Docking Study on Anti-HIV-1 Activity of Secondary Metabolites from Zingiberaceae Plants

Muhammad Sulaiman Zubair1, Saipul Maulana1, Agustinus Widodo1, Alwiya Mukaddas1, Ramadanil Pitopang2

1Department of Pharmacy, Science Faculty, Tadulako University, Palu 94118, Indonesia, 2Department of Biology, Science Faculty, Tadulako University, Palu 94118, Indonesia

INTRODUCTION

Human immunodeficiency virus type-1 (HIV-1) is a kind of retrovirus family that causes acquired immunodeficiency syndrome (AIDS), which creates health crisis worldwide today. Many researchers have extensively studied for discovering new anti-HIV-1 agents. However, effective agents with less side effect and high inhibition potency are still in demand. Recently, approximately 30 anti-HIV-1 drugs have been licensed for the treatment of AIDS therapy. The mechanism of these
Docking was conducted by some researchers. The chloroform extract of *Boesenbergia pandurata* rhizome and methanol extract of *Alpinia galanga* rhizome, showed potent inhibitory activity on HIV-1 PR. 5-Hydroxy-7-methoxyflavone and 5,7-dimethoxyflavone, isolated from *Kaempferia parviflora*, were reported to inhibit HIV-1 PR with half maximal inhibitory concentration \( IC_{50} \) values of 19.04 and 19.54 \( \mu M \), respectively. Zerumbone, the main compound from *Zingiber zerumbet* and *Zingiber aromaticum*, was reported to inhibit HIV with \( IC_{50} \) of 0.04 \( \mu g/mL \). \( 19S-19\)-acetoxychavicol acetate, isolated from *A. galanga*, was reported to block Rev transport that inhibits the replication of HIV type 1.\(^{[13]}\) A new diarylheptanoid; (3S,5S)-3,5-diacetoxy-1,7-bis(3,4,5-trimethoxyphenyl) heptanes, 5\( \alpha \)H-eudesmane-4\( \alpha \),11-diol, 5\( \alpha \)H-eudesmane-4\( \beta \),11-diol, 4\( \alpha \),10\( \beta \)-dihydroxy-1\( \beta \)H, 5\( \alpha \)-guai-6-ene (guaiamediol), and (+)-galanolactone from the rhizomes of *Zingiber mekongense* also showed anti-HIV activities.\(^{[14]}\) Although, the study of Zingiberaceae on anti-HIV-1 activity has been performed well, the comprehensive study of Zingiberaceae metabolite compounds against three HIV protein enzymes (PR, IN, and RT) by using docking molecular approach is still not reported yet. Therefore, in this study, the secondary metabolites of Zingiberaceae plants from the complete genera—*Alpinia, Curcuma, Etingeria, Hedychium, Boesenbergia*, and *Zingiber*—were docked into the three HIV-1 protein enzymes; protease, integrase, and reverse transcriptase.

**Materials and Methods**

**Hardware and software**

Docking molecular simulation was performed on Dell PC with Linux Fedora 3.6.11-4.fc16 i686 operating system, Intel® i5 CPU T5800 @ 2 GHz processor, and 4 GB of RAM. The software used for docking preparation was Chem3D Ultra 8.0, Ligprep, and AutoDock Tools (ADT) 1.4.5. Meanwhile, AutoDock 4.2.6 was used for running the docking simulation, and ADT 1.4.5 was used for analyzing the docking results.

**Docking preparation**

The secondary metabolites of Zingiberaceae plants were obtained from the KnapSack website (www.ka.naist.jp/knapsack_jsp/top.html) and literature.\(^{[9,14-17]}\) There are 1070 chemical structures comprising 165 compounds from *Zingiber*, 79 compounds from *Hedychium*, 7 compounds from *Etingeria*, 91 compounds from *Curcuma*, 251 compounds from *Boesenbergia* (*Kaempferia*), and 477 compounds from *Alpinia*. The structures were modeled with Chem3D Ultra 8.0 (ACD labs) and optimized by Ligprep using OPLS 2005 force field. The optimized structure was assigned by using Epik at the target pH value of 7.0 ± 2.0. These conformations were used as starting conformations to perform docking. All HIV-1 protein enzyme structures were obtained from the X-ray crystal on Brookhaven Protein Data Bank (http://www.rcsb.org/pdb). Receptors were prepared for docking by AutoDock Tools (ADT). All heteroatom nonreceptor atoms in protein such as water and ions were removed. Kollman charges and solvation parameters were assigned to the protein atoms. Grid boxes, which comprised 40 × 40 × 40 points (\( x = 16.751, y = 23.353, z = 17.611 \) spaced 0.375 Å apart, were used for PR enzymes; grid boxes, which comprised 28 × 42 × 28 points (\( x = -39.014, y = 31.127, z = -19.842 \) spaced 0.375 Å apart, were used for IN enzymes; and grid box, which comprised...
50 × 50 × 50 points (x = 10.35, y = 14.076, z = 18.252) spaced 0.375 Å apart, was used for RT enzymes.

**Docking calculation and validation**

Docking of each ligand was run by AutoDock 4.2.6 with a Lamarckian genetic algorithm. The parameters were as follows: population size of 150, 250,000 energy evaluations, a mutation rate of 0.02, crossover rate of 0.80, and 100 independent docking runs. A cluster tolerance of 2.0 Å in positional root-mean-square deviation (RMSD) was used to cluster the docking result. The ligand with the lowest free energy of binding was chosen as the best ligand conformation. Redocking and cross-docking experiments were used for validation of docking methods and parameter used. The redocking protocols are valid if the RMSD value is less than 2 Å. The docking energy of ligands was then compared to native ligands.

**Results**

The RMSD values obtained from cross-docking validation of HIV PR, HIV IN, and HIV RT are shown in Table 1. These RMSD values were from the best cluster conformations. Table 2 showed the top 10 docking results of the metabolite compounds from Zingiberaceae plants on HIV PR, IN, and RT enzymes.

**Discussion**

Current challenges on HIV-1 therapy have attracted many researchers to search for novel drugs from natural products. The available anti-HIV-1 drugs in the market now have limitations as they showed more side effects, poor drug–drug interactions, and were resistant against HIV-1. Although there are many studies regarding the HIV-1 inhibitory profiles of secondary metabolites from various plant species, it was reported that Zingiberaceae plants have less secondary metabolites for anti-HIV-1 activity compared to other plant species of different genera and family. Therefore, the Zingiberaceae plant secondary metabolites have more opportunity to be further studied by applying the molecular docking to predict the potency of the metabolites on inhibiting the HIV-1 protein enzymes.

In this study, the docking simulation was performed on 1070 chemical compounds of Zingiberaceae plants on PR, IN, and RT protein enzymes using AutoDock 4.2.6.[19] The docking validation was based on RMSD value on cross-docking methods by redocking the native ligand of each receptors on three different targets. The crystal structure with the lowest RMSD value was selected for further study. PDB code 3NU3 for PR enzymes with amprenavir as native ligand, 3OYA for IN protein with raltegravir as native ligands, and 3LP1 for RT with nevirapine as native ligand was chosen with the lowest value of RMSD as 1.403, 1.926, and 0.401 Å, respectively. Meanwhile, the docking energy of native ligands of amprenavir, raltegravir, and nevirapine were -18.02, -10.50, and -9.01 kcal/mol, respectively [Table 1].

On the basis of docking results [Table 2], plants of Hedychium coronarium, Zingiber officinale, Z. aromaticum, B. pandurata Roxb., Alpinia kathumadai, and Alpinia blepharocalyx were the potential plants in which their metabolite compounds could inhibit the PR enzyme. Noralpindenoside B and alpindenoside A, a norditerpene and labdane glycosides from A. densispica, were best docked on PR enzymes with docking energy of -18.02 and -17.9 kcal/mol, respectively, comparing native ligand amprenavir. These two compounds were reported to have NO inhibitory activity and no cytotoxic activity on four different cancer cell lines of HeLa, KB, Daoy, and WiDr.[20] Alpindenoside A was found to interact with ARG-8, GLY-127, ASP-125, ASP-130, ASP-30, ASP-29, and ARG-108. Meanwhile, noralpindenoside B was found to interact with ASP-130, ASP-129, ARG-8, GLY-148, VAL-82, THR-80, GLY-27, ASP-29, and ARG-108.

Panduratin E, a cyclonexenyl chalcone from B. pandurata Roxb., and 5α,8α-epidioxyergosta-6,22-dien-3β-ol, a steroid compound from Etlingera elatior, were found to have the lowest docking energy on IN enzyme (-11.97 and -11.41 kcal/mol, respectively) and also better interaction than raltegravir. Panduratin E showed interaction with

| Table 1: Root-mean square deviation value after redocking and cross-docking of three different ligands on HIV-1 protease, integrase, and reverse transcriptase enzymes |
|---------------------------------|----------|----------|---------------|-------------------------------|
| **Protein target**             | **PDB code** | **RMSD (Å)** | **Native ligand** | **Docking energy (kcal/mol)** | **Interaction with amino acids** |
| HIV-1 protease                 | 3NU3     | 1.403    | Amprenavir     | -14.61                        | ASP-25, ASP-125, GLY-127, ARG-8, ASP-129, ASP-130 |
| HIV-1 integrase                | 3OYA     | 1.926    | Raltegravir    | -10.50                        | TYR-212, ASP-185, ASP-128, GLU-221 |
| HIV-1 reverse transcriptase    | 3LP1     | 0.401    | Nevirapine     | -9.01                         | - |


Table 2: Top 10 docking result of Zingiberaceae plants metabolite compounds on HIV-protease, integrase, and reverse transcriptase enzymes

| No. | Metabolite compounds                                      | Docking energy (kcal/mol) | Interaction with amino acids | Plant source                                         |
|-----|----------------------------------------------------------|---------------------------|------------------------------|-----------------------------------------------------|
| HIV-1 protease enzyme                                                                                                                                          |
| 1   | Noralpindenoside B                                       | -18.02                    | ASP-130, ARG-8, GLY-148,    | Alpinia densespicata                                  |
|     |                                                          |                           | GLY-27, ASP-29, ARG-108     |                                                     |
| 2   | Alpindenoside A                                          | -17.90                    | ARG-8, GLY-127, ASP-129,    | A. densespicata                                      |
|     |                                                          |                           | ASP-130, ASP-30, ASP-29,    |                                                     |
|     |                                                          |                           | ARG-108                      |                                                     |
| 3   | Stigmasterol                                             | -17.48                    | ASP-30                       | Hedychium coronarium                                  |
| 4   | β-Sitosterol                                             | -17.30                    | ASP-30                       | Zingiber officinale                                  |
| 5   | Panduratin G                                             | -17.09                    | -                            | Boesenbergia pandurata Roxb.                         |
| 6   | Panduratin F                                             | -17.07                    | ASP-25                       | B. pandurata Roxb.                                   |
| 7   | Katsumadain A                                            | -17.04                    | ASP-129, VAL-82, THR-80     | Alpinia katsumadai                                   |
| 8   | Blepharocalyxin D                                        | -16.87                    | ARG-108, GLY-48, ASP-129    | Alpinia blepharocalyx                                |
| 9   | Epicalyxin G                                             | -16.87                    | ARG-108, ASP-130, ARG-8     | A. blepharocalyx                                     |
| 10  | Calyxin L                                                | -16.86                    | ARG-108, GLY-127, ASP-30,   | A. blepharocalyx                                     |
|     |                                                          |                           | ARG-8                        |                                                     |
| HIV-1 Integrase enzyme                                                                                                                                            |
| 1   | Panduratin E                                             | -11.97                    | GLU-221, ASP-128, Mg^{2+}   | B. pandurata Roxb.                                   |
| 2   | 5α,8α-Epidioxyergosta-6,22-dien-3β-ol                   | -11.41                    | TYR-212, GLN-186            | Etinglera elatior                                    |
| 3   | Rubraine                                                 | -11.22                    | DA-17, Mg^{2+}               | A. katsumadai                                        |
| 4   | Panduratin B1                                            | -10.77                    | Mg^{2+}                      | B. pandurata Roxb.                                   |
| 5   | (-)-Krachainz A                                          | -10.75                    | TYR-212, ASP-185, Mg^{2+}   | B. pandurata Roxb.                                   |
| 6   | Boesenbergin B                                           | -10.74                    | GLU-221, ASP-128, Mg^{2+}   | B. pandurata Roxb.                                   |
| 7   | Isorubraine                                              | -10.69                    | DA-17, Mg^{2+}               | A. katsumadai                                        |
| 8   | 3-O-B-D-Glucopyranosil sitosterol                        | -10.65                    | -                            | Alpinia pinnanensis                                  |
|     |                                                          |                           |                              | A. blepharocalyx                                     |
| 9   | Panduratin D                                             | -10.57                    | Mg^{2+}                      | B. pandurata Roxb.                                   |
| 10  | (2S)-7,8-dihydro-5-hydroxy-2-methyl-2-(4”-methyl-3”-pentenyl)-8-phenyl-2H,6H-benzo[1,2-b:5,4b’] dipyran-6-one | -10.56 | DA-17, Mg^{2+} | B. pandurata Roxb. |

HIV-1 Reverse transcriptase enzyme

| No. | Metabolite compounds                                      | Docking energy (kcal/mol) | Interaction with amino acids | Plant source                                         |
|-----|----------------------------------------------------------|---------------------------|------------------------------|-----------------------------------------------------|
| 1   | Pahangensin A                                            | -13.76                    | LYS-101                      | A. pahangensis Ridley                                |
| 2   | (+)-Krachainz A                                          | -12.75                    | LYS-101                      | B. pandurata Roxb.                                   |
| 3   | Shogasulfonic acid C                                     | -11.76                    | LYS-101, LYS-104, HIS-235    | Z. officinale                                        |
| 4   | Isorubraine                                              | -11.69                    | -                            | A. katsumadai                                        |
| 5   | Panduratin B1                                            | -11.43                    | -                            | B. pandurata Roxb.                                   |
| 6   | Calyxin L                                                | -11.42                    | GLN-91, LYS-101, ASN-103, HIS-235 | A. blepharocalyx                               |
| 7   | (+)-Panduratin A                                         | -11.41                    | -                            | B. pandurata Roxb.                                   |
| 8   | (-)-Krachainz A                                          | -11.41                    | LYS-101                      | B. pandurata Roxb.                                   |
| 9   | Blepharocalyxin D                                        | -11.28                    | LYS-101, HIS-235, TYR-318    | A. blepharocalyx                                     |
| 10  | Boesenbergin A                                           | -11.27                    | LYS-101                      | B. pandurata Roxb.                                   |

ASP-128, GLU-221, and Mg^{2+}. Meanwhile, 5α,8α-epidioxyergosta-6,22-dien-3β-ol interacted with TYR-212 and GLN-186 on IN enzymes. The interaction of 5α,8α-epidioxyergosta-6,22-dien-3β-ol found between its epidioxy chain and TYR-212, as well as the π-π interaction between oxadiazole ring of raltegravir and TYR-212. E. elatior was reported to posses antioxidant, antifungal, antibacterial, cytotoxic, hepatoprotective, and anti-tyrosinase properties, and still no report on HIV-1 activity is available.\[21\] Beside these plants, A. katsumadai, Alpinia pinnanensis, and A. blepharocalyx, were also the potential plants that might have potential compounds for inhibiting the HIV-1 IN enzyme.

On HIV-RT enzyme, A. pahangensis Ridley, B. pandurata Roxb., Z. officinale, A. katsumadai, and
A. blepharocalyx were plants that predicted to have potency in inhibiting HIV-1 RT enzyme. Pahangensin A, a diterpenoid isolated from A. pahangensis Ridley, showed best docked on RT enzyme with the docking energy of -13.76 kcal/mol and showed better interaction compared to nevirapine as well. It was found that the α,β-unsaturated γ-lactone ring possessed hydrogen bonding with LYS-101 at binding site of RT enzyme. Meanwhile, a part of labdanic diterpene inserted into the binding pocket of reverse transcriptase enzyme where nevirapine also interacted by hydrophobic interaction. This hydrogen bonding might contribute to the lowering score of docking energy of pahangensin A yielded for the more stable conformation than native ligand nevirapine. Pahangensin A was reported to possess antibacterial activity, and still no report on HIV-1 activity is available.22

Conclusion
Our docking study found that norditerpene and labdane glycosides, cyclohexenyl chalcone, steroid, and diterpenoids were the natural type compounds, predicted to possess inhibition activity on HIV-1 protein enzymes. Noralpindenoside B and alpindenoside A were suggested as inhibitors for HIV-1 PR enzyme, panduratin E and 5α,8α-epidioxoyergosta-6,22-dien-3β-ol as HIV-1 IN enzyme inhibitors, and pahangensin A as HIV-RT protein enzyme inhibitor.

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Conflicts of interest
There are no conflicts of interest.

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