

Prediction of Novel Bioactive Compound from *Zingiber officinale* as Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTIs) of HIV-1 through Computational Study

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ABSTRACT

Human immunodeficiency virus 1 (HIV-1) is one of the viruses that causes AIDS in humans and disease outbreaks in this modern era. Reverse transcriptase (RT) enzyme though to be the functional enzyme that play a role on the HIV-1 virus replication. Bioactive compounds contained on Ginger (*Zingiber officinale*) is known to inhibit viral replication. This study to determine the alternative bioactive compounds contained on Ginger (*Zingiber officinale*) as a non-nucleoside reverse transcriptase (NNRTIs) HIV-1 inhibitors through computational study. The reverse transcriptase (RT) enzyme model was retrieved from protein sequence database (PDB) and validated with Ramachandran Plot and the compound contained on Ginger was retrieved from database. Analysis of molecular docking, performed using PyRx and the interactions between Reverse Transcriptase (RT) enzyme of HIV-1 virus and *Zingiber officinale* active compound was analyzed by PyMol and LigPlot+, also the drug-likeness molecule properties with The Lipinski Rule's of Five. From 24 active compound which have been docked, β -sitosterol proven to be the most potential bioactive compound as inhibitors of Reverse Transcriptase (RT) enzyme because it has more negative binding energy and the same amino acid residue with the control. β -sitosterol is predicted to be used as non-nucleoside reverse transcriptase (NNRTIs) HIV-1 inhibitors.

Keywords: β -sitosterol, HIV-1, NNRTIs, *Zingiber officinale*

INTRODUCTION

Human immunodeficiency virus 1 (HIV-1) is one of the viruses of the retrovirus family that causes AIDS in humans and also the cause of disease outbreaks in this modern era, cases of HIV-1 virus spread and infection are more common than HIV-2. AIDS is known to cause immune system deficiency and infected patients will experience fatal opportunistic infections. This disease first appeared in 1981, and HIV-1 was isolated at the end of 1983. HIV has the characteristics of having round-shaped virions with a diameter of 80-100 nm, genetic material in the form of ssRNA, equipped with glycoproteins gp41 and gp120, and containing three type of enzyme for the replication process which consists of reverse transcriptase, integrase, and protease [1].

Replication of HIV-1 in the host cell will trigger the production of functional enzymes that play a role in the process of virions assembling, one of which is the reverse transcriptase (RT) enzyme. HIV has a type of genome in the form of RNA when inside the host cell the genome is converted by RT to complementary DNA (cDNA). These changes are related to the integration of the viral genome in the process of entering genetic material into the nucleus to produce essential proteins that play a role in the new virus maturation process. RTs play an important role in the cycle of HIV replication and can be used as targets in the prevention of HIV-1 replication [2].

The FDA states that HIV-1 reverse transcriptase inhibitors are categorized into three types, including nucleoside reverse transcriptase inhibitors (NRTIs), nucleotide reverse transcriptase inhibitors (NtRTIs),

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and non-nucleoside reverse transcriptase inhibitors (NNRTIs). One of the NNRTIs inhibitors that have been found today is nevirapine which is one of the inhibitors used in the treatment of HIV-1 infections [3]. NNRTIs such Nevirapine are different from other inhibitors because they directly bind to the hydrophobic pocket on the active side of the RT, so that the complex formed will inhibit the performance of the mechanism reverse transcription [4,21]. In the process of its molecular mechanism, nevirapine binds and inhibits the allosteric side of RT so that the enzyme cannot bind to the substrate in the form of viral RNA. NNRTIs bind to the hydrophobic pocket of the p66 subdomain at a distance of 10 Å from the active side. Therefore, binding pocket on NNRTIs will inhibit RT but differ in NRT [4]. Nevirapine resistance is caused by the mutation of amino acid residues in the pocket binding domain Y181C and K103N and is often found in some cases of NNRTIs resistance. NNRTIs resistance to nevirapine needs to be handled extensively in the search for bioactive compounds to effectively inhibit the process of HIV-1 replication [5,6].

Bioactive compounds are contained in some herbal plants, one of which is Ginger. Ginger (*Zingiber officinale*) is known to inhibit viral replication such HIV-1 infection, but the prediction of mechanism action of bioactive compound is still unknown [7]. Therefore, research on the potential predictions of bioactive compounds in ginger (*Zingiber officinale*) as a non-nucleoside reverse transcriptase HIV-1 inhibitors in a computational study can be used as a reference in the selection of drug compounds and a solution to cases of HIV-1 virus resistance.

MATERIALS AND METHODS

Sample Preparation

The 3D structure of the HIV-1 reverse transcriptase (Wild type) is obtained with the .pdb format from the databank (PDB) with Protein ID: 3LP1 [8]. Then, the 3D structure of the target protein is validated using the Ramachandran Plot Server [9]. Information about the bioactive compound content of *Zingiber officinale* is obtained from Sonale & Udaya [10] and the Herbaldb database [11]. About 25 compounds were obtained from PubChem database [12] to obtain samples with a Structure Data Format (.sdf), then minimization energy is carried out and converted into PDB format. The selection was carried out for nevirapine as control compound which is obtained from PDB [8], using

Pymol software [13] to obtain 3D structures from target proteins and compounds that have been docked.

Virtual Screening

Identification of small molecule which targets the HIV-1 reverse transcriptase (RT) (Wild type) is carried out by molecular docking with submitting 24 chemical compounds of *Zingiber officinale* that bind to pocket-binding domain control the same as control compound Nevirapine using the AutoDock Vina program in PyRx 0.8 (Virtual Screening Tools) [14]. We use specific docking, by directing the grid docking at the center position of the pocket-binding domain. Then, we determine the type of interaction between proteins and ligands and the types of amino acid residues with Lig-Plot+ software [15]. The Lipinski Rule's of Five are used to prove the potential of compounds that have low binding affinity also drug-likeness molecules properties [16].

Molecular Visualization

PyMol software [9] were used for visualizing docking results and 3D structure of target proteins. The molecular visualization is displayed by 3D protein structures in cartoon and surface shapes and to do the coloring selection to differentiate the domain between the chain in the target protein and the type of ligand attached. Ligplot+ were used to analyze the protein and ligand interactions that able to display the types of chemical bonds that contribute to the chemical interactions that occur, and also the position of amino acid residues.

RESULTS AND DISCUSSION

Preparation of Bioactive Compound Contained on *Zingiber officinale* and Structure Validation of HIV-1 Reverse Transcriptase (RT) Enzyme

About 24 compounds were obtained from the PubChem database in the .sdf format and then converted using the Open Babel tools in the PyRx software to obtain a 3D structure. The control compound (nevirapine) is obtained from PDB along with the target protein sample. The control binds to the target protein and then a selection process is performed using PyMol software. Before continuing to molecular docking stage, The proteins must be sterilized in PyMol software by removing the water molecules and ligands attached to the target protein, then visualized in rigid surface and cartoon structures with blue and red col-

ored to distinguish the chain that consist on protein (Figure 1).

The results of the Ramachandran plot analysis showed that the stereochemical quality of the protein

inputted by its core area had a score of more than 90%, indicating that the quality of the protein model used in this study was very good model.

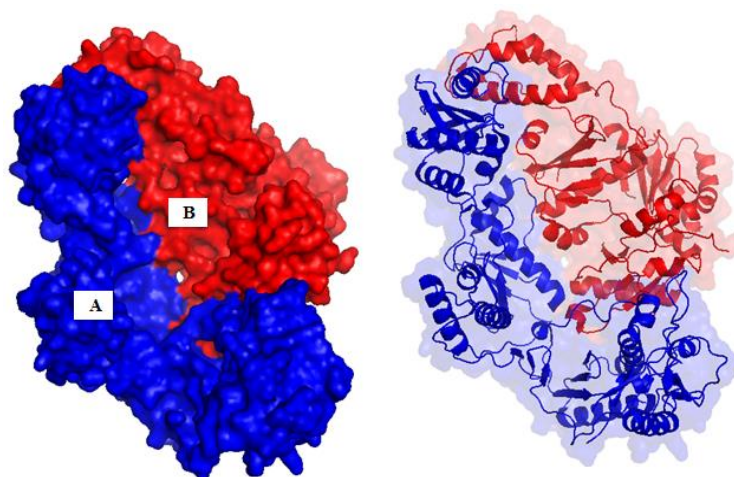


Figure 1. The structure of protein target (3LPI). Visualization of the reverse transcription (RT) target protein using a rigid surface (1) and cartoon (2). Coloring is done to differentiate between the chains (a,b) that compose the protein by PyMol software

Table 1. Binding affinity between query compound and control with RT enzymes. β – sitosterol has a more negative binding affinity than other compounds contained in *Zingiber officinale*

No	Ligand	Binding Affinity (Kcal/mol)	No	Ligand	Binding Affinity (Kcal/mol)
1	Nevirapine (Control)	-8.8	13	6-Gingediol	-7.7
2	β -sitosterol	-9.9	14	Methyl-6-Shogaol	-7.7
3	Germacrene	-8.9	15	6-Gingerol	-7.6
4	Cyclosativene	-8.7	16	Methyl-6-Gingerol	-7.6
5	Zingiberene	-8.2	17	8-Gingerol	-7.5
6	12-Gingediol	-8.1	18	4-Gingerol	-7.3
7	Hexahydrocurcumin	-8.1	19	Zingerone	-7.1
8	α -Farnesene	-7.9	20	Citral	-6.8
9	10-Gingerol	-7.9	21	Camphene	-6.2
10	Dehydrogingerdione	-7.8	22	α -Linalool	-6.1
11	6-Shogaol	-7.8	23	α -Pinene	-6.0
12	Gingerdione	-7.8	24	Pinene	-6.0
			25	Alcanfor	-5.7

Analysis of Bioactive Compound Contained on *Zingiber officinale* as Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTIs) for HIV-1 Virus

The docking in this study aims to determine the binding affinity generated when a query compound originating from *Zingiber officinale* interacts or binds to the control binding site domain on the target protein. The binding site of the control can be a grid reference, the side is located at the center (Å) X: 9.7 Y: 16.0 Z: 18.2 and dimensions (Å) X: 25.0 Y: 25.0 Z: 25.0. Compounds that have the ability to bind to target protein with the same binding side domain as control, indicate that the compound, have the capability as a control [17].

The number of binding affinities is an indicator of the ability of active compounds in the target protein. This includes spontaneous processes, the bonds between proteins and ligands occur, because of changes in Gibbs free energy (ΔG) on a negative system when it reaches an equilibrium state at con-

stant pressure and temperature, because the association between proteins and ligands determines a negative ΔG value, it can be considered to establish ΔG can provide stability to the protein-ligand complex [18]. β -Sitosterol is a plant sterol compound with a chemical structure similar to cholesterol, in humans, these compounds play a role in decreasing benign prostatic hyperplasia (BPH) and cholesterol level in the blood [22, 23]. Germacrene is a volatile hydrocarbon compound, this compound is also found most plant species and functions as an antimicrobial [24]. Based on the statement, it shows that the compounds contained in *Zingiber officinale*, β -sitosterol and germacren have binding affinity values of -9.9 and -8.9 kcal/mol lower than the control compound which is only -8.8 kcal/mol, this indicates that both compounds have a strong affinity for the target reverse transcriptase protein so that they can affect the physiological properties of the protein.

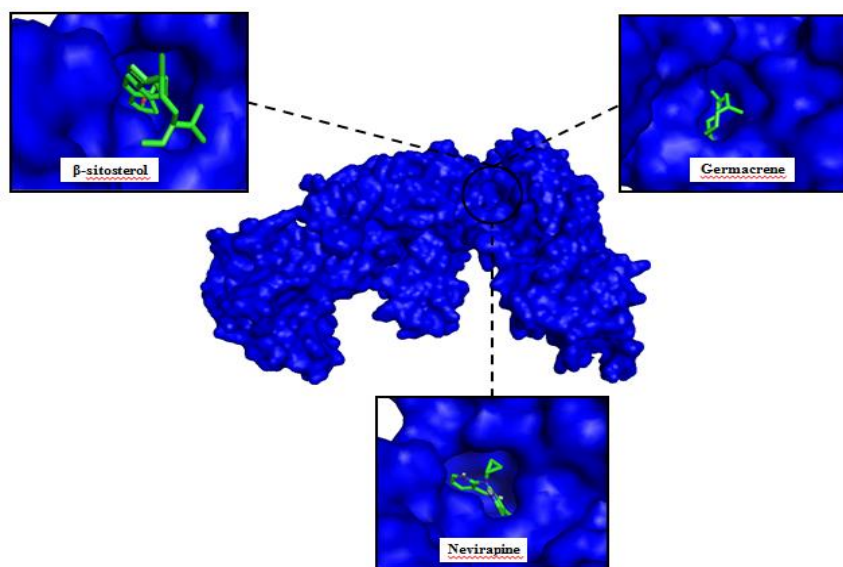


Figure 2. Visualization of the position of the ligand domain on the target protein (RT). All of the ligands have a binding pocket with the same pattern

After that to determine the bond distance between atoms, an RMSD value is formed. The root square deviation (RMSD) value obtained is referring to the distance of interaction between the atoms making up the ligand and the atom making up the amino acid residues in the RT domain, the RMSD obtained shows that the β -sitosterol chemical compound has a value of 1.11 Å lower than germacrene and nevirapine (control), respectively 3.04 and 2.94 Å. So, the distance of the interaction of the atoms making up the β -sitosterol compound with RT do-

main is closer than the germacrene and nevirapine (control) compounds [13].

Interaction Analysis Between Bioactive Compound Contained on *Zingiber Officinale* with Reverse Transcriptase Enzyme of HIV-1 Vire and the Druglike-ness Properties of the Compound

The predicted active compound has a strong bond to the target receptor if it has hydrogen bonds and can bind to the same amino acid residue on the active side and compared to the control or reference.

Active compounds are predicted to have a strong ability to inhibit receptors if they have the same binding position of amino acid residues as controls. Scores from binding affinity are indicators of the binding ability of active compounds to the target protein. Free energy is the enthalpy change needed to break certain bonds in 1 mole of gas inhibitor molecules [19].

The results of the protein-ligand interaction analysis showed that the two query ligands which have a binding affinity value lower than the control showed the similarity of the hydrophobic interaction position with the controls on the residues of Pro236, Gly190, Lys101, His235, Val106, Val179, Phe227, Leu100, Tyr181, Tyr318, Tyr188, Trp229, Leu234 and both do not have interactions using hydrogen bonds, and it can be ascertained that the bonding position of the two query compounds to the target protein lies in the same binding pocket. In general,

nevirapine resistance is caused by the mutation of amino acid residues in the pocket binding domain, namely Y181C and K103N, which is certainly also often found in cases of NNRTI resistance [22]. Nevirapine resistance will develop rapidly if no special treatment is found to inhibit the HIV replication process [20]. Active compounds are predicted to potentially have inhibitory properties and strong interactions if it has the same chemical interaction position on the target protein with the control [19].

NNRTIs such Nevirapine are Lipinski's rules are molecular mass less than 500 Dalton, high lipophilicity or LogP less than 5, the number of donor and acceptor hydrogen bonds less than 5 and 10, and molar reactivity must be between 40-130. Lipinski test results in this study indicate that the Germacrene compound is not following at least two Lipinski rules, so that not as drug like molecule.

Table 2. Interactions between ligands and proteins

Ligand	Interaction
β -sitosterol	Hydrogen bond : - Tyr181, Pro95, Leu100, Tyr188, Leu234, Phe227, Val106, Val179, Lys101, Asn103
Germacrene	Hydrogen bond :- : Tyr318, Trp229, Leu234, His235, Tyr188, Phe227, Tyr181, Leu100, Val106
Nevirapine (Kontrol)	Hydrogen bond : - Hydrophobic : Pro236, Gly190, Lys101, His235, Val106, Val179, Phe227, Leu100, Tyr181, Tyr318, Tyr188, Trp229, Leu234

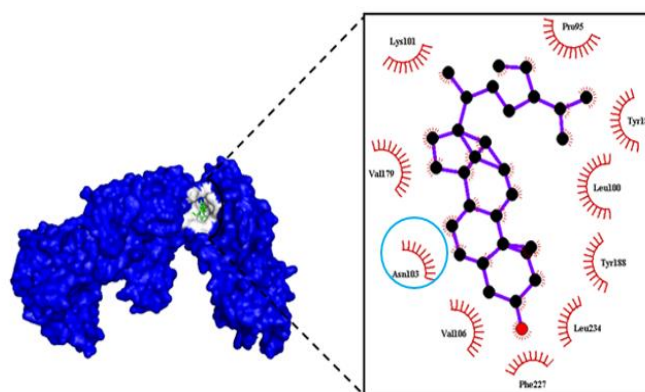


Figure 3. Visualization of protein and ligand interactions in β -sitosterol. The protein structure is displayed in the form of rigid surfaces which are dark blue, while the green sticks are for ligands and domains on white surfaces are pocket ligand bindings. The position of the Asn103 amino acid residue that interacts using hydrophobic with the ligand is shown in light blue circles.

β -Sitosterol compound has the lowest binding affinity value, fulfilling the five Lipinski rules, namely 414,000 Dalton, 1 donor, and acceptor hydrogen bonds, and LogP 8,124. So β -sitosterol can be categorized as a drug like a molecule.

CONCLUSION

The active compound contained in the *Zingiber officinale* are predicted to have a potential as an NNRTIs on HIV-1, especially β -sitosterol. This organic compound are predicted to be alternative compound to inhibit HIV as a synthesis drugs and its potential must be proven with wet lab study road to human clinical trial.

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REFERENCES

- Rosenthal VD et al., (2012) International Nosocomial Infection Control Consortium (INICC) Report, Data Summary of 36 Countries, for 2004-2009. American Journal Infect Control 40(5): 396-407. doi: 10.1016/j.ajic.2011.05.020.
- Richman DD, Margolis DM, Delaney M, Greene WC, Hazuda D, Pomerantz RJ. 2009. The challenge of finding cure for HIV infection. J. Sci 323 (5919):1304–1307.doi: 10.1126/science.1165706.
- Lassen K, Han Y, Zhou Y, Siliciano J, Siliciano RF. 2004. The multifactorial nature of HIV-1 latency.Mol. Med. 10 (11):525–531.doi:0.1016/j.molmed.2004.09.006.
- Iris U, Virgina M, Jose PE. 2013. Non-nucleoside reverse transcriptase inhibitors: a review on pharmacokinetics, pharmacodynamics, safety and tolerability. J. AIDS. Soc 16(1):18567.doi: 0.7448/IAS.16.1.18567.
- Schauer G, Huber D, Kelly D, Leuba SH, CN Sluis. 2014. Mechanism of allosteric inhibition of HIV-1 reverse transcriptase revealed by single-molecule and ensemble fluorescence. J. Nuc. Res 42(18): 11687–11696.doi: 10.1093/nar/gku819.
- Ren J, Bird LE, Chamberlain PP, Stewart GB, Stuart DI, Stammers DK. 2002. Structure of HIV-2 reverse transcriptase at 2.35-Å resolution and the mechanism of resistance to non-nucleoside inhibitors. J. Proc. Natl. Acad. Sci 99 (22): 14410 - 5.doi: 10.1073/pnas.222366699.
- Jung SC, Kuo WC, Chia YF, Den SE, Lien CC. 2012. Fresh ginger (*Zingiber officinale*) has anti-viral activity against human respiratory syncytial virus in human respiratory tract cell lines. J. Etno. Pharm. 1459(1):146-151.doi: 10.1016/j.jep.2012.10.043.
- The Protein Databank. <http://www.rcsb.org/pdb/home/home.do>. accessed 12 October 2018.
- Ramachandran Plot Analysis. <http://mordred.bioc.cam.ac.uk/~rapper/rampage.php>. accessed 12 October 2018.
- Sonale RS & Udaya SK. 2014. Characterization of gingerols analogues in supercritical carbon dioxide (SCCO₂) extract of ginger (*Zingiber officinale* R.). J. Food. Sci. Technol 51(11):3383-3389.doi: 10.1007/s13197-012-0851-4.
- Basis Data Tanaman Obat Indonesia. <http://herbaldb.farmasi.ui.ac.id/v3/>, accessed 12 October 2018
- Kim S, Thiessen PA, Bolton EE, Chen J, Fu G, Gindulyte A, Han L, He J, He S, Shoemaker BA, Wang J, Yu B, Zhang J, Bryant SH. 2016. PubChem Substance and Compound databases. Nucleic Acids Res. 44 (D1): D1202-13. doi: 10.1093/nar/gkv951.
- Seeliger D, & de Groot BL. 2010. Ligand docking and binding site analysis with PyMOL and Autodock/Vina. Journal of computer-aided molecular design 24(5): 417-422. doi: 10.1007/s10822-010-9352-6.
- Trott O & Olson AJ. 2010. AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization and multithreading. J. Comp. Chem. 31(2): 455-461.doi: 10.1002/jcc.21334.
- Laskowski RA & Swindells MB. 2011. LigPlot+: multiple ligand-protein interaction diagrams for drug discovery. J. Chem. Inf. Model 51: 2778-2786.doi: 10.1021/ci200227u.
- Lipinski CA. 2004. Lead- and drug-like compounds: the rule-of-five revolution. Drug Discovery Today: Technologies 1 (4): 337-341. doi: 10.1016/j.ddtec.2004.11.007.
- Syafrudin S, Septiadi L, Alfuruqi NTS, Wahyudi D, Kharisma, VD. 2018. The In Silico Analysis and Identification of Possible Inhibitor of H5N1 Virus. Bioinformatics and Biomedical Research Journal 1(2): 33-39.doi:10.11594/bbrj/01.02.02.
- Gilson MK & Zhou HX. 2007. Calculation of protein-ligand binding affinities. Annu. Rev. Biophys. Biomol. Struct 36:21-42. doi:10.1146/annurev.biophys.36.040306.132550.
- Zukhurullah M, Aswad M, Subehan S. 2012. Kajian beberapa senyawa antiinflamasi: docking terhadap siklooksigenase-2 secara in silico. Majalah Farmasi dan Farmakologi 16:37–44.
- Ren J, Bird LE, Chamberlain PP, Stewart GB, Stuart DI, Stammers DK. (2002). Structure of HIV-2 reverse transcriptase at 2.35-Å resolution and the mechanism of re-

- sistance to non-nucleoside inhibitors. J. Proc. Natl. Acad. Sci. 99(22): 14410-5.doi: 10.1073/pnas.222366699.
21. Jayaram B, Tanya S, Goutam M, Abhinav M, Shashank S, Vandana S. 2012. Freely accessible web-server for target directed lead molecule discovery. J. Bioin. 13: S7-S17.doi: 10.1186/1471-2105-13-S17-S7.
 22. Patel SS & Benfield P. 1996. New drug profile: nevirapine. J. Clin. Immunotherapeutics 6(4): 307–317.
 23. Kim TH, Lim HJ, Kim MS, Lee MS. 2012. Dietary supplements for benign prostatic hyperplasia: An overview of systematic reviews. J. Maturitas. 73(3): 180–5. doi: 10.1016/j.maturitas.2012.07.007.
 24. Rudkowska I, Abu Mweis SS, Nicolle, C, Jones PJ. 2008. Cholesterol-lowering efficacy of plant sterols in low-fat yogurt consumed as a snack or with a meal. J. Am. Coll. Nutr 27(5): 588–95.
 25. He X & Cane DE. 2004. Mechanism and stereochemistry of the germacradienol/germacrene D synthase of *Streptomyces coelicolor* A3(2). J. Am. Chem. Soc 126(9): 2678-9.doi: 10.1021/ja039929k.