Triple Inhibitor Mechanism of Antiretroviral from Sambucus nigra Phytochemical through Screening Docking

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ABSTRACT

Human immunodeficiency virus type 1 (HIV-1) has a higher infectious nature than HIV-2 because of the genetic drift mechanism. Several HIV-1 strains such as A, B, C, D, E, F, G, H, & J were obtained from Africa, Asia, Europe, Australia, and America. Endemic cases of HIV-1 are often found and HIV-2 is very rare, therefore the development of antiretroviral drugs is currently focused on treating HIV-1 infection5. Research on HIV-1 vaccines is still in the research phase and some patients are only taking antiretrovirals to survive. *Sambucus nigra* L. is used by the community for traditional medicine such as influenza A, B, C and SARS-CoV-2 infectious diseases. There are no research reports related to the potential of *Sambucus nigra* L. as an antiretroviral in the treatment of HIV-1 infection and the specific molecular mechanisms that explain it, therefore this research is important for the discovery of alternative antiretroviral drugs from natural materials. The in silico method used in this study consists of *Sambucus nigra* L. compounds retrieval, HIV-1 protein preparation, molecular docking, and 3D visualization. *Sambucus nigra* L. can have inhibitory activity on three enzymes of HIV-1 such as protease (PR), reverse transcriptase (RT), integrase (INT) through naringenin and Cyanidin-3,5-diglucoside compounds. Both compounds have more negative binding energy, form stable binding interactions in the target domain, and trigger inhibitory activity.

Keywords: Antiretroviral, HIV-1, *In Silico*, Molecular Docking, *Sambucus nigra*

INTRODUCTION

Human immunodeficiency virus type 1 (HIV-1) from the retrovirus family can trigger opportunistic diseases of the immune system or AIDS[1,2]. In these conditions the immune system fails due to infection with bacteria, protozoa, fungi, and other viruses. The identified types of HIV consist of HIV-1 & HIV-2, HIV-1 has a higher infectious nature than HIV-2 because of the genetic drift mechanism[3,4]. Several HIV-1 strains such as A, B, C, D, E, F, G, H, & J were obtained from Africa, Asia, Europe, Australia, and America[5,6]. HIV-1 has structural genes consisting of env, gag, and pol then essential elements & regulators such as rev, tat, nef, vpr, vpu, and vif that play a role in helping the virus to evade the immune system's response and pathogenesis in host cells[7.8].

Endemic cases of HIV-1 are often found and HIV-2 is very rare, therefore the development of antiretroviral drugs is currently focused on treating HIV-1 infection[9,10]. Research on HIV-1 vaccines is still in the research phase and some patients are only taking antiretrovirals to survive.

HIV-1 has several enzymes consisting of reverse transcriptase (RT), integrase (INT), & protease (PR), all of which play an important role in the replication process[11,12]. Reverse transcriptase plays a role in the formation of HIV-1 cDNA in several stages consisting of lysyl tRNA having a binding site, RT then adds a nucleotide to synthesize cDNA to the non-coding or U5 and R (repeat) region of the viral RNA[13,14]. The RT region known as RNAse H degrades U5 and R at the 5' end, then tRNA primers synthesize DNA strands. HIV-1 cDNA is carried by integrase to integrate with the host cell genome. Integrase enzymes play a role in the integration of HIV-1 cDNA into the host cell genome and protease enzymes cut viral polypeptides into peptides for the assembly process[15,16]. RT, INT, and PR activity has a crucial role in viral replication and is very likely to be a target in drug design.

Sambucus nigra L. has health benefits consisting of antidiabetic, anticancer, and antiviral[17,18]. The content of anthoxyanins in the flowers of Sambucus nigra L. is used by the community for traditional medicine such as influenza A, B, and C infectious diseases, the compound works as an antiviral through the mechanism of inhibiting replication activity, immunomodulatory, fever reduction, and anti-inflammatory[19,20]. *Sambucus nigra* L was reported to be an alternative treatment in cases of SARS-CoV-2 infection since 2019. There are no research reports related to the potential of *Sambucus nigra* L. as an antiretroviral in the treatment of HIV-1 infection and the specific molecular mechanisms that explain it, therefore this research is important for the discovery of alternative antiretroviral drugs from natural materials.

METHOD
Compound Retrieval
The chemical compounds of *Sambucus nigra* L. ligands in this study consist of Cyanidin-3-sambubioside, Cyanidin-3,5-diglucoside, Catechin, Epicatechin, Naringenin, and Quercetin. PubChem (https://pubchem.ncbi.nlm.nih.gov/) is used to retrieve data on ligands such as CID, molecular weight (g/mol), formula, and 3D structure with *structure data format* (sdf) files. Minimization of ligands and conversion to

protein databank (pdb) format were done through OpenBabel software to increase ligand flexibility[21,22].

Protein Preparation
This study uses targets from HIV-1 such as protease (PR) for polypeptide cutting, reverse transcriptase (RT) as reverse transcription of cDNA formation, and integrase (INT) which plays a role in the integration of HIV-1 cDNA into the host genome. All target samples were obtained from the RCSB PDB database (https://www.rcsb.org/) and then removed water molecules and native ligands through PyMol v2.5 software for optimization and docking preparation[23,24].

Molecular Docking
Docking aims to identify the ability of ligand binding to the target by referring to the binding affinity value and interaction pattern on the target domain. The type of docking used in this study is blind to screen the inhibitory activity of *Sambucus nigra* L. on all targets. Autogrid settings are done by covering the entire surface of the target to direct ligand binding which consists of centers and dimensions. Molecular docking in this study was performed by PyRx v.1.0.0 software with Vina Wizard plugin[25,26].

3D Visualization
The molecular complex with highest negative binding affinity value was displayed through PyMol v2.5 software with a 3D structure. The visualization method used in this study is structural selection and coloring based on C, H, and O. PyMol plays a role in molecular visualization of three-dimensional structures in proteins and chemical compounds through cartoons, surfaces, sticks, lines, and ribbons[27,28].

RESULTS AND DISCUSSION
Information such as name, CID, molecular weight (g/mol), formula, and links on chemical compounds from *Sambucus nigra* L. consisting of Cyanidin-3-sambubioside, Cyanidin-3,5-diglucoside, Catechin, Epicatechin, Naringenin, and Quercetin have been obtained from the PubChem database (Table 1). Previous research revealed that *Sambucus nigra* L can have potential as an antiviral, antibacterial, antitumor, and antidiabetic, the plant has polyphenol and lectins compounds that are strongly suspected to act as antivirals[29]. Extracts from *Sambucus nigra* L. can reduce viral load and suppress the spread of viral infection based on in vitro study data.

Table 1. *Sambucus nigra* L. compounds from database

Compound	CID	Molecular Weight (g/mol)	Formula	Link
Cyanidin-3-sambubioside	3084569	616.9	$C_{26}H_{29}ClO_{15}$	https://pubchem.ncbi.nlm.nih.gov/compound/3084569
Cyanidin-3,5-diglucoside	44256718	611.5	$C_{27}H_{31}O_{16}^{+}$	https://pubchem.ncbi.nlm.nih.gov/compound/44256718
Catechin	9064	290.27	$C_{15}H_{14}O_{6}$	https://pubchem.ncbi.nlm.nih.gov/compound/9064
Epicatechin	72276	290.27	$C_{15}H_{14}O_{6}$	https://pubchem.ncbi.nlm.nih.gov/compound/72276
Naringenin	439246	272.25	$C_{15}H_{12}O_{5}$	https://pubchem.ncbi.nlm.nih.gov/compound/439246
Quercetin	5280343	302.23	$C_{15}H_{10}O_{7}$	https://pubchem.ncbi.nlm.nih.gov/compound/5280343

Prediction of the binding mechanism of compounds from Sambucus nigra on the three HIV-1 proteins was carried out through molecular simulation. The simulation aims to determine the level of binding ability of a ligand to a protein domain based on the binding affinity value in a stable ligand-protein complex and produces negative energy. Binding affinity is formed when there is interaction between protein and ligand, this energy is formed through reversible reactions at constant temperature and pressure in accordance with the laws of thermodynamics[30,31,32]. The grid in the docking simulation plays a role to direct the ligand binding to the target protein. The simulation performed with refer to a docking grid consisting of Center (Å) X: 15.878 Y: 26.561 Z: 3.595 Dimensions (Å) X: 50.346 Y: 52.213 Z: 59.643 on HIV-1 PR Center (Å) X: 41.297 Y: -0.889 Z: 29.196 Dimensions (Å) X: 112.337 Y: 100.768 Z: 110.694 on HIV-1 RT Center (Å) X: -37.735 Y: 5.417 Z: -2.867 Dimensions (Å) X: 53.962 Y: 45.756 Z: 45.669 on HIV-1 INT. The docking results showed that Naringenin compound has a more negative binding affinity value on PR and Cyanidin-3,5-diglucoside on RT and INT HIV-1 (Table 2). This has answered the molecular mechanism of *Sambucus nigra* medicinal plants can potentially inhibit the activity or infection of the HIV-1 virus. Visualization of the ligand-protein complex structure consisting of Naringenin-PR, Cyanidin-3,5-diglucoside-RT, and Cyanidin-3,5-diglucoside-INT is displayed as sticks and cartoons with staining selection (Figure 1).

Table 2. The results of molecular docking simulation

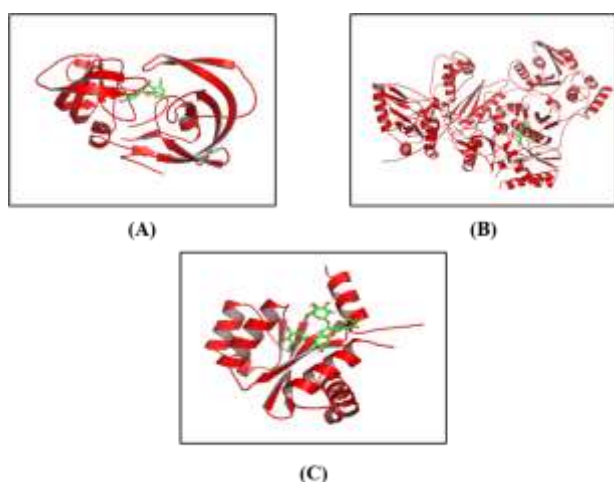| Compound	CID	Binding Affinity (kcal/mol)		
		PR (PDB ID: 1D4S)	RT (PDB ID: 8DX3)	INT (PDB ID: 6NCJ)
Cyanidin-3-sambubioside	3084569	-7.3	-8.1	-7.0
Cyanidin-3,5-diglucoside	44256718	-7.5	**-9.4**	**-7.8**
Catechin	9064	-7.9	-8.2	-6.4
Epicatechin	72276	-8.1	-7.7	-6,5
Naringenin	439246	**-8.3**	-8.1	-6.5
Quercetin	5280343	-8.2	-8.2	-6.3

Figure 1. Ligand-protein visualization from the docking results. (A) Naringenin-PR (B) Cyanidin-3,5-diglucoside-RT (C) Cyanidin-3,5-diglucoside-INT.

CONCLUSION

Sambucus nigra L. can have inhibitory activity on three enzymes of HIV-1 such as protease (PR), reverse transcriptase (RT), integrase (INT) through naringenin and Cyanidin-3,5-diglucoside compounds. Both compounds have more negative binding energy, form stable binding interactions in the target domain, and trigger inhibitory activity. Further research needs to be conducted such as validation through *in vivo* and *in vitro* approaches for additional scientific evidence.

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