


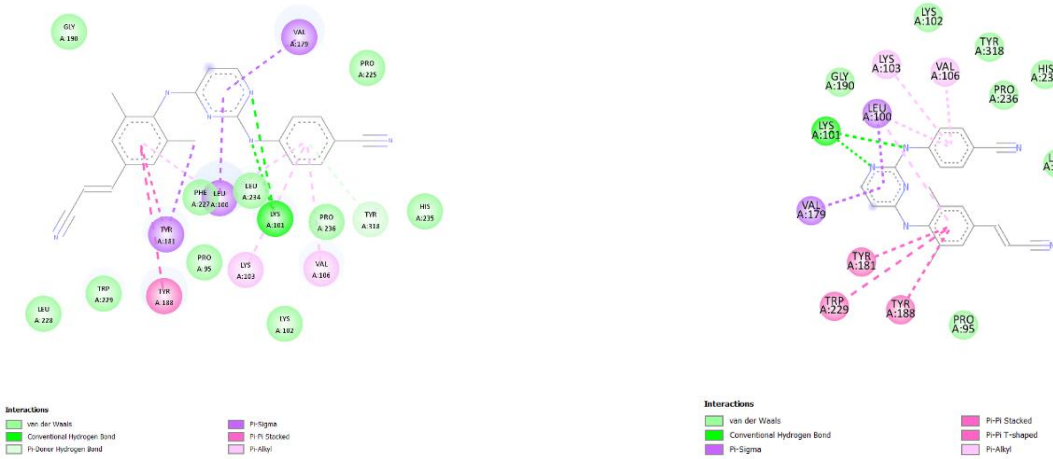

**Fig. 5: *M. oleifera* compounds–HIV-1 disease protein interaction**

**Molecular docking**

Molecular docking, using Autodock Vina, was applied to predict ligands and HIV-1 RT binding affinity. Docking simulations were conducted using Autodock Vina, resulting in 9 ligand conformations bound to the receptor and ranked based on their binding energy [32]. The HIV-1 RT X-Ray crystal structures (3MEE: 2,4 A) in complex with TMC278 (Rilpivirine) a second generation of Non-

Nucleoside Reverse Transcriptase Inhibitors (NNRTI) [33–35] were downloaded from the Protein Data Bank (https://www.rcsb.org/ accessed on 23 July 2023). Molecular docking was validated by ensuring that the grid_box encompassed the receptor and ligand binding site. Fig. 6 demonstrated that the native ligand before and after a docking overlapped and had the same amino acid arrangement (fig. 7 and table 3). Confirmation results through the Yasara application showed that the generated RMSD was 0.5159 Å. Such validation of a grid box is expected to improve the results of molecular docking with a flexible ligand/protein binding site and distinguish the compounds that can bind to a protein target from false positives [25].

**Fig. 6: Visualization of native ligands before (blue molecules) and after docking (yellow molecules)**




**(a)** **(b)**

**Fig. 7: Visualization of the interaction of receptor amino acids and native ligands before docking (a) and after docking (b)**

**Table 3: Amino acid interaction of native ligand and macromolecule (3MEE)**

| No | Native ligand | Amino acid |
|---|---|---|
| 1 | TMC278 before docking | GLY A: 190, VAL A: 179, PRO A: 225, PHE A: 227, LEU A: 100, LEU A: 234, TYR A: 181, LYS A: 101, PRO A: 236, TYR A: 318, HIS A 235, PRO A: 95, LYS A: 103, VAL A: 106, LEU A: 228, TRP A: 229, TYR A: 188, LYS A: 102 |
| 2 | TMC278 after docking | LYS A: 102, LYS A: 103, TYR A: 318, HIS A: 235, PRO A: 225, VAL A: 106, PRO A: 236, LEU A: 234, PHE A: 227, GLY A: 190, LEU A: 100, LEU A: 228, LYS A: 101, PRO A: 95, VAL A: 179, TYR A: 181, TRP A: 229, TYR A: 188 |

**Table 4: Molecular docking result**

| No | Compound name | Binding affinity (kcal/mol) |
|---|---|---|
| 1 | Native Ligand | -12.1 |
| 2 | Nevirapine | -10.2 |
| 3 | Apigenin | -9.5 |
| 4 | Kaempferol | -9.5 |
| 5 | Luteolin | -9.3 |
| 6 | Epicatechin | -9.0 |
| 7 | Ferulic acid | -9.0 |
| 8 | Caffeic acid | -7.0 |
| 9 | Benzyl isothiocyanate | -6.7 |
| 10 | Syringic acid | -6.5 |
| 11 | Gallic acid | -6.2 |
| 12 | Vanillin | -5.9 |
| 13 | Benzylamine | -5.8 |

In general, a lower energy level in the conformation of the receptor and ligand increases the likelihood of interaction, and an affinity energy of ≤ −5 kcal/mol is considered indicative of a strong binding affinity [21]. Table 4 indicates that our ligands exhibited binding affinities with HIV-1 RT in the range of -5.8 to -9.5 kcal/mol. These results demonstrated that all selected ligands have strong binding affinity with HIV-1 RT as target molecules. Among them, apigenin and kaempferol showed the highest binding affinity to HIV-1 RT at-9.5 kcal/mol. The binding occurs in the subunit p66, which plays a catalytic role in the polymerase domain [36] and forms hydrogen bonding with Lys 101 residues of HIV-1 RT. This binding affinity can be stabilized by van der Waals, pi-sigma and pi-alkyl amino acid interaction (fig. 8). These results show that the docked ligands have a stable complex with protein and have antiviral activity.

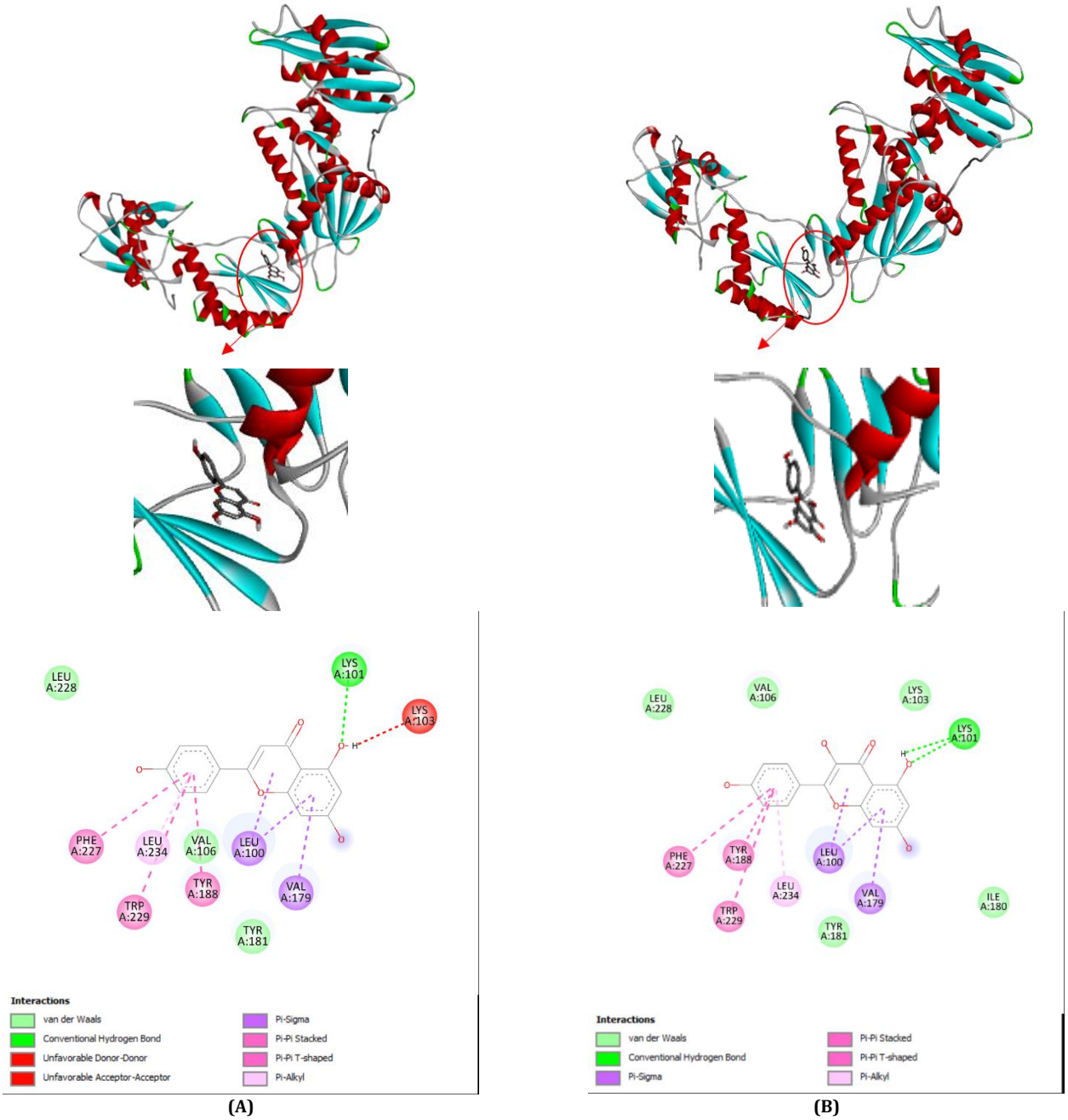



**Fig. 8: Protein and ligands interaction (A) apigenin (B) kaempferol**

## DISCUSSION

*M. oleifera* was used for HIV treatment in South Africa [6] and widely used for its high nutritional content and various medicinal properties. It had antioxidant, antimicrobial, anti-inflammatory, antihypertensive activities, immunomodulatory, antiglycemic activity and antiviral activity [6, 8, 37]. In Indonesia, *M. oleifera* leaf is traditionally used to treat skin disorders like ringworm and rashes, beriberi, and gout [38]. The potential for its utilization could have been further developed, including as an antiviral agent. Research regarding the activity of *M. oleifera* leaf extracts against HIV has been conducted on the HIV-1RT enzyme, showing that both water and 50% methanol extracts exhibited inhibitory activity against the HIV-1 RT enzyme with $IC_{50}$ values of 9 µg/ml and 79 µg/ml, respectively [3]. However, the active compounds responsible for inhibiting the HIV-1 RT enzyme in *M. oleifera* leaf extracts have not been identified. Therefore, pharmacological network and molecular docking methods can be utilized to determine the active compounds. Nowadays, network pharmacology is widely used in the drug development and utilization process. Through this method, essential drug characteristics can be optimized and, speeding up and simplifying the drug discovery process [39].

First, screening for physicochemical properties was carried out using Lipinski's rule of five, an approach based on the principle of drug-likeness that relates to the bioavailability of oral drugs, including molecular weight, log P, and the number of hydrogen donors and acceptors [21, 40]. Molecular weight shows the size, density, and mass/volume of active compound, which modulates ligand binding [41]. Low molecular weight, typically 300-500 Da or less can facilitate efficient binding interactions [42, 43]. Small molecules possess high permeability and affect membrane transport. This membrane transport is also affected by Log-P, which determines whether compounds are lipophilic or lipophobic [43]. The H-bond donor and H-bond acceptor present in the structure of active compound also play a vital role in membrane transport,

protein-compound interaction, distribution, and solubility [41]. Based on our study there are 35 active compounds were obtained from *M. oleifera* that meet the rules (table 1) and have the potential for future analysis.

In addition, other crucial properties in drug discovery are related to absorption, distribution, metabolism, excretion, and toxicity. The causes of some failures in the discovery of new chemical entities (NCEs), accounting for 50%-60%, are poor ADMET properties and the occurrence of drug side effects. The bioavailability of a drug is determined by various factors, such as its ability to dissolve within the digestive system, its permeability across intestinal membranes, and its metabolic interactions in the liver. In the case of the majority of compounds, roughly 64% of their bioavailability is predominantly shaped by the process of intestinal absorption. Consequently, forecasting intestinal absorption serves as the initial stage in anticipating the bioavailability of these compounds [44]. The assessment of HIA involves a comparison between the amount reaching the vein and the oral dose, resulting in categorization as follows: HIA+(HIA ≥ 70%) denotes strong absorption, HIA±(30%<HIA<70%) indicates moderate absorption and HIA–(HIA ≤ 30%) signifies limited absorption [45]. In this study, an HIA threshold of>30% is used to obtain compounds with moderate and strong absorption, aiming for a favorable level of bioavailability. CYP2D6 is an enzyme that plays a key role in the metabolism of various drugs and chemical compounds within the human body. This enzyme is essential for drug metabolism and can influence on the effectiveness and safety of pharmaceuticals. Consequently, inhibiting CYP2D6 may raise the potential for toxicity. Hence, in this research, compounds that do not hinder the CYP2D6 enzyme were selected [46]. According to the Globally Harmonized System of Classification and Labelling of Chemicals (GHS), there are six acute toxicity classes. The choice of these toxicity classes from classes 4-6 is based on their classification as non-toxic, with LD50 values in class 4 ranging from 300 mg/kg to 2000 mg/kg, class 5 ranging from 2000 mg/kg to 5000 mg/kg, and class 6 exceeding>5000 mg/kg, all falling within the non-toxic category [15].

The interaction between active compound targets and diseases is constructed through protein-protein analysis, thus providing an overview of potential protein targets in HIV treatment. The HIV-1 life cycle is an intricate sequence of events that includes various stages, starting from the viral entry into the host cell and culminating in the assembly of new viral particles. At the core of this cycle is the activity of various viral proteins, notably reverse transcriptase, integrase, and protease enzymes. The process initiates with the attachment of the viral envelope protein, glycoprotein 120 (gp120), to the CD4 receptor on host cells. This is followed by the attachment to chemokine receptors (such as CCR5 or CXCR4) and subsequent merging of the viral and host cell membranes. The viral capsid containing the genome and enzymes then enters the cell, and undergoes transcription, integration, and translation, leading to the assembly of new viral particles [36]. In our study, we focused on the critical stage of transcription, particularly the action of the HIV-1 RT enzyme. This enzyme converts the single-stranded viral RNA into double-stranded DNA, making it a prime target for antiretroviral therapy.

Our network pharmacology and molecular docking analyses revealed two potential inhibitors of HIV-1 RT from *M. oleifera*: apigenin and kaempferol. Both compounds exhibited the lowest binding affinity compared to others (table 4). This binding affinity is associated with the amino interaction of both compounds in the hydrophobic pocket of the allosteric site, which contains the amino acids Leu A: 234, Leu A: 100, Lys A: 101, Lys A: 103, Val A: 106, Val A: 179, Phe A: 227, Trp A: 229, Tyr A: 188, and Tyr A: 181. These amino acids are essential in the HIV-1 RT binding site and these interactions are similar to the NNRTIs group, including nevirapine, efavirenz, etravirine, and rilpivirine [25]. These results align with the study conducted by Muruganantham [47], that showed apigenin from *Hybanthus enneaspermus* had the best binding affinity at-7.21 kcal/mol and interacted with the amino acid Lys: 102 through a hydrogen bond [47]. These results suggest their promising potential as anti-HIV agents.

Apigenin has antiviral activity, such as inhibiting several viruses, including enterovirus 71 (EV71), herpes simplex viruses HSV-1 and HSV-2, hepatitis C virus, and influenza virus. The inhibitory mechanism involves inhibiting viral replication by inhibiting intracellular ROS (reactive oxygen species) and disrupting cytokine regulation, resulting in apoptosis. Apigenin disrupts hepatitis C virus replication by interfering with the function of the viral internal ribosome entry sites (IRES) and preventing the activation of the N-terminal c-Jun (JNK) kinase, which is essential for viral replication triggered by EV71 [48]. In the HIV, apigenin contained in *Punica granatum* leaf extract exhibits HIV-1 RT-RNase inhibitor activity with an $IC_{50}$ value of 3.7±0.5 μM [49]. Apigenin compound was also tested on the HIV-1 RT enzyme and showing a high percentage of inhibition, at>100% and an $IC_{50}$ value of 8.5 μg/ml [50].

Kaempferol exhibits a wide range of pharmacological effects, including anti-inflammatory properties via blocking the NF-KB pathway and suppressing the release of pro-inflammatory cytokines, including IL-8 and TNF-alpha in cell line-based *in vitro* experiments. Apart from that, the anti-inflammatory mechanism of kaempferol also targets the Mitogen-Activated Protein Kinases (MAPK) activation pathway, thereby inhibiting the formation of inflammatory cytokines [51]. In addition, kaempferol isolated from *Securigera securidaca* has acted as an HIV-1 RT inhibitor with an $IC_{50}$ value of 50 μg/ml [52]. Kaempferol compound was also tested on HIV-1 RT enzyme and showed a good percentage of inhibition at 88.08 % and an $IC_{50}$ value of 41.4 μg/ml [50].

Apigenin and kaempferol, identified from *M. oleifera*, demonstrated inhibitory potential against HIV-1 RT. Apigenin exhibited anti-HIV activity by inhibiting the activation of BAX, CASP9, and CASP3, critical players in the apoptotic pathway [53, 54]. Additionally, both compounds displayed inhibitory effects on HIV-1 RT, a crucial enzyme in viral replication, by binding to the enzyme's active site. These findings suggest that *M. oleifera* possesses a dual mechanism against HIV, targeting both host cells and the virus itself [36].

Based on the results of GO and KEGG enrichment analysis (fig. 3 and 4), it appears that the pathway includes a fusion of virus membrane, apoptosis signaling pathway, virus receptor activity, protein domain specific binding, apoptosis pathway, and HIV-1 infection pathway. Apoptosis is a form of cell death that involves a series of activation processes, including intrinsic and extrinsic pathways The extrinsic pathway begins with activating apoptotic receptors on the cell surface, which belong to the TNF receptor family. The main pathways in the apoptosis process that have an important role in HIV infection include the Fas/FaL pathway, TNF-related apoptosis-inducing ligand (TRAIL), and TNF pathway. Meanwhile, the BCL2 gene family tightly regulates the intrinsic pathway, which consists of proteins that control the release of special factors and caspases from mitochondria. The main effect of this pathway is to release cytochrome-c from mitochondria, which forms a complex with Apoptotic Protease Activating Factor 1 (APAF1). This complex triggers the activation of CASP9, which then activates the executive caspase responsible for triggering the apoptosis process. HIV infection is characterized by viral proteins that have proapoptotic and antiapoptotic properties, including the proteins gp120, Tat, Vpr, Vpu, and Nef [55].

In infectious pathways such as cancer and viral infection, apigenin increases the sensitivity of TNF-alpha, which can cause apoptosis by inhibiting NFKB. The induction of the apoptosis process occurs through increased production of Reactive Oxygen Species (ROS) and calcium (Ca2+), elevating BAX levels and inhibiting BCL2 levels, changing the BAX/BCL2 ratio. This eventually leads to a decrease in mitochondrial membrane permeability, followed by the release of cytochrome C, AIF, and Endo G from mitochondria, and induces activation of CASP9 and CASP3 [54]. The apoptotic pathway through mitochondria is mostly caused by non-receptor disorders, including the presence of toxins, hyperthermia, hypoxia, oxidants, and viral infections, which can affect the permeability of the mitochondrial membrane, causing the pores of the mitochondria to open and release pro-apoptosis factors. Pro-apoptosis includes cytochrome-c, the Second mitochondria-derived activator of caspase (Smac)/direct IAP binding protein with low pI (DIABLO), and the serine protease HtrA2/Omi. This protein activates caspases, including CASP9, which ultimately causes cell apoptosis. Apart from activated caspases, there is activation of the BCL2 protein group, which plays a pro-apoptotic

role, such as BAX, and BCL10 [55]. This is followed by the activity of *M. oleifera* in signaling the apoptotic pathway, where the *M. oleifera* fraction inhibits the proliferation of the SCC15 cell line and causes apoptosis by involving the activation of CASP3 and BAX [56].

Our study sheds light on potential HIV-1 RT inhibitors derived from *M. oleifera*. However, it is crucial to recognize the study's limitations. The study's *in silico* approach heavily relies on computational predictions and molecular docking, necessitating validation through robust experimental investigations. Moreover, the study focused on a specific subset of compounds, yet *M. oleifera* encompasses a diverse array of compounds that may harbor further promising candidates for combating HIV. To mitigate these limitations, future research should encompass experimental validation of the identified compounds' inhibitory effects of HIV-1 RT, employing both *in vitro* and *in vivo* studies. Additionally, a deeper understanding of potential synergistic effects among compounds within the *M. oleifera* extract is vital for a thorough comprehension its anti-HIV properties.

## CONCLUSION

In summary, our study utilized a combination of network pharmacology and molecular docking analyses to shed light on the relationship between *M. oleifera* and the HIV-1 RT enzyme. The finding identified apigenin and kaempferol as potential marker compounds within *M. oleifera* with inhibitory effects on HIV-RT inhibitors.

These compounds display dual mechanisms against HIV. Firstly, *M. oleifera* activates the apoptosis signaling pathway and involves in HIV-1 infection signaling pathway on host cells. Secondly, it exhibits inhibitory activity on the HIV-1 RT enzyme, thwarting viral replication by hindering double-stranded DNA formation. These findings offer a valuable foundation for future research in developing natural HIV treatments, which should be further validated through *in vitro* and *in vivo* studies.

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## AUTHORS CONTRIBUTIONS

Conceptualization: MF, Formal analysis: MF, AM, F, WA, Methodology: MF, Writing–review and editing: MF, AM, F, WA

## CONFLICT OF INTERESTS

There is no conflict of interest in the study.

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