

## **In Silico Study of the Active Compound of the Sambiloto Plant (*Andrographis Paniculata* Ness.) on Hiv-1 Protease Receptors**

**Rosario Trijuliamos Manalu<sup>1\*</sup>, Tiah Rachmatiah<sup>2</sup>, Tri Maratuzzakiah<sup>3</sup>**  
Faculty of Pharmacy, National Institute of Science and Technology, Jakarta

**ABSTRACT:** *Human Immunodeficiency Virus* (HIV) is a virus that attacks the immune system. Sambiloto (*Andrographis Paniculata* Ness) is a medicinal plant that has some active compounds and is used to prevent and treat some diseases. The purpose of this study was to determine candidate active compounds from sambiloto plant that able to inhibit the work of HIV-1 protease enzyme using the insilico method. The data of chemical compound of sambiloto was obtained from *pubchem site* and the structure of HIV-1 protease receptor got from Protein Data Bank with the code PDB 1D4H. The molecular docking results showed bisandrographolide and phytol have potential as anti-HIV drugs compared to amprenavir as a comparison ligand.

**Keywords:** HIV-1, Insilico, Protease HIV-1, *Andrographis paniculata* Ness.

*Submitted: 09-07-2022 ; Revised: 17-07-2022 ; Accepted: 23-07-2022*

**\*Corresponding Author :** [rio@istn.ac.id](mailto:rio@istn.ac.id)

## INTRODUCTION

HIV (*Human Immunodeficiency Virus*) is a virus that attacks white blood cells which causes a decrease of human immunity (Zubair *et al.*, 2020). Data on cases of *Human Immunodeficiency Virus / Acquired Immuno Deficiency Syndrome* (HIV / AIDS) in Indonesia continuously increase from year upon year it looks that during last eleven years the number of HIV cases in Indonesia reached its peak in 2019, there were 78% of new HIV infections in the Asia Pacific region. The highest of AIDS case during last eleven years was in 2013, totaled 12.214 cases (Anonim, 2020).

At this time the treatment for HIV is using high activity antiretroviral therapy. The administration of antiretroviral drugs (ARV) so far hasn't been effective for killing HIV. Antiretroviral therapy is only able to inhibit the development of virus. ARV drugs work by pressurizes replication of virus sufferer, and inhibiting the progression of infection. One of work mechanisms of ARV drugs is by inhibiting the action of protease enzymes that play a role in virus maturation. Inhibition of protease enzymes affects the *immature* virus so that the virus becomes a non infectious form. Therefore, the importance of HIV protease enzymes in the virus life cycle makes protease enzymes the main target for the treatment of HIV disease (Zubair *et al.*, 2020).

*Andrographis paniculata* (Burm f.) Nees (Acanthacease) is a medicinal plant used in many countries, including Indonesia, known as Sambiloto. Among the single compounds extracted from *Andrographis paniculata*, andrographolide was the main compound in terms of a bioactive properties and abundance. Phase 1 of andrographolide clinical trial, with increasing doses in HIV-POSITIVE patients showed a significant increase in the average CD4+ lymphocyte levels of HIV patients. Andrographolide inhibits HIV induced cell cycle dysregulation, leading to increased levels of CD4+ lymphocytes in HIV-1 individuals (Churiyah *et al.*, 2015). In this study, an insilico test was carried from the bitter plant as many as 22 compounds.

## THEORETICAL REVIEW

### *Molecular Docking*

Molecular Docking is a genetic based method that can be used to looking for the most precise and implicated pattern of interaction between two molecules, it is receptor and ligand. Ligand is signal molecule involved in both inorganic and biochemical processes. Docking is bely interaction between a ligand and a protein that is used to predict the position and ligand orientation when it is bound to the protein receptor. And the docking process will obtain bond energy ( $\Delta G$ ) which is a parameter of conformational stability between the ligand and the receptor.

## METHODOLOGY

#### *Protein Structure Preparation*

The preparation of target protein was carried out by downloading the 1D4H protein through the website (<https://www.rcsb.org/>). Proteins are separated from non standard ligands or residues using YASARA application (edit>all>residue), removing water molecules (edit>delete>water>) and adding hydrogen (edit>all>hydrogen to all). The results of separation are stored with the name protein with format .mol2.

#### *Ligand Structure Preparation*

The ligand structure is downloaded on the site (<https://pubchem.ncbi.nlm.nih.gov/>) in 2D form, protonated at Ph 7,4 using *Marvinsketch* software, the data obtained is saved in mrv format, the file is reopened and a search is carried out conformation into 20 structures for each compound (*Tools>>conformation>conformer*) and then saved in .mol2 format (Manalu, Safitri, et al., 2021).

#### *Molecular Docking with PLANTS*

Docking was carried out on the Windows operating system, the result of ligand and protein preparation were transferred in .mol2 format. In the next step to find the binding side obtained with the command "plants -mode bind ref\_ligand.mol2 5 protein.mol2". for run the docking process, enter the command "plants -mode screen pc\_kodepdb.txt". the results of the docking can be viewed in the terminal by entering the command "cd results" followed by "more bestranking.csv". The ligands used in this study were negative control, positive control, and ligand from 22 compounds of bitter plant (*Andrographis paniculata* Ness). Each ligand compound was docked with 1D4H protein using PLANTS.

#### *Docking Analysis and Visualization*

To view the relationship of the ligand and receptor used the software that able to visualize the structure either in two dimensions or three dimensions so that observations are easier to do. The result of molecular docking of the best compounds were combined with YASARA, file that has been prepared by YASARA then loaded on Discovery Studio 2021 in .pdf format.

#### *Lipinski's Rule of Five Analysis*

Lipinski's Rule of Five was carried out to determine the physicochemical properties of a ligand when it crosses cell membranes in the body. Analysis can be done with plant compound files and then uploaded to Lipinski's Rule of Five webserver.

#### *Pre-ADMET Test*

Prediction of pharmacokinetic properties (ADMET: absorption, distribution, metabolism, excretion and toxicity) was performed using the pkCSM online tool.

## RESULTS

The initial stage of the docking process is the preparation of protein structures, the selection of proteins at the PDB site is based on the protein to be tested. The structure with the downloaded 1D4H identity is the original homodimer structure, the HIV-1 protease available in PDB is a macromolecular structure bound to ligands, water and other residues.

### *Molecular Docking*

Based on the results of the docking score between the ligand and the receptor, the ligand conformation with the smallest energy can be seen in table 1. The results of docking score. The less of free energy of a molecule, so the more stable the molecule and reaction will proceed spontaneously. This is called thermodynamic equilibrium, the more negative of free energy, the more spontaneous the reaction or will quickly from a stable conformation (Manalu, Meheda, et al., 2021; Suhadi et al., 2019).

**Table 1.** Score docking results between Sambiloto and comparison ligands with 1D4H using PLANTS

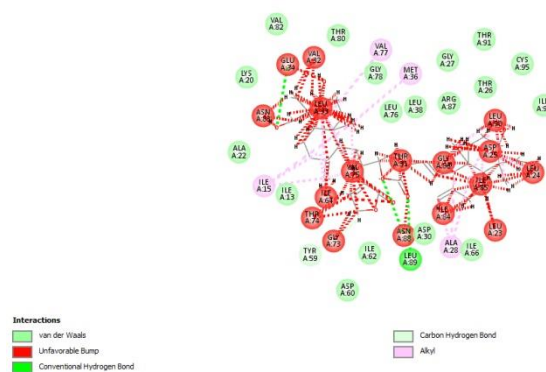
No	Ligand	Score Docking
1	Negative control	-114.762
2	Positive control	-101.217
3	Bisandrographolide	-98.238
4	Phytol	-88.522
5	3,14,19-triacetylandrographolide	-86.210
6	Deoxyandrographolide	-83.181
7	14-acetylandrographolide	-82.323
8	Andrograpanin	-80.057
9	14-deoxyandrographolide	-79.651
10	14-deoksi-14,15-didehydroandrographolide	-78.878
11	2-cis-6-trans farnesol	-78.381
12	2-trans-6-trans farnesol	-78.315
13	Andrographolide	-78.159
14	Panikulida B	-77.998
15	Apigenin	-77.518
16	14-deoksi-11-oxoandrographolide	-76.438
17	3,19-isopropylideneandrographolide	-76.139
18	14-deoksi-11,12-didehydroandrographolide	-75.447
19	Panikulida A	-75.159
20	5-hydroxy-7,8-dimetoxyflavone	-75.026

21	19-O-acetyl-14-deoxy-11,12 didehydroandrographolide	-73.779
22	Panikulida C	-72.851
23	Onysilin	-71.627
24	7-O methylwogonin	-70.415

Docking scores of two best compounds, is bisandrographolide and phytol had docking scores of -98,238 and -88,522, it is means that the score is close to the score of the positive control -101.217.

#### Docking Result Visualization

Visualization of docking results using Discovery Studio 2019 was carried out to see amino acid residues that bind to proteins. Figure 1 shows the interaction between protein and bisandrographolide ligands with one amino acid residue on hydrogen bonds. The presence of hydrogen bonds provides conformational stability in the interaction between the ligand and the receptor.



**Figure 1.** Visualization of the interaction of bisandrographolide with proteins using *Discovery Studio 2021*

#### Lipinski's Rule of Five Analysis

**Table 2.** Lipinski's analysis results

No	Ligand	Molecular Weight	Log P	H-Donor	H-Acceptor	Molar Refractivity
1	Bisandrographolide	312.000000	0.053101	5	6	77.145782
2	Phytol	296.000000	5.721511	1	1	109.166779
3	3,14,19-triacetylandrographolide	476.000000	5.255140	0	5	129.621964
4	Deoxyandrographolide	334.000000	4.205599	2	4	101.130585
5	14-acetylandrographolide	392.000000	4.355579	2	6	111.213577
6	Andrograpanin	318.000000	4.505400	1	3	100.251785
7	14-deoxyandrographolide	334.000000	4.205599	2	4	101.130585

8	14-deoksi-14,15-didehydroandrographolide	332.000000	4.029109	2	4	98.190582
9	2-cis-6-trans farnesol	222.000000	3.750559	1	1	77.328789
10	2-trans-6-trans farnesol	222.000000	3.750559	1	1	77.328789
11	Andrographolide	350.000000	3.905799	3	5	102.009384
12	Panikulida B	233.000000	2.650409	1	2	66.484291
13	Apigenin	270.000000	0.956370	3	5	61.890392
14	14-deoksi-11-oxoandrographolide	348.000000	3.683809	2	5	98.926086
15	3,19-isopropylideneandrographolide	390.000000	4.961670	1	5	115.801773
16	14-deoksi-11,12-didehydroandrographolide	332.000000	3.980599	2	4	98.906586
17	Panikulida A	231.000000	2.697610	1	2	63.973694
18	5-hydroxy-7,8-dimetoxyflavone	300.000000	2.653470	1	5	73.408798
19	19-O-acetyl-14-deoxy-11,12-didehydroandrographolide	374.000000	4.430379	1	5	108.110779
20	Panikulida C	203.000000	2.516109	2	1	60.897491
21	Onysilin	300.000000	2.653470	1	5	73.408798
22	7-O methylwogonin	298.000000	2.438970	1	5	71.129799

Molecular weight that exceed 500 daltons will be difficult to penetrate through membranes either in the skin or digestion. Based on Table 2, it can be seen that all the test ligands met the molecular weight limit requirements. However, in order to be used orally, the drug should have a log P value of more than 1 and less 5 (Kelutur et al., 2020). So it can be said that three are four test ligands that are not suitable to be given or need modification for oral use. Log P value more than 5 also have the potential to cause toxic effects because of their low solubility in water, so that they are difficult to excrete and accumulate, easily bind to hydrophobic targets compared to their intended targets, and are difficult to metabolize (Kelutur et al., 2020).

#### *Pre-ADMET Test*

In developing a new drug, it is necessary to study absorption, distribution, metabolism, excretion, and toxicity aspects before conducting clinical trials. Parameters that can be predicted through *in silico* include pharmacokinetic properties and prediction of toxicity. According to Chander et al. (2017), a compound is said to have good absorption if the absorption value is >80% and the absorption is not good when <30%. The intestine is the main place for absorption of drugs given orally. And Table 3 can be seen that the intestinal absorption (human) value of bisandrographolide and phytol compounds can be predicted that bisandrographolide and phytol compounds will be absorbed very well in the intestine.

The parameters of skin permeability are very important in the drug delivery. According to Pires et al. (2015), the compound is said to have relatively skin permeability low if it has a log value of  $K_p > -2.5$  in table 3 that bisandrographolide and phytol compounds have skin permeability (log  $K_p$ ) values of -2.2826 and -2,576. So it can be predicted that bisandrographolide compounds have good skin permeability.

Caco2 cell monolayer permeability is often used as an in vitro model of the intestinal mucosa so that it can predict the absorption of orally administered drugs. According to Pires et al. (2015) compounds are considered to have high Caco2 permeability if  $P_{app} > 8 \times 10^{-6}$  cm/s. However, in the predictions using pkCSM permeability will be translated into  $P_{app}$  log which is declared high if it has a value  $> 0,90$ . The log P values for bisandrographolide and phytol compounds were 0,451 and 1,515 which means that phytol compounds have high Caco2 permeability. Prediction of P-glycoprotein substrates and inhibitors I and II states that bisandrographolide compounds will be absorbed through P-glycoprotein and P-glycoprotein inhibitors I and II. In phytol compounds will only be absorbed through P-glycoprotein II only. Bisandrographolide compounds have good single layer cell permeability and bisandrographolide compounds show that they can be absorbed through P-glycoprotein and P-glycoprotein I and II.

Volume of distribution ( $VD_{ss}$ ) is the theoretical volume that the total dose of drug needs to be distributed to give the same concentration as on blood plasma. The higher the VD value, the more drugs are consumed distributed than plasma (Hardjono, 2017). The compound is said to have distribution volume is low when the Log VD value is  $< -0,15$ , and high when  $> 0,45$ . From both these compounds do not bind to the drug fraction in plasma. Compound said able to penetrate the BBB (Blood Brain Barrier) of the brain well when it has value Log BB  $> 0,3$  and not well distributed when Log BB  $< -1$  phytol. Compound able to penetrate the brain's Blood Brain Barrier well. Compound bisandrographolide has a log value of PS -2,984 and phytol -1,563 which means bisandrographolide compounds can penetrate the Central Nervous System (CNS) whereas phytol compounds do not, compound can be said to penetrate with Central Nervous System (CNS) when the PS log is  $> 2$  and if the PS log  $< 3$  is considered not penetrate the CNS.

Metabolism is a chemical process in which drugs are converted in the body to form a metabolite. The organ responsible is the liver. Bisandrographolide compound can only be metabolized on CYP3A4 (cytochrome P450) substrates while phytol compounds can be metabolized by CYP3A4 substrates and CYP1A2 inhibitors. To predict the process of compound excretion, it can be done by measuring the Total Clearance constant (CL<sub>TOT</sub>) and Renal Organic Cation Transporter 2 (OCT)2 substrate. CL<sub>TOT</sub> is a combination of hepatic clearance (metabolism in the liver and bile) and renal clearance (excretion through flow). This is related to bioavailability, and it is important to determine the dose level to achieve steady-state concentrations. From Table 3, it can be seen that the compound bisandrographolide 0,221 and the phytol compound 1,686. It can be

predicted that phytol compounds have the highest value so that they are excreted the fastest from the body.

Ames Toxicity test is a widely used method for assessing the mutagenic compound potential using bacteria. Ames toxicity in these two compounds that are not mutagenic. Both compounds are categorized as low because they have a value  $<0,447$  safe dose limit for humans (Hasnaa et al., 2022). hERG gene is one of the important factors in the discovery of new drugs. If the hERG gene is inhibited, cardiac arrhythmias will occur which can be fatal. Acute toxicity is exposure to a substance in less than 24 hours. One method of measuring the level of acute toxicity is to determine the value of the Oral Rate Acute Toxicity (LD50). Based on the results in Table 3, the LD50 value of the bisandrographolide compound is 2,715 indicating a relatively low toxicity and for the phytol compound 1,607. The higher the LD50 value, the lower the toxicity. So that bisandrographolide compounds have lower toxicity than phytol compounds.

**Table 3.** ADMET prediction Bisandrographolide and Phytol compounds using pkCSM

Prediction Test		Prediction Result	
		Bisandrographolide	Phytol
Absorption	Water solubility	-5.144 log mol/L	-7.554 log mol/L
	Caco2 permeability	0.451 log Papp in $10^{-6}$ cm/s	1.515 log Papp in $10^{-6}$ cm/s
	Intestinal absorption (human)	86.55 %	90.71 %
	Skin permeability	-2.826 log Kp	-2.576 log Kp
	P-glicoprotein substrate	Yes	No
	P-glicoprotein I inhibitor	Yes	No
	P-glicoprotein II inhibitor	Yes	Yes
Distribution	VDss (human)	-1.438 log L/kg	0.468 log L/kg
	Fraction unbound (human)	0	0
	BBB permeability	-1.187 log BB	0.806 log BB
	CNS permeability	-2.984 log PS	-1.563 log PS
Metabolism	CYP2D6 substrate	No	No
	CYP3A4 substrate	Yes	Yes
	CYP1A2 inhibitor	No	Yes
	CYP2C19 inhibitor	No	No

	CYP2C9 inhibitor	No	No
	CYP2D6 inhibitor	No	No
	CYP3A4 inhibitor	No	No
Excretion	Total clearance	0.221 log ml	1.686 log ml
	Renal OCT2 substrate	No	No
	AMES toxicity	No	No
	Max. Tolerated dose (human)	-0.561	0.05
	hERG I inhibitor	No	No
	hERG II inhibitor	No	Yes
Toxicity	Oral Rate Acute Toxicity (LD50)	2.715 mol/kg	1.607 mol/kg
	Oral Rate Choric Toxicity (LOAEL)	2.296 log mg	1.043 log mg
	Hepatotoxicity	Yes	No
	Skin Sensitisation	No	Yes
	<i>T.Pyriiformis</i> toxicity	0.285 log ug/L	1.884 log ug/L
	Minnow toxicity	-0.095 log Mm	-1.504 log Mm

## CONCLUSIONS

Based on the results of molecular anchoring using 22 active compounds from sambiloto plant to the HIV-1 protease target, it showed that two compounds with the best affinity, namely bisandrographolide and phytol with docking scores of 98,238 and 88,522 which were found in conformation 20 and conformation 10. This indicates that these compounds have potential for activity as an anti-HIV drug. The results of protein-ligand visualization showed that bisandrographolide compounds has good stability interactions. ADMET prediction shows that bisandrographolide compounds better than phytol compounds.

## REFERENCES

- Anonim. (2020). Infodatin HIV AIDS. *Kemenkes*, 1–12.
- Chander, S., Tang, C. R., Al-Maqtari, H. M., Jamalis, J., Penta, A., Hadda, T. Ben, Sirat, H. M., Zheng, Y. T., & Sankaranarayanan, M. (2017). Synthesis and study of anti-HIV-1 RT activity of 5-benzoyl-4-methyl-1,3,4,5-tetrahydro-2H-1,5-benzodiazepin-2-one derivatives. *Bioorganic Chemistry*, 72. <https://doi.org/10.1016/j.bioorg.2017.03.013>
- Churiyah, Pongtuluran, O. B., Rofaani, E., & Tarwadi. (2015). Antiviral and Immunostimulant Activities of *Andrographis paniculata*. *HAYATI Journal of Biosciences*, 22(2), 67–72. <https://doi.org/10.4308/hjb.22.2.67>
- Hardjono, S. (2017). Prediksi Sifat Farmakokinetik, Toksisitas dan Aktivitas Sitotoksik Turunan N-Benzoil-N'-(4-fluorofenil)tiourea sebagai Calon Obat Antikanker melalui Pemodelan Molekul. *Jurnal Ilmu Kefarmasian Indonesia*, 14(2), 246–255.
- Hasnaa, N. R., Manalu, R. T., & Subaryanti. (2022). Molecular Docking of Turmeric Active Compounds (*Curcuma longa* L.) against Main Protease in Covid-19 Disease. *East Asian Journal of Multidisciplinary Research*, 1(3), 353–364.
- Kelutur, F. J., Mustarichie, R., & Umar, A. K. (2020). Virtual Screening Kandungan Senyawa Kipas Laut (*Gorgonia mariae*) sebagai Anti-Asma. *ALCHEMY Jurnal Penelitian Kimia*, 16(2), 48. <https://doi.org/10.20961/alchemistry.16.2.39965.48-59>
- Manalu, R. T., Meheda, I. O., & Octaviani, C. (2021). Inhibition of HMG-CoA Reductase Activity from Active Compounds of Ginger (*Zingiber officinale*): In-Silico Study. *Jurnal Farmasi Etam (JFE)*, 1(1), 32–38.
- Manalu, R. T., Safitri, H., Sari, H. P., Devina, R., Irnawati, I., & Liliwana, E. A. (2021). Analisis In-Silico Penghambatan Main Protease (Mpro) Pada Sars-Cov-2 Oleh Senyawa Aktif Teh Hijau (*Camelia sinensis*). *Jurnal Farmagazine*, 8(2), 1–7.
- Pires, D. E. V., Blundell, T. L., & Ascher, D. B. (2015). pkCSM: Predicting small-molecule pharmacokinetic and toxicity properties using graph-based signatures. *Journal of Medicinal Chemistry*, 58(9), 4066–4072. <https://doi.org/10.1021/acs.jmedchem.5b00104>
- Suhadi, A., Rizarullah, R., & Feriyani, F. (2019). Simulasi Docking Senyawa Aktif Daun Binahong Sebagai Inhibitor Enzyme Aldose Reductase. *Sel Jurnal Penelitian Kesehatan*, 6(2), 55–65. <https://doi.org/10.22435/sel.v6i2.1651>
- Zubair, M. S., Maulana, S., & Mukaddas, A. (2020). Penambatan Molekuler dan Simulasi Dinamika Molekuler Senyawa Dari Genus *Nigella* Terhadap Penghambatan Aktivitas Enzim Protease HIV-1. *Jurnal Farmasi Galenika (Galenika Journal of Pharmacy) (e-Journal)*, 6(1), 132–140. <https://doi.org/10.22487/j24428744.2020.v6.i1.14982>