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LC-MS Identification of Bioactive Compounds from the Methanol Extract of *Veitchia merrillii* Seeds as Potential Antiviral Agents Targeting HIV-1 Protease, Integrase, and Reverse Transcriptase: An *In Silico* Study

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ABSTRACT

Human Immunodeficiency Virus type 1 (HIV-1) is one of the most dangerous viruses that attacks the immune system, causing acquired immune deficiency syndrome (AIDS). Enzymes such as protease (PR), integrase (IN), and reverse transcriptase (RT) are protein receptors that play crucial roles in the HIV replication process. *Veitchia merrillii* is a common ornamental plant in the Arecaceae family, with a high content of phenolic and flavonoid compounds. This study aimed to identify the compounds in the methanol extract of *V. merrillii* seeds that could be potential inhibitors of three receptors (protease, integrase, and reverse transcriptase) involved in HIV-1 replication. *V. merrillii* seed was extracted by maceration in methanol. Bioactive compounds in the extract were identified using ultra-performance liquid chromatography-mass spectrometry (UPLC-MS). The identified compounds were tested for their inhibitory activities against three HIV-1 enzymes using *in silico* studies. UPLC-MS analysis identified 39 compounds in the methanol extract of *V. merrillii* seeds. Molecular docking study revealed three compounds in the methanol extract of *V. merrillii* seeds, including epicatechin-3-*O*-gallate, 3-*O*-acetyl-16-α-hydroxydehydrotrametenol acid, and epigallocatechin gallate as potential inhibitors of three HIV-1 enzymes (protease, integrase, and reverse transcriptase) with binding affinities more stable than the control drugs. PASSOnline analysis found that these three potentially bioactive compounds have anti-HIV activity, thereby supporting the docking results. Further research, including *in vitro* and *in vivo* studies, are needed to substantiate the potential of this compound as an antiretroviral agent against HIV-1.

Keywords: Antiretroviral, Bioactivity, Human Immunodeficiency Virus-1, *Veitchia merrillii*, Molecular docking, Liquid Chromatography-Mass Spectrometry

Introduction

Veitchia merrillii (or *Adonidia merrillii*) commonly known as the "Christmas Palm," is a member of the Arecaceae family that is cultivated for its ornamental value.[1,2] Despite its popularity, the plant was placed on the IUCN Red List as a vulnerable species in 2020, due to the population decrease of the plant.[3] The fruits which grow in the leaf axils, are oval with a smooth surface, green when unripe, and bright red when ripe.[4] In addition to their ornamental use, much attention is being paid to the seeds of *V. merrillii* due to their unique phytochemical profile and bioactivities. Research has shown that the seeds of *V. merrillii* are rich in phenolic and flavonoid compounds, with strong antioxidant activity.[5]

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Phytochemical screening has identified a number of phytochemicals including tannins, alkaloids, flavonoids, triterpenoids, and saponins.[6] The seed oil contains essential fatty acids such as oleic, stearic, linoleic, palmitic, and myristic acids.[7] High-performance liquid chromatography (HPLC) profiling of the methanol, ethyl acetate, and aqueous extracts of the seeds confirmed the presence of various bioactive phenolic and flavonoid derivatives, including pyrogallol, gallic acid, vanillic acid, caffeic acid, syringic acid, rutin, and naringin.[8,9] An acute toxicity evaluation indicated a median lethal dose (LD_{50}) of 866.03 mg/kg, categorizing the extract as slightly toxic.[9,10] These findings collectively underscore the considerable phytochemical richness, antioxidant potential, and industrial significance of *V. merrillii* seeds, illustrating their promise as a source of natural bioactive compounds, while simultaneously highlighting the imperative for further research and conservation of this endangered palm species. The Human Immunodeficiency Virus (HIV) is recognized as one of the most dangerous viruses because of its capacity to progressively weaken the human immune system and lead to acquired immunodeficiency syndrome (AIDS) once left untreated.[11] The virus predominantly infects $CD4^+$ T-lymphocytes by binding to surface receptors via glycoproteins that facilitate viral entry and subsequent replication.[12] In 2020, over 39 million individuals globally were reported to be living with HIV.[13] The inability to eradicate AIDS mostly caused by the complexity of HIV pathogenesis and the critical role of viral enzymes in the replication process. Three enzymes; protease (PR), integrase (IN), and reverse transcriptase (RT), are crucial protein receptors that facilitate the completion of the virus's life cycle.[14-16] HIV protease (PR) cleaves viral

polyproteins into smaller functional proteins that assemble with the viral DNA to form mature, infectious particles.[14] Integrase (IN) facilitates the incorporation of viral DNA into host DNA, a crucial step for the virus's persistence within the host genome.[15] Retroviruses introduce nucleoprotein complexes into the cytoplasm of host cells during infection. These complexes have two copies of viral RNA and essential enzymes, including integrase (IN) and reverse transcriptase (RT). Subsequently, reverse transcriptase (RT) synthesizes a complementary DNA copy from the viral RNA.[16] This produces the double-stranded DNA required for integration. PR, IN, and RT are all essential for the viral replication cycle. Inhibition of any of these enzymes will prevent HIV proliferation, making them effective targets for therapeutic intervention.

Antiretroviral therapy (ART) is currently the primary method for treating HIV. It consists of combinations of drugs that inhibit essential viral enzymes, thereby preventing viral replication and reducing mortality risk.[21] The U.S. Food and Drug Administration (FDA) has sanctioned various antiretroviral therapy (ART) drugs that target specific stages of the replication cycle. Darunavir functions as a protease inhibitor, elvitegravir serves as an integrase inhibitor, and nevirapine acts as a reverse transcriptase inhibitor.[21–23] Antiretroviral therapy (ART) has significantly enhanced both the life expectancy and quality of life of HIV patients, transforming the disease into a manageable chronic condition. Nonetheless, prolonged usage may result in significant adverse effects, including an increased risk of cancer, metabolic disorders, and cardiovascular disease.[24–26] These limitations highlight the essential need for safer and more sustainable treatment alternatives that maintain efficacy while minimizing toxicity. This study aimed to discover the bioactive chemicals in the methanol extract of *V. merrillii* seeds as retroviral inhibitors of HIV-1. The identification of bioactive chemicals was conducted using ultra performance liquid chromatography-mass spectrometry (UPLC-MS) analysis because of its high sensitivity and ability to separate and identify molecules at minimal quantities. Molecular docking was employed to forecast the efficacy of *V. merrillii* seeds as an HIV-1 antiviral via molecular interaction simulations, serving as an effective preliminary step prior to further laboratory testing.

Materials and Methods

Plant collection and identification

Veitchia merrillii seeds were randomly collected from Gayungan District, Surabaya City, Indonesia (7°19′44″S; 112°43′35″E) in June 2023. The samples were identified at the School of Life Sciences and Technology, Bandung Institute of Technology, Bandung, Jawa Barat 40132, Indonesia. Herbarium specimen was deposited with voucher number B/51108/UN38.1.2/TU.00/2023.

Preparation of extract

The seeds were removed from their shell, and dried in the oven for 24 h. The dried seeds were blended into a fine powder. The powdered seeds (200 g) was extracted by maceration in 800 mL methanol (Merck, Germany) at room temperature for 24 h with three replications. The extract was filtered through a Buchner funnel connected to a vacuum pump. The filtrate was evaporated using a rotary vacuum evaporator (Rotavapor® R-300 Buchi, Swiss).

UPLC-QToF-MS analysis

Identification of bioactive compounds in the methanol extract of *V. merrillii* seeds was carried out using an Ultra-Performance Liquid Chromatography (UPLC) unit (LC: ACQUITY UPLC® H-Class System, Waters, USA) with C18 (1.8 µm 2.1 x 100 mm) (ACQUITY Premier HSS, Waters, USA) and a Mass Spectrometer (Xevo G2-S QTof, Waters, USA) at the Prime Police Cooperative (Primkopol) Central Forensic Laboratory of the Republic of Indonesia. The sample was dissolved with methanol (LC-MS Grade Merck, Germany) to prepare a 1000 mg/L solution. The sample (5 µL) was pipetted with a microsyringe, then injected into the UPLC column in four replicates. UPLC-MS analysis was performed by electrospray ionization (ESI) in positive mode. The ions produced were analyzed by the Quadrupole Time-of-Flight (Q-ToF) analyzer. The mobile phase consisted of a mixture of formic acid and water (0.1:99.9) and a mixture of formic acid and acetonitrile (0.1:99.9) at a flow rate of 0.2 mL/min. Compounds were identified by their peak areas with reference to FSTP-NUS library.

Molecular docking

Bioactive compounds from the methanol extract of *V. merrillii* seeds were used as ligands. FDA-approved antiretrovirals, darunavir (protease inhibitor),[27] elvitegravir (integrase inhibitor),[28] and nevirapine (non-nucleoside reverse transcriptase inhibitor),[29] served as controls. The 3D structures of the Ligands were retrieved from PubChem database and minimized using OpenBabel in PyRx (v0.8, 2011) to improve stability and flexibility,[30,31] then converted from *.sdf* to *.pdb* format. Receptor proteins involved in HIV-1 replication, protease (PDB ID: 3SO9),[32] integrase (PDB ID: 4NYF),[23] and reverse transcriptase (PDB ID: 1REV),[33] were obtained from RCSB web server, prepared with PyMOL (v1.5.7),[34] and their active sites identified using BIOVIA Discovery Studio (v4.5).[35] Docking coordinates were: PR (X = 12.5499, Y = −8.4349, Z = −8.3764), IN (X = 21.7696, Y = 63.6600, Z = 9.5478), and RT (X = 1.7994, Y = −37.1162, Z = 22.0755). Molecular docking was performed using AutoDock Vina via PyRx.[36] Compounds with lower binding affinities than the controls were further analyzed. Interactions were visualized in 2D and 3D using PyMOL and Discovery Studio.

Drug-likeness and toxicity prediction

Ligands were analyzed to determine their potential as a medicinal compound using Lipinski's rule of five. SCFBio is a web server that can be used to predict drug-likeness components (http://www.scfbio-iitd.res.in/software/drugdesign/lipinski.jsp).[37,38] The emerging analog design module generates a virtual chemical library and score each point of drug-likeness parameters. Lipinski's rule of five include molecular weight ≤ 500 Daltons, hydrogen bond acceptors ≤ 10, hydrogen bond donors ≤ 5, molar refraction between 40 and 130, and lipophilicity (log P) less than five.[38] Toxicity predictions were carried out using ProTox-II, a web-based server capable of producing very high accuracy (≥ 80%) and sensitivity (https://tox-new.charite.de/protoxII/).[39] Toxicity parameters used in this research included hepatotoxicity, carcinogenicity, immunotoxicity, and the expected median lethal dose (LD_{50}) prediction in mg/kg.[40]

PASSOnline prediction

The evaluation of potential compounds with HIV-1 antiviral activity was performed by the PASSOnline web server.[41] The examination results were reported in terms of the probabilities; Pa (probability of activity) and Pi (probability of inactivity), with a values ranging from 0 to 1.[42] A compound was predicted to have potential antiviral activity when the predicted Pa > 0.3 (moderate probability).[35]

Results and Discussion

Compounds identified from UPLC-MS analysis of V. merrillii seed extract

The bioactive compounds in *Veitchia merrillii* seeds were analyzed using Ultra-Performance Liquid Chromatography-Mass Spectrophotometry (UPLC-MS). UPLC-MS is an instrument that is used to identify components of a mixture based on the principle of separation by LC and identification by MS detector.[43] The mixture is injected with a mobile phase so that separation occurs in the column according to the polarity of the components. The MS ionizes the sample molecules and separates them according to their mass-to-charge ratios (m/z) using electric and/or magnetic fields.[44] The compound fragments are then analyzed using existing compound libraries to determine the compound identity in the extract. LC-MS is generally used for the analysis of large, ionic, polar, thermally unstable, and non-volatile compounds.[44] LC/MS data is used to provide information about the molecular weight, identity, structure, and relative amount of specific sample component.[45] The chromatogram of the LC analysis is presented in Figure 1, and data of the identified compounds are presented in Table 1. From the LC-MS data, 39 compounds were identified in the methanol extract of *V. merrillii* seeds, with the dominant compound being

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Table 1: Compounds identified from the UPLC-MS analysis of methanol extract of *Veitchia merrillii* seeds

Compound Name	RT (min)	Molecular Formula	Molecular Mass	Phytochemical Group	Percentage (%)
8-Hydroxy-5-*O*-*β*-*D*-glucopyranosylpsoralen	1.232	$C_{17}H_{16}O_{10}$	381.07825	Flavonoid	2.81
Kojic acid	1.851	$C_6H_6O_4$	143.0343	Phenolic	1.05
Flavone-*O*-glycosides	3.742	$C_{27}H_{26}O_{11}$	527.17462	Flavonoid	3.89
Procyanidin B2	3.742	$C_{30}H_{26}O_{12}$	579.15173	Flavonoid	4.28
3,4,5-Trimethoxycinnamic acid	4.270	$C_{12}H_{14}O_5$	239.21155	Phenolic	1.76
Catechin	4.487	$C_{15}H_{14}O_6$	291.22778	Flavonoid	2.15
Desaminotyrosine	5.521	$C_9H_{10}O_3$	167.0700	Phenolic	1.23
2,4,5-Trimethoxybenzaldehyde	5.977	$C_{11}H_{16}O_3$	197.1172	Phenolic	1.45
Epicatecin-3-*O*-gallate	5.977	$C_{22}H_{18}O_{10}$	443.1318	Flavonoid	3.27
2',4-Dihydroxychalcone	6.308	$C_{15}H_{12}O_3$	241.0687	Flavonoid	1.78
Naringenin-7-*O*-glucoside	6.329	$C_{21}H_{22}O_{10}$	435.1666	Flavonoid	3.21
Resveratrol	6.906	$C_{14}H_{12}O_3$	229.1411	Flavonoid	1.69
7-β-galactopyranosyl-oxycoumarin-4-acetic acid methyl ester	7.103	$C_{18}H_{20}O_{10}$	397.20773	Flavonoid	2.93
Apigenin	7.785	$C_{15}H_{10}O_5$	271.05875	Flavonoid	2.00
Epigallocatechin gallate	7.982	$C_{22}H_{18}O_{11}$	459.1304	Flavonoid	3.39
Formononetin	8.312	$C_{16}H_{12}O_4$	269.17554	Flavonoid	1.98
4-methylumbelliferyl glucuronide	8.396	$C_{16}H_{16}O_9$	353.22049	Flavonoid	2.61
Methyl 4-hydroxycinnamate	8.881	$C_{10}H_{10}O_3$	179.0706	Phenolic	1.32
Feruloyl quinic acid	9.191	$C_{17}H_{20}O_9$	369.2244	Flavonoid	2.72
2'-Hydroxy-2,4,4'-trimethoxychalcone	9.296	$C_{18}H_{18}O_5$	315.0872	Flavonoid	2.32
Gallocatechin	9.781	$C_{15}H_{14}O_7$	307.1874	Flavonoid	2.26
Pachymic acid	10.618	$C_{33}H_{52}O_5$	529.32477	Triterpenoid	3.91
3-*O*-Acetyl-16-α-hydroxydehydrotrametenolic acid	10.899	$C_{32}H_{48}O_5$	513.33002	Triterpenoid	3.79
3',7-Dimethoxy-3-hydroxyflavone	11.869	$C_{17}H_{14}O_5$	299.25922	Flavonoid	2.20
Piperlongumine	12.108	$C_{17}H_{19}NO_5$	318.30133	Phenolic	2.35
Gingerol	12.811	$C_{17}H_{26}O_4$	295.2272	Phenolic	2.18
Capensine	13.255	$C_{15}H_{16}O_5$	277.21835	Flavonoid	2.04
Naringenin chalcone	13.409	$C_{15}H_{12}O_5$	273.18573	Flavonoid	2.01
Naringenine	13.409	$C_{15}H_{12}O_5$	273.1668	Flavonoid	2.01
3-β-Androstanediol	14.330	$C_{19}H_{32}O_2$	293.2481	Steroid	2.16
Demethoxycurcumin	14.485	$C_{20}H_{18}O_5$	339.28998	Flavonoid	2.50
Isosilybin	14.619	$C_{25}H_{22}O_{10}$	483.40628	Flavonoid	3.57
(2E)-3-(3-Hydroxy-4-methoxyphenyl)-1-[4-(2-methyl-2-propanyl)phenyl]-2-propen-1-one	14.920	$C_{20}H_{22}O_3$	311.1636	Flavonoid	2.29
Hirsutrin	15.364	$C_{21}H_{20}O_{12}$	465.38159	Flavonoid	3.43
Rotenone	15.602	$C_{23}H_{22}O_6$	395.3909	Flavonoid	2.92
Dihydroflavonol + 2-*O*, 2Prenyl	16.397	$C_{25}H_{28}O_5$	409.3677	Flavonoid	3.02
Diferuloyl putrescine	16.481	$C_{24}H_{28}N_2O_6$	441.432	Phenolic	3.26
Tephrosin	16.925	$C_{23}H_{22}O_7$	411.38434	Flavonoid	3.03
Ferocaulicin	17.121	$C_{26}H_{30}O_6$	439.3789	Flavonoid	3.24

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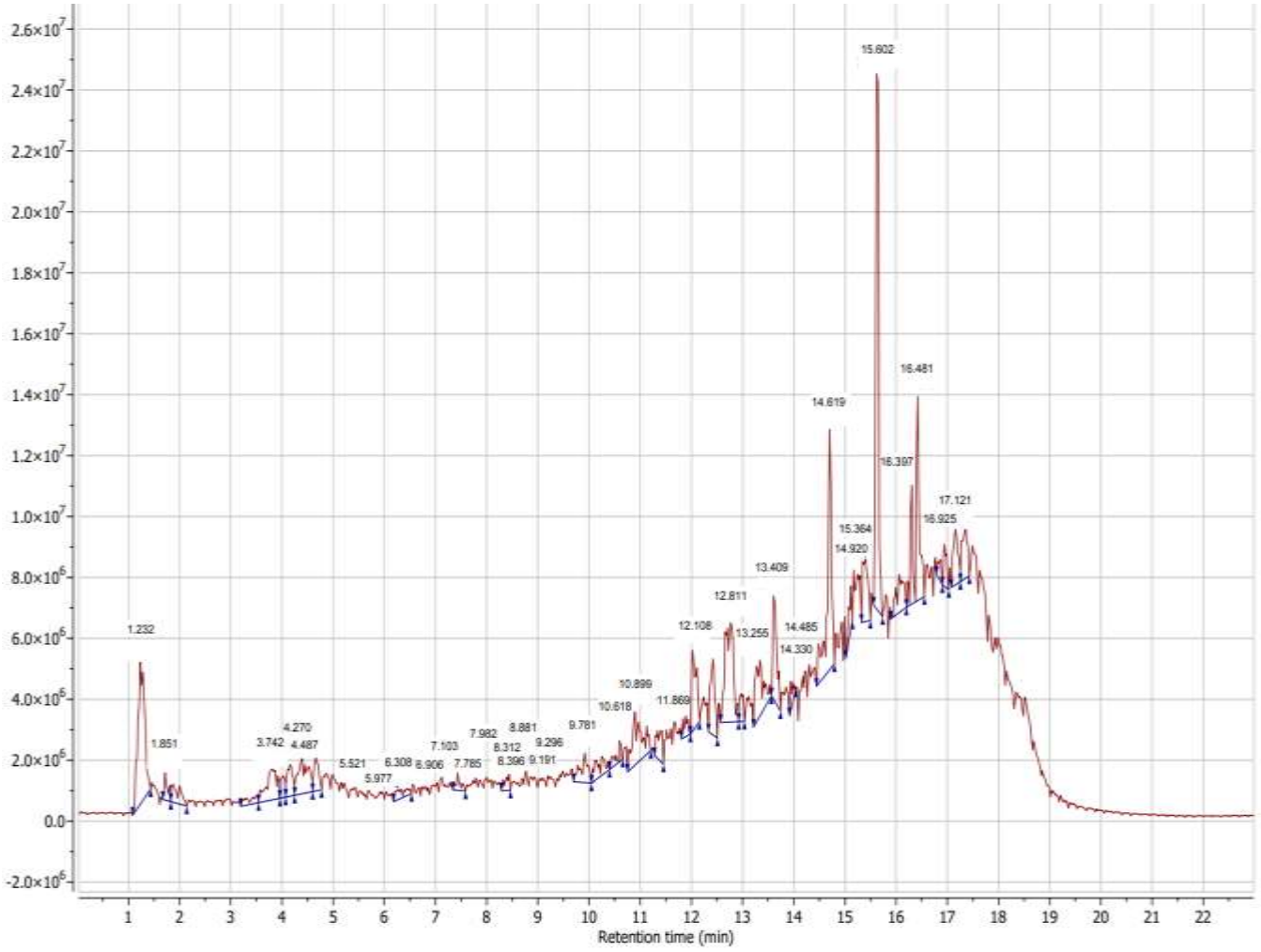

Figure 1: UPLC-MS Chromatogram of *Veitchia merrillii* seed methanol extract

procyanidin B2 (4.28%) at a retention time of 3.742 min. The compounds identified were 28 flavonoids, 8 phenolic compounds, 2 triterpenoids, and 1 steroid.

Molecular docking result

The potential antiretroviral activity of compounds identified from the UPLC-MS analysis were evaluated using molecular docking simulations. Molecular docking analysis is an *in silico* approach that utilizes modern computing technology to predict the bioactivity of a compound as a candidate of drug.[31] Molecular docking aims to interact a compound as a ligand with the active site of a receptor that play a role in a particular disease. In this study, the receptors used were HIV-1 protease (PR), HIV-1 integrase (IN), and HIV-1 reverse transcriptase (RT); these three receptors play a role in the replication mechanism of the HIV-1 virus.

Therefore, a promising antiretroviral drug candidate should possess inhibitory activity against all three receptors and work together to effectively prevent the replication of the HIV-1 virus. Molecular docking analysis result (Table 2) of the compounds identified in the methanol extract of *V. merrillii* seeds showed that 3 out of the 39 compounds identified had binding affinities more negative than the HIV-1 antiretroviral control drugs used for the three receptors. The PR-darunavir complex had a binding affinity of -9.3 kcal/mol; in the RT-nevirapine complex, the binding affinity was -9.7 kcal/mol; and the IN-elvitegravir complex had a binding affinity of -6.8 kcal/mol. In this study, a compound was regarded as having potential antiretroviral activity if it has a binding affinity lower than the control drugs at the three receptors. The three compounds with lower binding affinities than the three control drugs were epicatechin-3-*O*-gallate, 3-*O*-acetyl-16-*α*-hydroxydehydrotrametanolic acid, and epigallocatechin gallatepicatechin-3-*O*-gallate, with epicatechin-3-*O*-gallate having the lowest binding affinity at all three receptors. Thus, these three compounds have the potential to act as antiretrovirals against HIV-1 by inhibiting PR, RT, and IN in the replication process.

Interactions and visualization of the potential compounds were carried out to determine the type and position of interaction formed between the proteins and the ligands. The visualization results (Figure 2a-c) showed that various types of interactions were formed, namely favorable interactions, including hydrogen bonds, electrostatic bonds, hydrophobic bonds, and halogen bonds, as well as unfavorable interactions. Hydrogen bonds are strong bonds formed from the interaction of hydrogen atoms with nitrogen (N), oxygen (O), and fluorine (F) atoms.[46] Hydrophobic bonds are interactions between hydrophobic groups such as carbon chains and benzene rings.[47] Electrostatic bonds are strong bonds due to the difference in charge. Halogen bonds are interactions between halogen atoms (as Lewis acids) and donor atoms (Lewis bases).[48] On the other hand, unfavorable bonds are repulsive interactions caused by interference such as steric interference, similarity of charge, and others.[49] Subsequently, the similarity of interactions between control drugs and the potential antiretroviral compounds was analyzed. The similarity of inhibitory activity of potential bioactive compounds with control drugs was supported by similar amino acid residues.

In the PR-ligand complex (Figure 2a), it was found that the compound epicatechin-3-*O*-gallate has five amino acid residues, namely; Ala:A 28, Ile:A 50, Val:B 84, Ala:B 28, and Ile:B 50 in common with darunavir, while 3-*O*-acetyl-16-α-hydroxydehydrotrametenolic acid has six similar amino acid residues with the control

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Table 2: Binding affinity of *Veitchia merrillii* bioactive compounds with three HIV-1 receptors

No.	Ligand	Binding Affinity (kcal/mol)		
		Protease	Reverse Transcriptase	Integrase
1.	Darunavir (Control)	-9.3	-	-
2.	Nevirapine (Control)	-	-9.7	-
3.	Elvitegravir (Control)	-	-	-6.8
4.	8-Hydroxy-5-*O*-β-*D*-glucopyranosylpsoralen	-7.7	-8.1	-7
5.	Kojic Acid	-8.4	-8.7	-4.7
6.	Flavone-*O*-glycosides	-8.8	-10.3	-7
7.	Procyanidin B2	-7.9	-9.7	-7.3
8.	3,4,5-Trimethoxycinnamic acid	-6.2	-7	-5.5
9.	Catechin	-7.7	-9.2	-7.8
10.	Desaminotyrosine	-5.7	-5.5	-5.6
11.	2,4,5-Trimethoxybenzaldehyde	-9.1	-11.9	-5
12.	Epicatecin-3-*O*-gallate	-9.5	-9.9	-8.2
13.	2,4-Dihydroxychalcone	-7.7	-9	-6.9
14.	Naringenin-7-*O*-glucoside	-10	-8.8	-7.7
15.	Resveratrol	-6.9	-8.1	-6.8
16.	7-β-galactopyranosyl-oxycoumarin-4-acetic acid methyl ester	-9.9	-7.9	-7.5
17.	Apigenin	-8	-8.7	-7.8
18.	Epigallocatechin gallate	-9.5	-9.8	-7.9
19.	Formononetin	-8.4	-10.7	-7.6
20.	4-methylumbelliferyl glucuronide	-8.3	-8.5	-7.5
21.	Methyl 4-hydroxycinnamate	-8.2	-7.7	-5.6
22.	Feruloyl quinic acid	-7.7	-8.9	-6.6
23.	2'-Hydroxy-2,4,4'-trimethoxychalcone	-7.4	-7.7	-6.8
24.	Gallocatechin	-8	-9	-7.6
25.	Pachymic acid	-8.4	-12.1	-7.7
26.	3-*O*-Acetyl-16-α-hydroxydehydrotrametenolic acid	-9.4	-9.8	-7.8
27.	3',7-Dimethoxy-3-hydroxyflavone	-8.2	-9.5	-7.6
28.	Piperlongumine	-7.4	-7.5	-6.6
29.	Gingerol	-8.1	-10.1	-6.2
30.	Capensine	-7.6	-8,8	-7
31.	Naringenin chalcone	-9.6	-8.9	-6.7
32.	Naringenine	-4.9	-5.2	-7.8
33.	3-β-Androstanediol	-9.4	-8.8	-7.5
34.	Demethoxycurcumin	-8.7	-9.7	-6.8
35.	Isosilybin	-5.3	-6	-8
36.	(2E)-3-(3-Hydroxy-4-methoxyphenyl)-1-[4-(2-methyl-2-propanyl)phenyl]-2-propen-1-one	-8.7	-9.7	-7.7
37.	Hirsutrin	-8	-10	-7.7
38.	Rotenone	-8.1	-8.8	-7.5
39.	Dihydroflavonol + 2-*O*, 2Prenyl	-8.6	-9.4	-7.1
40.	Diferuloyl putrescine	-6.1	-7.5	-5.9
41.	Tephrosin	-7.7	-9.4	-6.8
42.	Ferocaulicin	-7.9	-9.7	-7.7

these amino acids include Ile:A 50, Ala:B 28, Ala:A 28, Ile:B 50, Pro:B 81, and Val:B 84, lastly, epigallocatechin gallate has five amino acid residues, namely; Ile:A 50, Ala:B 28, Val:B 84, Ile:B 50, and Ala:A 28 in common with the control. The 3D visualization is presented in Figure 3a.

In the RT-ligand complex (Figure 2b), the compound epicatechin-3-*O*-gallate was found to have five amino acid residues, including Glu:A 170, His:A 171, Gln:A 168, Lys:A 173, and Gln:B 95 similar to the control (Nevirapine). For the compound 3-*O*-acetyl-16-α-hydroxydehydrotrametenolic acid, three amino acid residues, including Gln:B 95, His:A 171, and Lys:A 173 were in common with that of the control, while epigallocatechin gallate has three similar amino acid residues, including Gln:B 95, Glu:A 170, and Gln:B 95 with the control. The 3D visualization is presented in Figure 3b.

In the IN-ligand complex (Figure 2c), it was found that epicatechin-3-*O*-gallate has the amino acid residues Lys:A 101, Tyr:A 318, Val:A 179, Lys:A 103, Val:A 106, Leu:A 100, and Tyr:A 318 similar to the control (Elvitegravir), while 3-*O*-acetyl-16-α-hydroxydehydrotrametenolic acid has the following amino acid residues; Tyr:A 318, Lys:A 103, Leu:A 100, Val:A 106, Val:A 179, and Tyr:A 181 similar to the control, and epigallocatechin gallate has the amino acid residues Lys:A 101, Val:A 106, Tyr:A 318, Leu:A 100, and Val:A 179 similar to the control. The 3D visualization is presented in Figure 3c.

This similarity in amino acid residues supports the validation that the potential bioactive ligands has comparable retroviral inhibitory activity as the control drugs.[50] The control drugs inhibit the receptor at its active site by inhibiting critical amino acid residues that bind specific ligands for its metabolic function. When compounds have high similarities in their amino acid residues, their activity will also be similar. The type of interaction affects the stability of the formed complex, which also influences the binding affinity. A stable complex is a complex with a large number of favorable interactions and few unfavorable bonds. The bonds formed between the receptor and ligand also influence the stability of the complex. The stability of the complex, and inhibitory activity increase as more interactions are formed.[35,51–53]

Drug-likeness and toxicity of test ligands

The potential bioactive compounds were analyzed for Lipinski's drug-likeness profile and toxicity to support its potential as an HIV-1 antiretroviral drug candidate. Lipinski drug-likeness analysis is carried out via the SCF-Bio web server to determine the potential of a compound as a medicinal compound.[54] Lipinski's Rule of five helps distinguish between drug-like and non-drug-like molecules.[51] A molecule is said to be drug-like according to Lipinski's Rule of Five if

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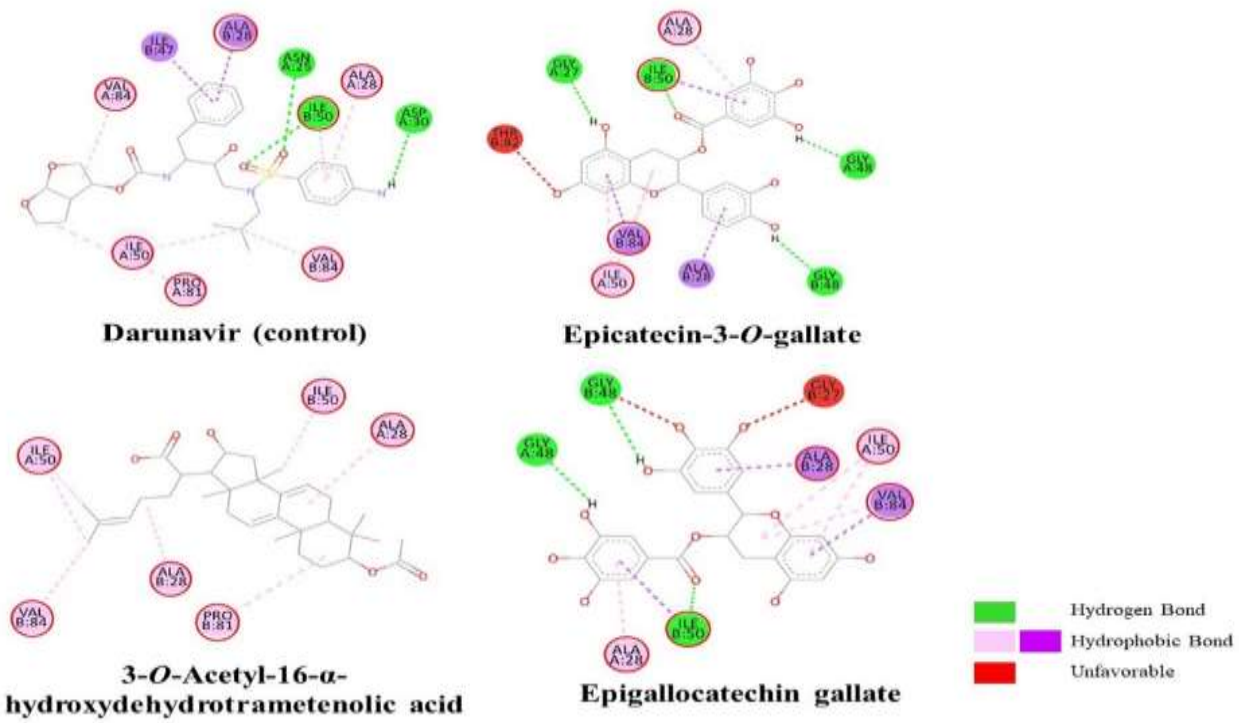

Figure 2a: 2D visualization of protease-ligands complexes

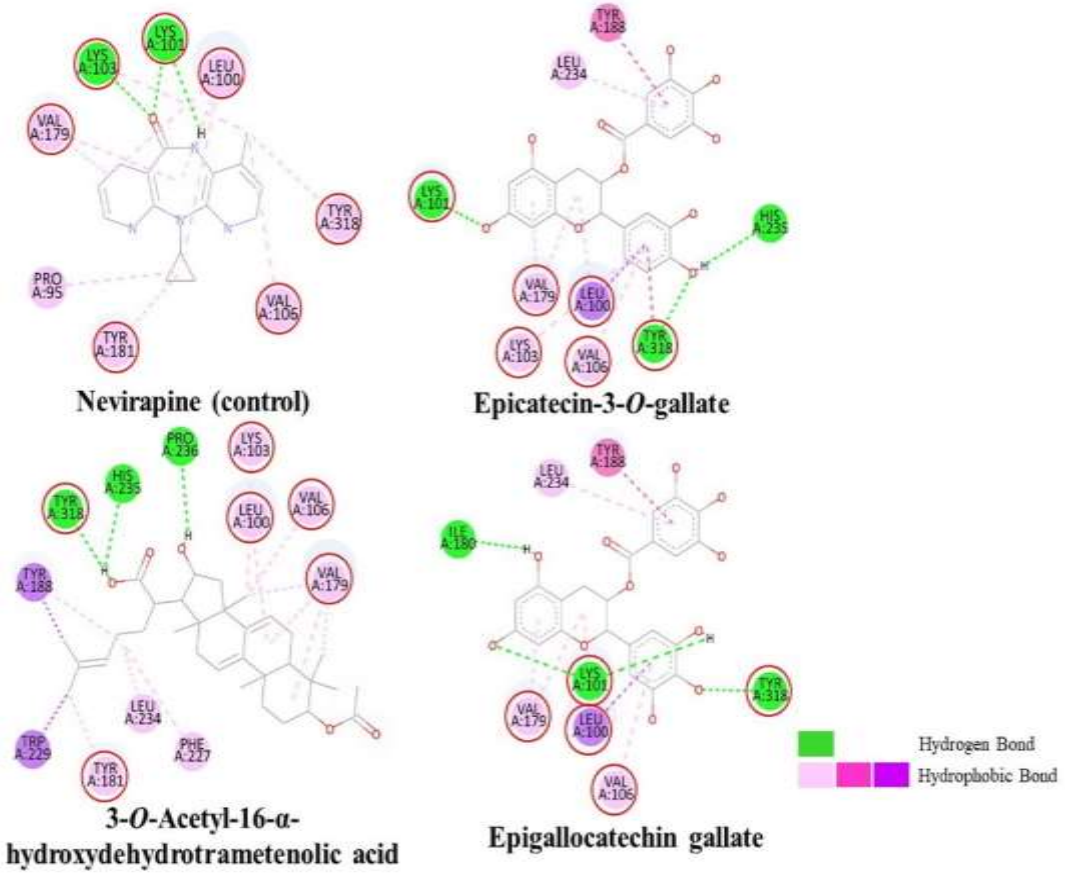

Figure 2b: 2D visualization of reverse transcriptase-ligands complexes

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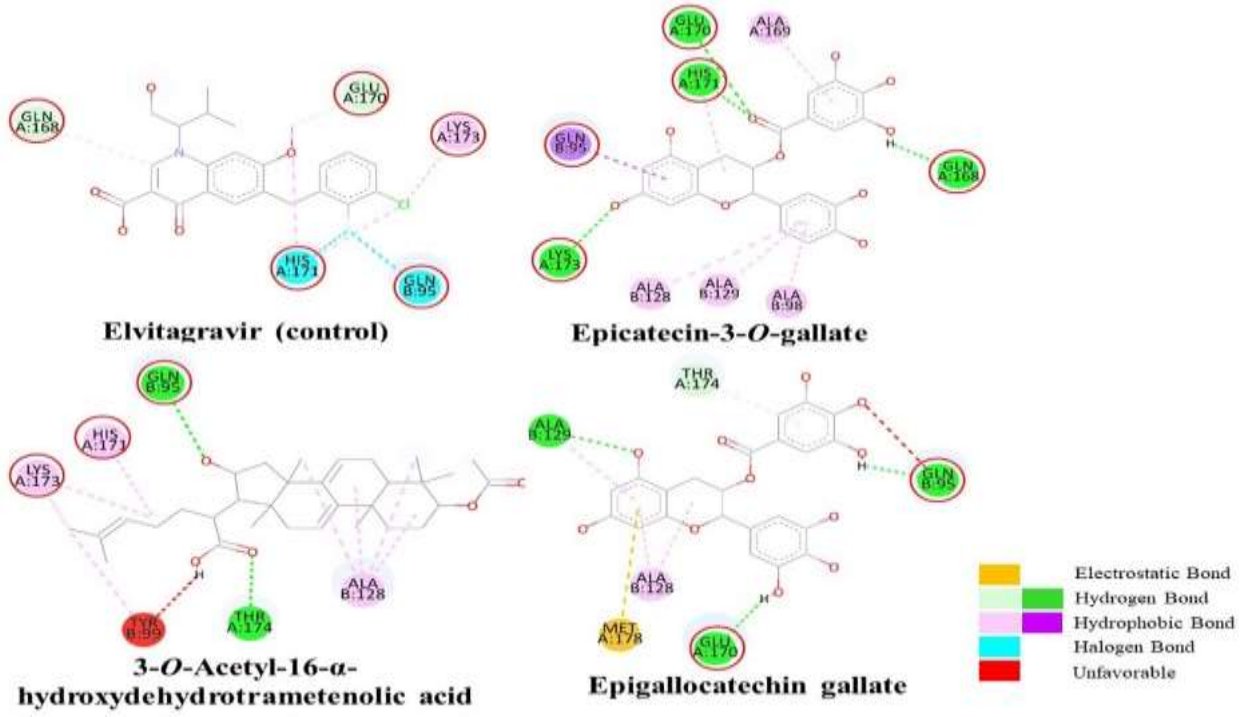

Figure 2c: 2D visualization of integrase-ligands complexes

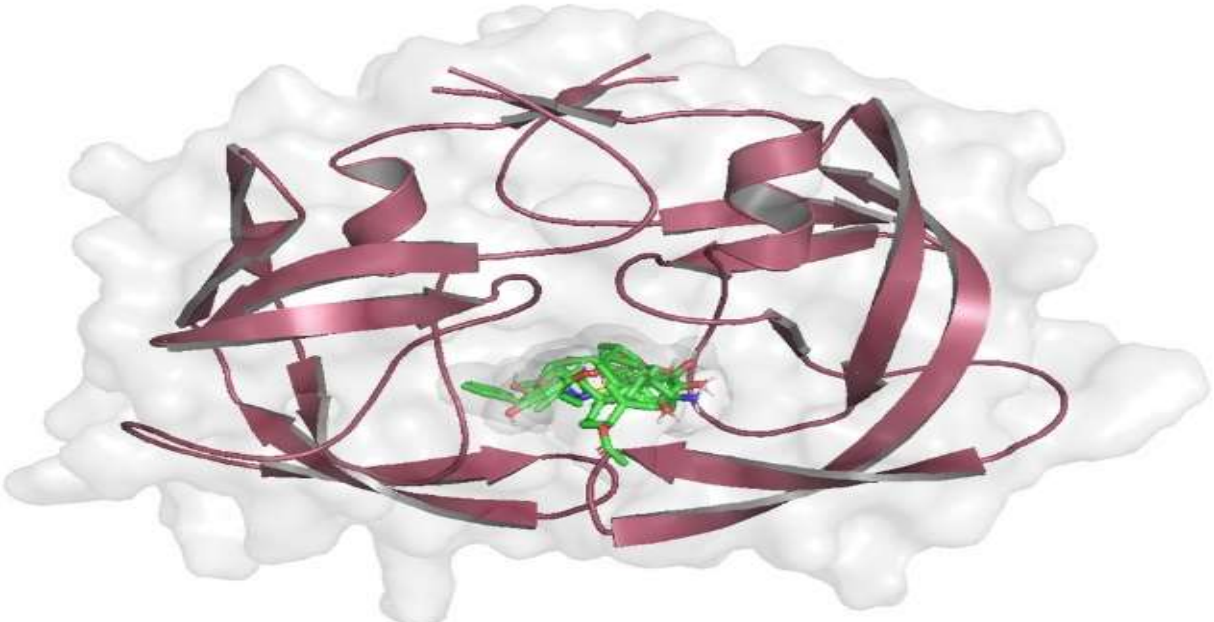

Figure 3a: 3D visualization of protease-ligands complexes

it meets 3 or more of Lipinski's 5 rules. Lipinski's rule of five guidelines were used to evaluate the drug-likeness of three compounds that were selected from molecular docking study.

The results as shown in Table 3 indicated that the three compounds possess the potential as drug candidates, as they complied with at least three of Lipinski's requirements. Furthermore, the toxicity of the three compounds was predicted using the ProTox-II server to determine their toxic profiles.[39] The toxicity parameters chosen in this study were hepatotoxicity, carcinogenicity, immunotoxicity, and LD_{50} prediction. Hepatotoxicity is the degree to which a substance can cause liver damage.[53] Carcinogenicity is a complex, uncontrolled process of abnormal cell growth and differentiation that can lead to cancer.[52] Immunotoxicity is hypersensitivity, decreased immunity, and uncontrolled immune cell proliferation due to the administration of a substance. LD_{50} is acute toxicity parameter, which measures the lethal effects within a short time of administering a single dose of a chemical.[40,55]

The results of the toxicity prediction (Table 3) show that two out of the three selected potentially active compounds were predicted not to have hepatotoxic, carcinogenic, and immunotoxic properties, thus possessing a good profile as a drug. In addition, these two compounds had predicted LD_{50} in class 4. These two compounds were epicatechin-3-*O*-gallate and epigallocatechin gallate. The remaining one potentially active compound, namely; 3-*O*-Acetyl-16-α-hydroxyhydrotrametenolic acid was predicted to have carcinogenic and immunotoxic properties

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Table 3: Lipinski's rule of five (Drug-likeness) and toxicity prediction

Parameter	Epicatecin-3-*O*-gallate	3-*O*-Acetyl-16-α-hydroxydehydrotrametenolic acid	Epigallocatechin gallate
Molecular Weight (g/mol)	442	466	458
Hydrogen bond donor	7*	2	8*
Hydrogen bond acceptor	10	5	11*
Lipophilicity (LOG P)	2.527600	6.861401*	2.233201
Molar refractivity	107.256042	145.684647*	108.920845
Drug-likeness Lipinski	Yes (1 violation)	Yes (2 violations)	Yes (2 violations)
Hepatotoxicity	Inactive	Inactive	Inactive
Carcinogenicity	Inactive	Active	Inactive
Immunotoxicity	Inactive	Active	Inactive
LD_{50} (mg/kg)	1000	5000	1000

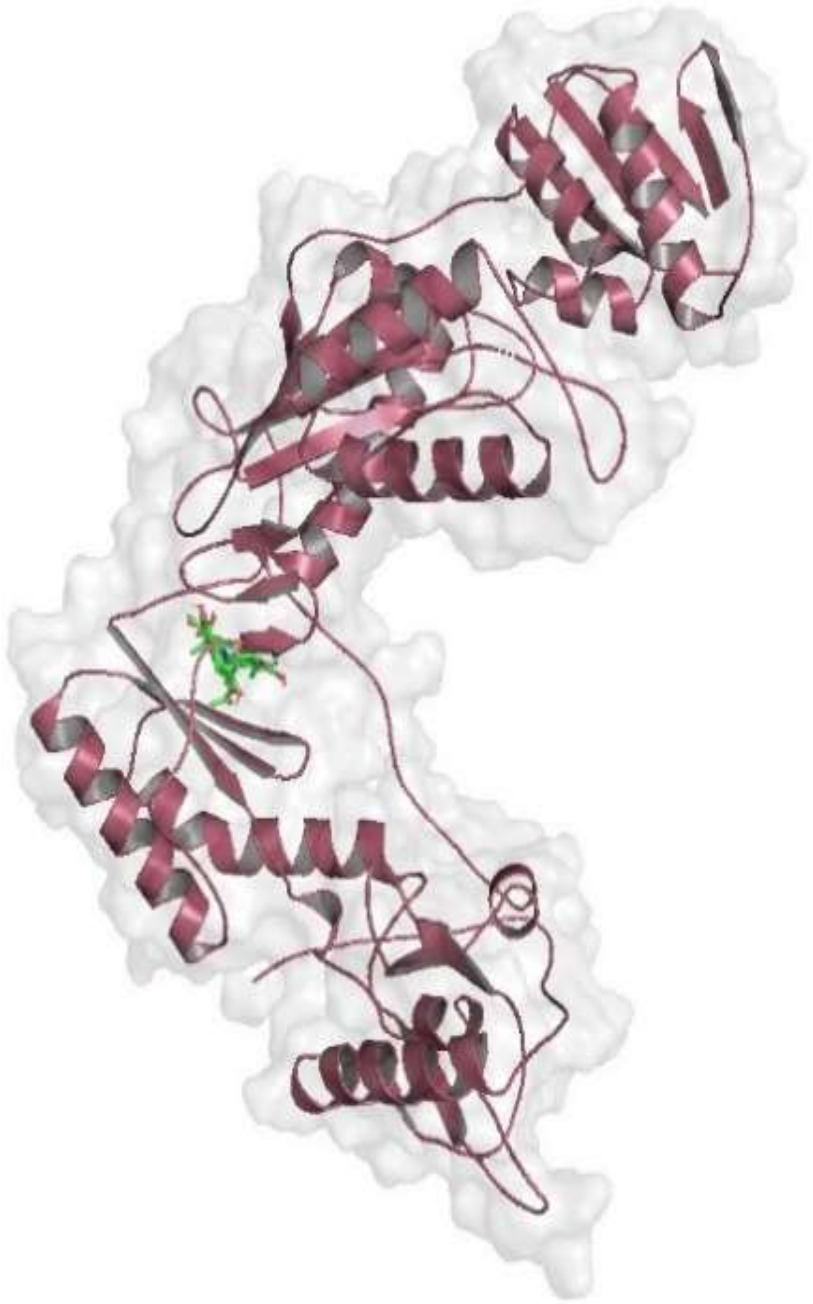

Figure 3b: 3D visualization of reverse transcriptase-ligands complexes

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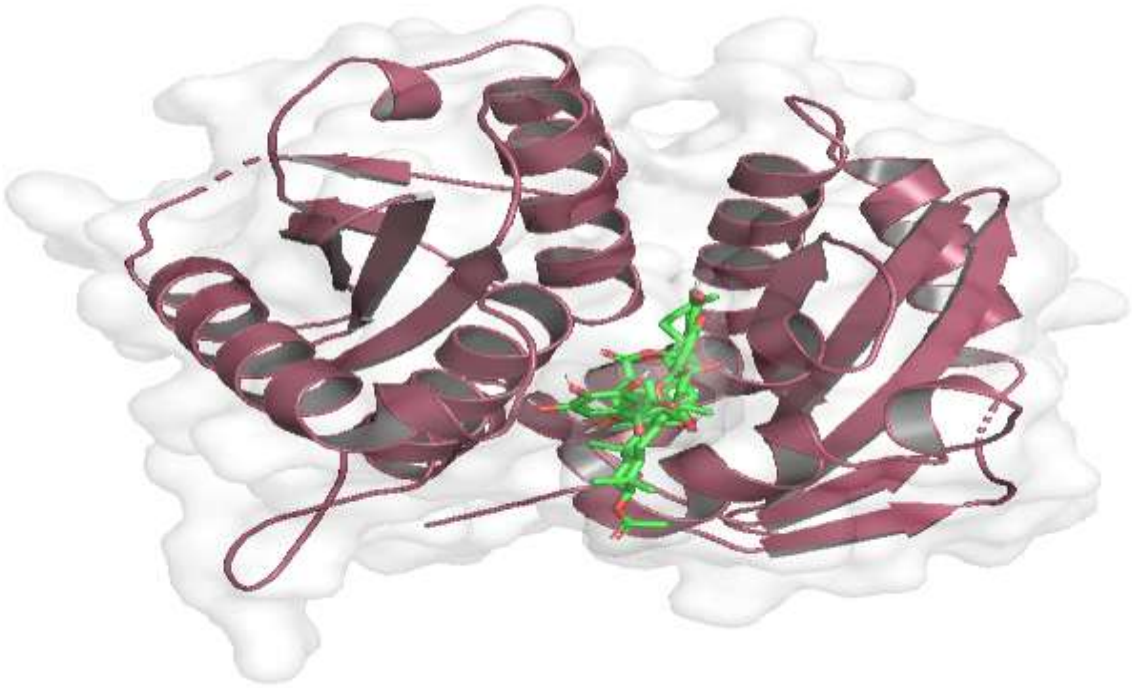

Figure 3c: 3D visualization of integrase-ligands complexes

and has a predicted LD_{50} in class 5, thus eliminating its potential as a good drug candidate. Based on these results, epicatechin-3-*O*-gallate and epigallocatechin gallate were selected as potential antiretrovirals against HIV-1 with good drug-likeness and toxicity profiles.

PASSOnline prediction

The potential bioactive compounds with good profile as medicinal agents were analyzed for their possibility as HIV-1 antivirals via PASSOnline server. PASSOnline is a predictive tool used to determine the bioactivity of a compound when it enters the body.[41] The results of the analysis are presented as the possibility of being active (Pa) and the possibility of being inactive (Pi). The Pa and Pi values predict a compound's potential to have certain bioactivity in the body. A Pa value of more than 0.7 is included in the high category, while a Pa value of more than 0.3 is included in the medium category.[35] The analysis results (Table 4) showed that the two potential bioactive compounds with good profiles as medicinal compounds have Pa values that support them as antiretroviral drug candidates for HIV-1.[56]

Table 4: PASS-Online Prediction

Potential Compound	Pa	Pi	Biological Activity
Epicatechin-3-O-gallate	0.284	0.010	Antiviral (HIV)
	0.235	0.007	HIV-1 integrase (3'-Processing) inhibitor
	0.206	0.007	HIV-1 integrase inhibitor
	0.190	0.009	HIV-1 integrase (Strand Transfer) inhibitor
	0.039	0.027	HIV-1 protease inhibitor
Epigallocatechin gallate	0.300	0.008	Antiviral (HIV)
	0.243	0.006	HIV-1 integrase (3'-Processing) inhibitor
	0.214	0.006	HIV-1 integrase inhibitor
	0.198	0.008	HIV-1 integrase (Strand Transfer) inhibitor
	0.041	0.023	HIV-1 protease inhibitor

The findings from this study suggest that *V. merrillii* seed extract, particularly its notable constituents; epicatechin-3-*O*-gallate and epigallocatechin gallate have antiviral properties against HIV-1 by inhibiting three receptors (PR, IN, and RT). The drug-likeness and toxicity profiles suggest that these compounds hold promise as oral medication. Furthermore, the medicinal potential of the plant is supported by its reported biological activity, including antioxidant,[5] anthelminthic,[57] and anticancer activities.[58] It is important to note that research on antivirals derived from *V. merrillii* remains scarce, highlighting the significance of the present study, and the potential for development of natural antiviral agents from *V. merrillii* seeds.

Conclusion

The findings from the present study show that *V. merrillii* seeds have potential as an antiviral agent against HIV by inhibiting the three main receptors of the HIV-1 virus, with the most potent compounds being epicatechin-3-*O*-gallate and epigallocatechin gallate out of the 39 compounds identified by UPLC-MS analysis of the plant extract. Drug-likeness and PASSOnline analysis provided good predictions of the selected compounds as good antiviral drug candidates against HIV-1. Further *in vitro* and *in vivo* studies should be conducted to provide clarity on the potential of *V. merrillii* seeds as an antiviral agent against HIV-1.

Conflict of interest
The authors declare no conflicts of interest.

Authors' Declaration
The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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