Antiretroviral activity from elderberry (*Sambucus nigra* L.) flowers against HIV-2 infection *via* reverse transcriptase inhibition: a viroinformatics study

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Abstract

HIV-2 infection is a unique concern with fewer cases than HIV-1, but it poses a high mortality rate due to its resistance to all HIV-1 antiretroviral treatments. This study focuses on one type of antiretroviral, reverse transcriptase (RT) inhibitors, as they play an important role in HIV-2 replication. The screening of potential HIV-2 antiretroviral candidates was carried out using compounds from elderberry (*Sambucus nigra* L.) flower extract. There is a lack of research on the antiviral potential of elderberry flower extracts, particularly in HIV-2; therefore, this study is important to explain the molecular mechanism underlying the potential of elderberry (*Sambucus nigra* L.) flower extracts to inhibit RT activity in HIV-2 through bioinformatics simulations. This study uses the *in silico* method, involving sample preparation in the database, drug-like molecular prediction through the server, molecular docking simulation, chemical bond interaction analysis, and three-dimensional structure visualization. Isorhamnetin has the most negative binding affinity of -9.9 kcal/mol compared to other compounds. It interacts with the HIV-2 RT domain at residues Trp4(B), Pro25(B), Asn137(B), Pro133(B), Gln23(B), Pro140(B), Leu21(B), Ile90(A), Thr131(B), Asn57(B), Arg22(B), and Glu89(A) with hydrophobic bond interactions. Hydrogen bond interactions are formed at the positions of Ser134(B), Gly141(B), and Thr88(A). Isorhamnetin from elderberry (*Sambucus nigra* L.) flower extract could be a potential HIV-2 antiretroviral candidate because it has the most negative binding affinity and the formation of hydrophobic hydrogen bond interactions on the RT domain.

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Introduction

HIV is an RNA virus that infects/attacks lymphocyte cells in the body, causing a decrease in the body's system of recognition.[1] HIV-2, sharing similarities with HIV-1 in its envelope, is predominantly found in West Africa but carries the potential to become a global epidemic.[2] Approximately 1 to 2 million people are living with minimally treated HIV-2, resulting in high mortality and morbidity. The virus, initially identified in 2018, has affected an estimated 38 million people worldwide.[3] In 2020, globally, an estimated 37.7 million people were living with HIV and 1.5 million became newly infected with HIV.[4] The Ministry of Health of Indonesia in 2020 reported that only 14% were known to be virally suppressed after 6 months of ART.[5] HIV-2 is endemic in several West African countries, namely Guinea-Bissau, Senegal, Cape Verde, Gambia, Mali, Sierra Leone, Cote d'Ivoire, and Nigeria, with a prevalence rate of 1%. Beyond West Africa, cases have also surfaced in South America, Europe, Asia, and the United States.[6] In Indonesia, the death rate due to HIV/AIDS is still high.[7] The Ministry of Health of the Republic of Indonesia stated that in Indonesia, in the last 12 years (data for 2010-2022, for those aged over 15 years), projections of new infections have shown a positive trend, namely from 56,187 new cases to 25,740.[8]

Patients with HIV/AIDS experience various problems, both physical and psychological, one of which is health-related quality of life.[9] People living with HIV require a focus on their quality of life to prevent deterioration.[10] Treatment for patients diagnosed with HIV-2 involves antiretrovirals, comprising a combination of nucleoside reverse transcriptase (RT) inhibitors, integrase strand transfer inhibitors, and protease inhibitors.[11] However, the virus poses a challenge by demonstrating resistance to all three types of antiretrovirals commonly used for HIV-1 treatment. While RT inhibitors have proven effective against HIV-1, their impact on HIV-2 replication is negligible. Consequently, there is a pressing need to screen new inhibitor candidates to develop treatments for HIV-2. Despite exhibiting a lower viral load compared to HIV-1, HIV-2 has developed resistance to commonly used antiretrovirals for HIV-1 treatment.[11] As an enveloped virus with double-stranded RNA (dsRNA) genetic material, HIV-2 encodes several enzymes such as RT, protease, and integrase.[12] This study specifically focuses on RT due to its pivotal role in HIV-2 replication.[13] The RT enzyme in HIV-2 serves a similar function to HIV-1, facilitating the formation of viral complementary DNA (cDNA) that integrates into the host cell genome.[2] Reported mutations of RT in HIV-2 result in resistance to specific antiretroviral types, notably non-nucleoside RT inhibitors (NNRTIs).[13] The resistance pattern positions the RT enzyme as the ideal target for designing antiretrovirals to combat HIV-2 by inhibiting its enzymatic activity.

Elderberry (*Sambucus nigra* L.) has been utilized in alternative medicine due to its potential as an antiviral, antidiabetic, antibacterial, antitumor, and antioxidant agent.[14] Polyphenolic compounds in the plant are also predicted to inhibit virus replication.[15] The flower of elderberry (*Sambucus nigra* L.) is rarely used as an alternative medicine; however, it contains several chemical compounds. These include quinic acid, caffeoylquinic acid, 1-caffeoylquinic acid, coumaroylquinic acid, feruloylquinic acid, isorhamnetin, quercetin-3-rutinoside, quercetin-acetyl glucoside, kaempferol rutinoside, and isorhamnetin acetylhexoside.[16] Previous studies have shown that flower extracts from elderberry (*Sambucus nigra* L.) can inhibit gram-positive bacteria such as *Staphylococcus aureus* and *S. epidermidis*.[17] Currently, there is a lack of research exploring the potential of flower extracts from elderberry (*Sambucus nigra* L.) as an antiviral, especially for HIV-2. This research is important for understanding the molecular mechanism underlying the potential of elderberry (*Sambucus nigra* L.) flower extracts to inhibit RT activity in HIV-2 using bioinformatics simulations.

Materials and Methods

Sample preparation

This study used compounds extracted from elderberry (*Sambucus nigra* L.) flowers. Ten compounds from *Sambucus nigra* L. flower were selected from the chromatographic results and their data presence in the PubChem database. These compounds are quinic acid, caffeoylquinic acid, 1-caffeoylquinic acid, coumaroylquinic acid, feruloylquinic acid, isorhamnetin, quercetin-3-rutinoside, quercetin-acetyl glucoside, kaempferol rutinoside, and isorhamnetin acetylhexoside, as ligands.[16] Information such as compound identification, simplified molecular input line entry system canonical, formula, and structure data format files were obtained from PubChem (https://pubchem.ncbi.nlm.nih.gov/). Minimization of ligands was performed using OpenBabel v2.3.1 software to convert files into protein databank format (PDB). The target chosen in this study was RT HIV-2 from RCSB PDB (https://www.rcsb.org/) with ID 1MU2 (https://www.rcsb.org/structure/1mu2), obtained in PDB format. Subsequently, water molecules and native ligands were removed through PyMOL software v.2.5.2 (Schrödinger, Inc., USA) with an academic license.[18,19]

Drug-like molecule prediction

The objective of drug-like molecule prediction is to identify the similarity of properties in chemical compounds from elderberry flower extract (*Sambucus nigra* L.) with those found in drug molecules. In this study, the drug-like molecule prediction method refers to the Lipinski rule of five through the SCFBio server (http://www.scfbio-iitd.res.in/software/drugdesign/lipinski.jsp). The rules include molecular mass, high lipophilicity, molar refractivity, and acceptor-donor hydrogen bonds. For a compound to be categorized as a drug-like molecule, it must meet at least two of these rules.[20,21]

Molecular docking simulation

In this study, molecular docking simulations are conducted to predict the ligand binding strength of elderberry flower extract (*Sambucus nigra* L.) on HIV-2 RT. Molecular docking refers to the interaction between the ligand and the target, resulting in the formation of a molecular complex with a stable bond, and the output of this process is the binding affinity. Binding affinity is the energy generated in the ligand-protein complex, which is characterized by a negative value. The more negative the value, the higher the probability of triggering specific activities, such as inhibition.[22] The PyRx 0.9.9 software (Scripps Research, USA) uses a screening docking method, covering the entire target surface with a directed grid and selecting ligands based on their more negative binding affinity energy.[23]

Chemical interaction

Chemical bonding interactions are formed in ligand-protein complexes, consisting of both hydrophobic and hydrogen bonds. These weak bonds collectively contribute to the stability of drug binding to the target and can subsequently trigger specific activities, such as inhibition. The software used to identify the position and type of chemical bond interactions in this study is LigPlot+v.2.2.[24,25]

Structural visualization

Three-dimensional visualization of docking results was displayed through PyMOL software v.2.5.2 (Schrödinger, Inc., USA) with an academic license. Protein structures were displayed in the form of cartoons and transparent surfaces with publication standards, ligands were displayed through stick structures with coloring based on C, H, N, O, and F atoms.[26]

Results

In this study, we revealed that the compounds retrieved from the database include quinic acid, caffeoylquinic acid, 1-caffeoylquinic acid, coumaroylquinic acid, feruloylquinic acid, isorhamnetin, kuersetin-3-rutinoside, kuersetin-acetyl glucoside, kaempferol rutinoside, and isorhamnetin acetylhexoside (Table 1). Next, the two-dimensional structures of all compounds of elderberry (*Sambucus nigra* L.) flower extract are shown in Figure 1. Then, the druglikeness prediction results indicated two compounds not

meeting the criteria for drug-like molecules, while eight compounds received positive predictions as drug-like molecules (Table 2). Ligands with the most negative binding affinity values are predicted to trigger inhibitory activity on the target. The molecular docking simulation results indicated that isorhamnetin has the most negative binding affinity of -9.9 kcal/mol compared to other compounds (Table 3). Three-dimensional structures on ligands and proteins from docking results are visualized with transparent surfaces, cartoons, and sticks using specific coloring selections (Figure 2). The analysis of the position and type of interaction in the ligand-protein complex revealed that isorhamnetin interacts with HIV-2 RT domain at residues Trp4(B), Pro25(B), Asn137(B), Pro133(B), Gln23(B), Pro140(B), Leu21(B), Ile90(A), Thr131(B), Asn57(B), Arg22(B), and Glu89(A) through hydrophobic bond interactions. Hydrogen bond interactions are formed at the positions of Ser134(B), Gly141(B), and Thr88(A) (Figure 3).

Discussion

Elderberry (*Sambucus nigra* L.) extract shows potential as an antiviral for treating influenza infection.[27] Recent research indicates that flower extracts from elderberry (*Sambucus nigra* L.) can be used for cold flu symptoms, such as pain, fever, cough, and congestion.[28] The antiviral activity of elderberry (*Sambucus nigra* L.) was demonstrated *in vitro* at 400 µg/mL, inhibiting DENV-2 repli-

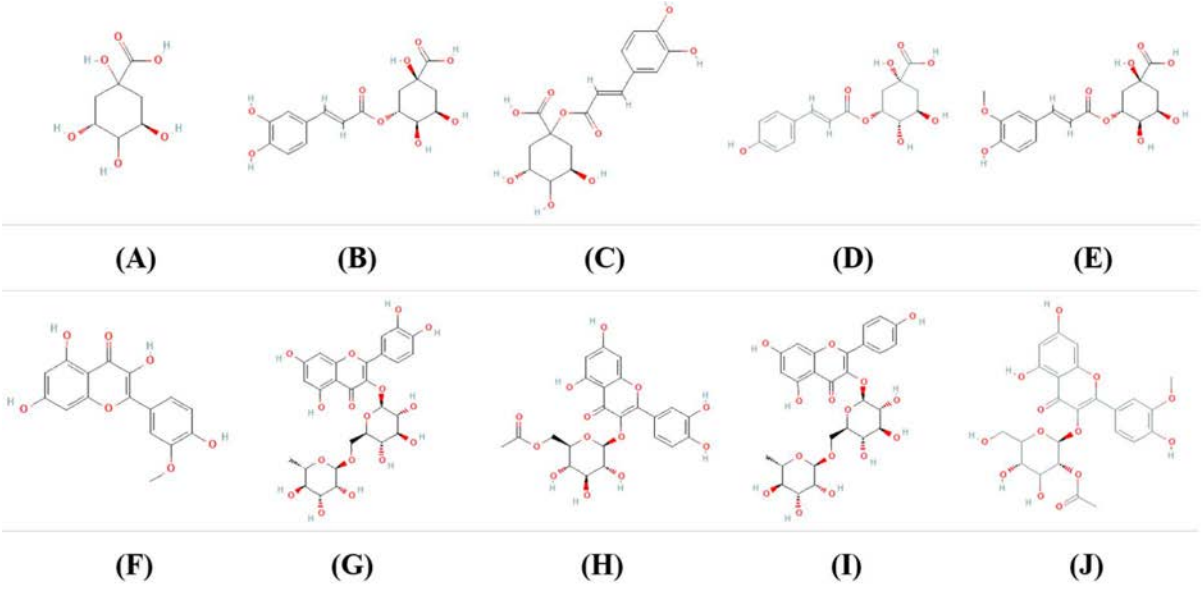

Figure 1. Elderberry (*Sambucus* nigra L.) flower extract compounds from database. A) Quinic acid; B) caffeoylquinic acid; C) 1-caffeoylquinic acid; D) coumaroylquinic acid; E) feruloylquinic acid; F) isorhamnetin; G) quercetin-3-rutinoside; H) quercetin-acetyl glucoside; I) kaempferol rutinoside; J) isorhamnetin acetylhexoside.

Table 1. Ligand retrieval from PubChem.

No	Compounds	CID	SMILE Canonical	Formula
1.	Quinic acid	6508	C1C(C(C(CC1(C(=O)O)O)O)O)O	$C_7H_{12}O_6$
2.	Caffeoylquinic acid	1794427	C1C(C(C(CC1(C(=O)O)O)OC(=O)C=CC2=CC(=C(C=C2)O)O)O)O	$C_{16}H_{18}O_9$
3.	1-Caffeoylquinic acid	10155076	C1C(C(C(CC1(C(=O)O)O)OC(=O)C=CC2=CC(=C(C=C2)O)O)O)O)O	$C_{16}H_{18}O_9$
4.	Coumaroylquinic acid	9945785	C1C(C(C(CC1(C(=O)O)O)OC(=O)C=CC2=CC=C(C=C2)O)O)O	$C_{16}H_{18}O_8$
5.	Feruloylquinic acid	9799386	COC1=C(C=CC(=C1)C=CC(=O)OC2CC(CC(C2O)O)(C(=O)O)O)O	$C_{17}H_{20}O_9$
6.	Isorhamnetin	5281654	COC1=C(C=CC(=C1)C2=C(C(=O)C3=C(C=C(C=C3O2)O)O)O)O	$C_{16}H_{12}O_7$
7.	Quercetin-3-rutinoside	5280805	CC1C(C(C(C(O1)OCC2C(C(C(C(O2)OC3=C(OC4=CC(=CC(=C4C3=O)O)O)C5=CC(=C(C=C5)O)O)O)O)O)O)O)O	$C_{27}H_{30}O_{16}$
8.	Quercetin-acetyl glucoside	10006384	CC(=O)OCC1C(C(C(C(O1)OC2=C(OC3=CC(=CC(=C3C2=O)O)O)C4=CC(=C(C=C4)O)O)O)O)O	$C_{23}H_{22}O_{13}$
9.	Kaempferol rutinoside	5318767	CC1C(C(C(C(O1)OCC2C(C(C(C(O2)OC3=C(OC4=CC(=CC(=C4C3=O)O)O)C5=CC=C(C=C5)O)O)O)O)O)O)O	$C_{27}H_{30}O_{15}$
10.	Isorhamnetin acetylhexoside	44259375	CC(=O)OC1C(C(C(OC1OC2=C(OC3=CC(=CC(=C3C2=O)O)O)C4=CC(=C(C=C4)O)OC)CO)O)O	$C_{24}H_{24}O_{13}$

CID, compound identification; SMILE, simplified molecular input line entry.

cation.[29] The plant's polyphenolic compounds are also predicted to have inhibitory effects on virus replication.[15] This study is important to reveal the potential of elderberry (*Sambucus nigra* L.) flower extract as an antiviral for HIV-2. The compounds retrieved from the database include quinic acid, caffeoylquinic acid, 1-caffeoylquinic acid, coumaroylquinic acid, feruloylquinic acid, isorhamnetin, kuersetin-3-rutinoside, kuersetin-acetyl glucoside, kaempferol rutinoside, and isorhamnetin acetylhexoside. The two-dimensional structures of all compounds of elderberry (*Sambucus nigra* L.) flower extract are shown.

Table 2. Drug-like molecule properties.

No	Compounds	Molecular mass	LogP	HBD	HBA	Molar refractivity	Probable
1.	Quinic Acid	192.000	-2.321	5	6	39.839	Drug-like Molecule
2.	Caffeoylquinic Acid	354.000	-0.645	6	9	82.518	Drug-like Molecule
3.	3-p-Coumaroylquinic acid	354.000	-0.645	6	9	82.518	Drug-like Molecule
4.	Coumaroylquinic Acid	338.000	-0.351	5	8	80.853	Drug-like Molecule
5.	Feruloylquinic Acid	368.000	-0.342	5	9	87.405	Drug-like Molecule
6.	Isorhamnetin	316.000	2.313	4	7	78.937	Drug-like Molecule
7.	Quercetin-3-rutinoside	610.000	-1.878	10	16	137.495	Non drug-like Molecule
8.	Quercetin-acetyl glucoside	506.000	-0.159	7	13	115.821	Drug-like Molecule
9.	Kaempferol rutinoside	594.000	-1.584	9	15	135.830	Non drug-like Molecule
10.	Isorhamnetin acetylhexoside	520.000	0.143	6	13	120.708	Drug-like Molecule

HBD, hydrogen bond donor; HBA, hydrogen bond acceptor.

Table 3. The binding affinity score.

Compounds	CID	Target PDB ID	Binding affinity (kcal/mol)
Quinic acid	6508	1MU2	-6.8
Caffeoylquinic acid	1794427	1MU2	-8.6
3-p-Coumaroylquinic acid	10155076	1MU2	-7.9
Coumaroylquinic acid	9945785	1MU2	-8.5
Feruloylquinic acid	9799386	1MU2	-7.3
Isorhamnetin	5281654	1MU2	-9.9
Quercetin-acetyl glucoside	10006384	1MU2	-8.0
Isorhamnetin acetylhexoside	44259375	1MU2	-9.3

CID, compound identification; PDB, protein databank format.

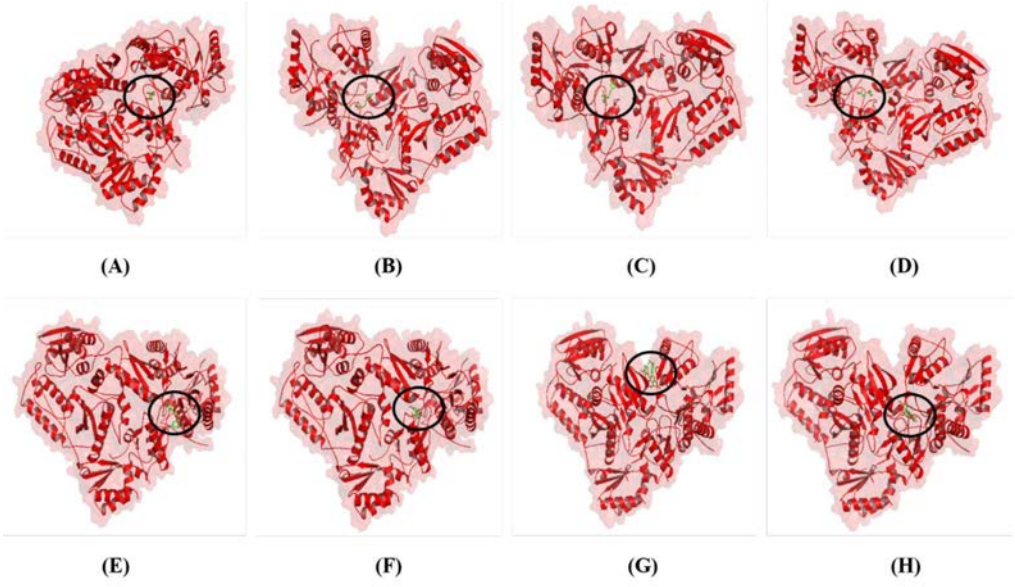

Figure 2. Structural visualization from the molecular docking simulation. A) Quinic acid_RT HIV-2; B) caffeoylquinic acid_RT HIV-2; C) 3-p-coumaroylquinic acid_RT HIV-2; D) coumaroylquinic acid_RT HIV-2; E) feruloylquinic acid_RT HIV-2; F) isorhamnetin_RT HIV-2; G) quercetin-acetyl glucoside_RT HIV-2; H) isorhamnetin acetylhexoside_RT HIV-2. RT, reverse transcriptase.

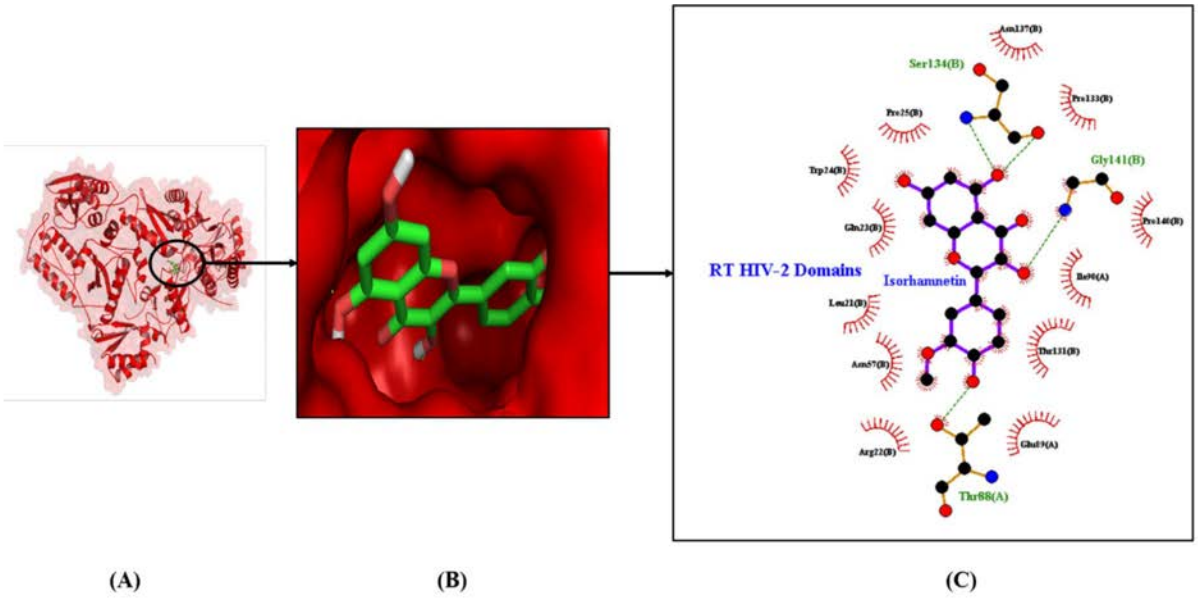

Figure 3. Molecular interaction analysis of isorhamnetin on RT HIV-2 domains. A) Isorhamnetin_RT HIV-2; B) isorhamnetin pocket binding cave; C) two-dimensional plot of protein-ligand molecular interactions. RT, reverse transcriptase.

The prediction of druglikeness in compounds from elderberry (*Sambucus nigra* L.) flower extract aimed to identify the physicochemical characteristics of a drug-like molecule. The prediction uses Lipinski's rules of five, which stipulate that compound with drug-like molecule characteristics must fulfill at least two rules.[30] The druglikeness prediction results indicated two compounds not meeting the criteria for drug-like molecules, while eight compounds received positive predictions as drug-like molecules. Compounds exhibiting drug-like molecule characteristics are predicted to induce biological activity and possess the ability to penetrate the cell membrane, reaching targets within the cytoplasmic environment.[31]

Molecular docking aims to identify how ligands interact with the target domain.[32] In this study, grid docking is used with the position set at Center (Å) X: 2.746, Y: -25.031, Z: 16.706, Dimensions (Å) X: 105.798, Y: 83.933, Z: 106.561 to orient the ligand on the target. The ligand consists of compounds from elderberry (*Sambucus nigra* L.) flower extract characterized as a drug-like molecule, and the target is RT HIV-2 (PDB ID: 1MU2). A screening docking method is employed to identify or screen the ligand activity on the target with the most negative binding affinity.[33] Ligands with the most negative binding affinity values are predicted to trigger inhibitory activity on the target. The molecular docking simulation results indicated that isorhamnetin has the most negative binding affinity of -9.9 kcal/mol compared to other compounds. A more negative binding affinity value signifies a stronger influence produced by ligand binding on the target.[34] Isohamnetin from elderberry (*Sambucus nigra* L.) flower extract is predicted to be a good antiretroviral candidate through inhibition of HIV-2 RT activity. Three-dimensional structures on ligands and proteins from docking results are visualized with transparent surfaces, cartoons, and sticks using specific coloring selection.

The molecular interactions formed in the docking result complex consist of hydrogen and hydrophobic bonds.[35] Both bonds contribute to the strength of the interaction and the stability of the drug molecule.[36] The analysis of the position and type of interaction in the ligand-protein complex revealed that isorhamnetin interacts with the HIV-2 RT domain at residues Trp4(B), Pro25(B), Asn137(B), Pro133(B), Gln23(B), Pro140(B), Leu21(B), Ile90(A), Thr131(B), Asn57(B), Arg22(B), and Glu89(A) through hydrophobic bond interactions. Hydrogen bond interactions are formed at the positions of Ser134(B), Gly141(B), and Thr88(A). Isorhamnetin is predicted to interact through hydrogen bonding and hydrophobicity on the RT domain of HIV-2, potentially triggering inhibitory activity on the target. The predicted mechanism of HIV-2 RT inhibitors in this study refers to NNRTIs in HIV-1 by targeting a hydrophobic pocket for inhibition of polymerization reactions.[37] In contrast to HIV-1, HIV-2 has mutations in RT with positions K65R, K70E, L74V, Q151M, M184I/V; RT mutations in HIV-2 trigger significant antiretroviral resistance compared to HIV-1.[11] The position of amino acid residues in the pocket binding domain of isorhamnetin from elderberry (*Sambucus nigra* L.) has a type of hydrophobic bond interaction at the position of amino acid residues that do not include mutation regions, allowing isorhamnetin to be predicted as a potential HIV-2 RT inhibitor.

Conclusions

Isorhamnetin from elderberry (*Sambucus nigra* L.) flower extract shows promise as a potential HIV-2 antiretroviral candidate because it has the most negative binding affinity and the formation of hydrophobic hydrogen bond interactions on the RT domain. We recommend positions on the HIV-2 RT consisting of Trp4(B), Pro25(B), Asn137(B), Pro133(B), Gln23(B), Pro140(B), Leu21(B), Ile90(A), Thr131(B), Asn57(B), Arg22(B), Glu89(A), Ser134(B), Gly141(B), and Thr88(A) as potential targets for designing future HIV-2 drug candidates.

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