

Virtual screening of Indonesian herbal database as HIV-1 reverse transcriptase inhibitor

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Abstract:

HIV-1 (Human immunodeficiency virus type 1) is a member of retrovirus family that could infect human and causing AIDS disease. AIDS epidemic is one of most destructive diseases in modern era. There were more than 33 million people infected by HIV until 2010. Various studies have been widely employed to design drugs that target the essential enzymes of HIV-1 that is, reverse transcriptase, protease and integrase. In this study, *in silico* virtual screening approach is used to find lead molecules from the library or database of natural compounds as HIV-1 reverse transcriptase inhibitor. Virtual screening against Indonesian Herbal Database using AutoDock4 performed on HIV-1 reverse transcriptase. From the virtual screening, top ten compounds were mulberrin, plucheoside A, vitexilactone, brucine N-oxide, cyanidin 3-arabinoside, alpha-mangostin, guaijaverin, erycristagallin, morusin and sanggenol N.

Keywords: Indonesian herbal database, HIV-1, molecular docking, virtual screening.

Background:

HIV-1 is a member of retrovirus family that cause AIDS in human. AIDS epidemic is considered as one of the most destructive disease in the modern era. It will make immune system weakened and leading into lethal opportunistic infection [1, 2]. More than 33 million people have been infected by AIDS until 2010 and this number increases approximately by 2-3 million per year [3].

When replicating in the host cell, HIV utilizes vital production enzymes to create mature virions. One of these enzymes is reverse transcriptase (RT). HIV contains RNA genomic in its virion, and within host cell it will be converted by reverse transcriptase to cDNA (complimentary DNA) which in turn will be integrated into the host cell to produce essential protein of new viral maturation [4]. Because of RT essential role in the HIV life cycle, it has been considered as major anti HIV-1 drug targets [5]. FDA clinically approved HIV-1 reverse transcriptase inhibitors could be categorized into nucleoside reverse transcriptase inhibitors (NRTIs), nucleotide reverse

transcriptase inhibitors (NtRTIs), and non-nucleoside reverse transcriptase inhibitors (NNRTIs). NRTIs were the first HIV drug approved for clinical use; it has structure similar to reverse transcriptase's substrate but lack hydroxyl functional group at position 3'. In the other hand, NNRTIs target the binding site of HIV-1 reverse transcriptase directly [6].

HIV has high mutation rate as it lacks the ability to proofread its reverse transcription process. If the mutation occurred at the HIV binding pocket, it will more likely to develop resistances. Because of that reason, there is constant need for new drugs [7, 8]. Novel drugs could be developed from synthetic molecules or natural resources. Natural products have been reported as HIV-1 RT potent inhibitor, most notably from flavonoid groups [9-11]. The development of natural products compound as lead could provide benefits for HIV future treatment.

Indonesia has been considered as one of the most varied biodiversity center in the world, only outranked by Brazil in the first place. There are more than 30,000 plant species in

Indonesia from approximately 40,000 species known on earth. At least 9,600 species have pharmacological activity [12]. Previous research has collected few of the natural product compounds and turns it into database [13]. This database could provide us valuable and potential lead compounds for various diseases and targets, including HIV-1 RT.

Virtual screening is known to be useful method for selecting hits and searching lead from vast database. It has many advantages such as less cost, and time consuming. It also reduces the number of compound to be extracted or synthesized, and assayed by a factor of 10 to 1000 [14]. It could be applied to screen a large set of library compounds and complement HTS technique to identify hits [15]. Because of its applicability, virtual screening will be done to search potential HIV-1 RT inhibitors from herbal database of Indonesia.

The aim of this research was to apply the virtual screening to the Indonesian herbal database as HIV-1 reverse transcriptase inhibitors. This research employed structure based screening (docking) with AutoDock 4 [16] and PyRx [17] as tools of the virtual screening. Potential hits from the natural compounds will be represented as ranks. Top ten compounds will be considered as hits.

Methodology:

This research was done using literature study and virtual screening by molecular docking (structure-based virtual screening). The experimental design applied was as follow.

Preparing the HIV-1 RT protein structure

This research started by selecting HIV-1 reverse transcriptase structure suitable as docking target. The HIV-1 reverse transcriptase structure consists of p66 and p51 subunit was obtained from protein databank website [18]. Inclusion criteria used to choose the target are: wild type HIV-RT, binding with ligand or inhibitor, complete chain information and has resolution less than 2.5 Å. The chosen protein structure was 3LP1, HIV-1 RT bound with NVP (nevirapine) [19]. The macromolecule downloaded as *.pdb file format.

The structure was optimized using VegaZZ [20]. The optimization includes addition of hydrogen with "protein" and "each residue ends" option, separation of solvent molecules and cofactor or ligand continued by adding partial charges (Gasteiger), applying AutoDock forcefield with AutoDockTools in MGLTools 1.5.4.

Preparing Ligand File Format

Indonesian herbal compounds structure were obtained from the Herbaldb [13]. The database 3D structure was generated using VegaZZ script "2D to 3D". This structure then optimized by adding hydrogen with "generic organic" and "after each heavy atom" option, After adding hydrogen to the structure, the minimization was done using steepest descent and conjugate gradient 1,000 steps for each method. The last step was adding Gasteiger partial charges, and applying AutoDock force field with AutoDockTools.

Validating of Molecular Docking Protocol

Using 3LP1 crystal structure containing nevirapine as ligand reference, the ligand binding site coordinates were defined by

AutoDockTools. This binding site will be used as the center target of molecular docking for the virtual screening. The coordinates of binding site center were $x = 10.350$; $y = 14.076$; and $z = 18.252$ in NNRTI pocket.

After defining the binding site, preliminary docking was done to validate the protocol using 14 control compounds given in the **Table 1** (see supplementary material). The control used was referring to the FDA approved drugs. NNRTIs used as positive controls, while protease inhibitors (PIs) and integrase inhibitor as negative controls [21]. Molecular docking was attempted in triplicate using AutoDock 4.

Virtual Screening of Indonesian Herbal Database

Virtual screening was done using PyRx software with best parameters of the control compounds orientation docking. Grid parameter used was 50 x 50 x 50 units with 0.375 Å per unit. Docking parameter was set at 250,000 calculations; 27,000 generations; 150 populations; mutation and crossover rate at default. Virtual screening was applied five times.

Refinement Analysis

Top ten compounds screened were refined by afterward docking. The parameter was set at 2,500,000 calculations. Best pose then analyzed to observe its binding site and interactions using PyMOL [22]. Binding site was defined as residues in proximity of 5 Å from the docked ligand pose.

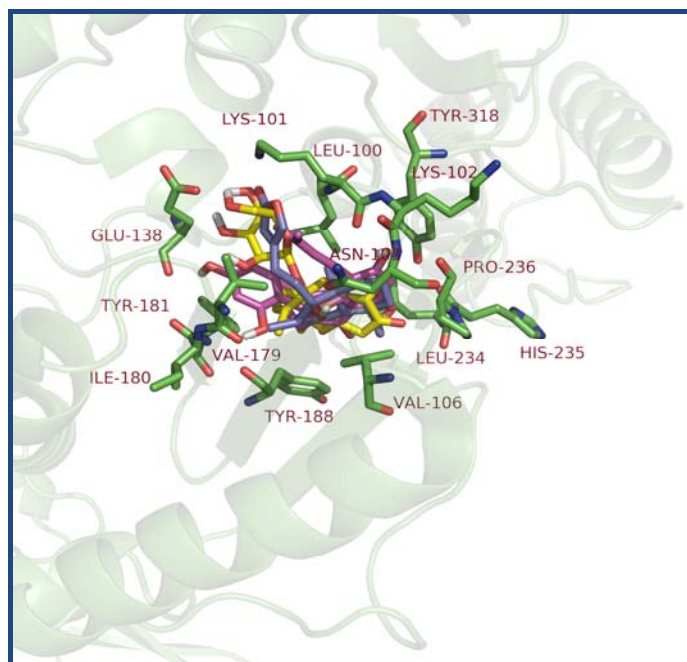


Figure 1: Best pose of top three rank from virtual screening with HIV-1 RT. Magenta: 1st rank (mulberrin), yellow: 2nd rank (plucheoside A), bluish purple: 3rd rank (vitexilactone).

Results & Discussion:

The preliminary docking was done three times (N=3) with 14 control compounds listed. Five compounds as positive controls which were known to have inhibition activity to HIV-1 RT: etravirine, nevirapine, rilpivirine, efavirenz and delavirdine. The docking results were ranked based on its best docked binding energy and shown in (**Table 1**). Using optimized parameter, positive controls outrank all negative controls.

After obtaining the parameter from preliminary docking, virtual screening was applied to the Indonesian Herbal Database (<http://herbaldb.farmasi.ui.ac.id>) [13]. Top ten compounds were given in the **Table 2 (see supplementary material)**. The average binding energies varies from -11.28 kcal/mol to -10.36 kcal/mol. From five virtual screening runs, three of the compounds were consistently ranked as top ten from each run. The three compounds are vitexilactone, brucine N-oxide, and morusin. The first ranked compound, mulberrin, alongside with morusin and sanggenol N (ranked 9 and 10, respectively) were flavonoids from Moraceae family, notably from *Artocarpus fretessi*, *A. gomezianus* Wallich ex Trexcul, *A. heterophyllus*, *Morus alba*, *M. australis* Poir, and *M. mongolica* [13, 23-27]. Recent research shows that flavonoids from *Morus alba* (e.g morusin, kuvanon H and morusin-4'-glucoside) have anti-HIV activities [28]. However, we have not found any research that shows the anti-HIV activities of sanggenol N.

Plucheoside A ranked as second compound. It is terpene glycoside from *Pluchea indica* [13, 23, 29]. Locher *et al* have studied the anti HIV-1 activities of Hawaiian plants. From that research, it was concluded that *Pluchea indica* could inhibit HIV-1 activities, but the mechanism of inhibition was not revealed further [30]. Compound ranked on third position is vitexilactone, a diterpene from *Vitex cannabifolia*, *V. cannabinifolia*, *V. trifolia* and *Tinospora rumphii* [13, 23, 31-33]. This plant is known well as a treatment for HIV-AIDS and shown anti HIV-1 RT activities [34]. However, there was not any recent research that directly connects vitexilactone with HIV-1 RT inhibitor activities.

Brucine N-oxide as fourth ranked compound is an indole alkaloid from *Strychnos atlantica*, *S. lucida* R. Br, *S. Spinosa*, and *S. wallichiana* [13, 23, 35]. The compound itself has no recent study which shows its activities as HIV inhibitor. Nevertheless, indole alkaloids have been researched because of its antiviral activities. Some research also show indole derived alkaloid activities as anti HIV, such as the study of derivatives of isatisine A done by Ji-Feng Liu *et al* which shown moderate anti-HIV activity [36]. The appearance of brucine N-oxide as hits could suggest us a possibility of new indole alkaloids to be anti HIV-1 RT lead compound.

Cyanidin 3-arabinoside is a flavonoid compound that spreads among plants of Ericaceae familia such as *Empetrum nigrum* and *Vaccinium spp.* Also spread amongst other plant like *Theobroma cacao*, *Malus sylvestris*, *Mangifera indica*, and *Camellia japonica* [13, 23, 37, 38]. The aglycone of this compound, cyanidin and its derivatives predicted to have antiretroviral activities [39, 40].

Alpha-mangostin, ranked sixth, is a xanthone compound from *Allanbackia monticola* STANER L.C., *Garcinia cowa*, *G. dulcis*, *G. echinocarpa*, *G. fusca*, *G. mangostana*, *G. terpnophylla*, and *Cratoxylum cochinchinense* [13, 23, 41-46]. Mangostin was known for its anti HIV activities, but the suggested inhibition mechanism was noncompetitive HIV protease inhibitor as shown in study done by Chen *et al* [47].

Guaijaverin is a 3-arabinopyranoside glycoside of quercetine from *Psidium guajava*, *Taxodium distichum*, and Ericaceae familia [13, 23, 48]. Eventhough it is more famous for its bacteriostatic and antiplaque activities, its aglycone was also exhibit antiviral

and HIV-1 inhibitor [10, 49], erycristagallin is a pterocarpane from *Erythrina* genus plants such as *Erythrina orientalis* [13, 23]. The plant species of *Erythrina abyssinica* was suggested to have anti-HIV activities [50]. However, no recent research seems to directly relate the plant activities with erycristagallin.

Out of top ten compounds, five compounds were belong to flavonoid group or its glycoside. This could give a new view on potential development of flavonoids derived compound as anti-HIV by their inhibition mechanism of HIV-1 reverse transcriptase. A research in the future should be done to refine and enhance the better criteria for making flavonoid as HIV-1 RT inhibitor lead compound.

Mulberrin, morusin, sanggenol N, erycristagallin, and plucheoside A were amongst the top ten compounds from plants that show anti HIV-activities but not specifically show RT inhibitor activities. Further *in vitro* anti-RT research should be done with these compounds to find the direct connection between the plants' compounds with their activities as anti-HIV. Anti-HIV activities of brucine N-oxide and its source plant never been reported on recent research. But as a member of indole alkaloid group, it still show some promise to be researched by *in vitro* study as a member of indole alkaloid group. Alpha mangostin, vitexilactone, guaijaverin and cyanidin 3-arabinoside has shown anti HIV activities from previous experiment, but they could be developed further by initiating medicinal chemistry studies to improve the molecules as leads.

From further docking of the top ten compounds, we can find residues contributing as binding site and might be referred in future research. The interaction analysis of these compounds was observed on docked pose with lowest energy. All the compounds in docking process involves in hydrogen interaction with Lys101, where it serves as the hydrogen bond donor or acceptor. Some of the compounds have more than one hydrogen bond with Lys101 notably cyanidin 3-arabinoside, guaijaverin, and sanggenol N. Pose of top three best dock compound shown at (**Figure 1**).

Other residues that likely to have hydrogen bond with more than one top ranked compound in the best docking pose were as follow: Asn103 with vitexilactone, cyanidine 3-arabinoside, and guaijaverin; Val179 with mulberrin; Tyr181 with guaijaverin, morusin and sanggenol N; Tyr318 with mulberrin and plucheoside A; and also Glu318 from chain B with mulberrin, plucheoside A, cyanidine 3-arabinoside, and erycristagallin.

Residues that consistently within 5 angstrom boundaries from docked ligand were Leu100, Lys101, Lys102, Asn103, Val106, Val179, Ile180, Tyr181, Tyr188, Leu234, His235, Pro236, Tyr318 of p66 subunit in chain A and Glu138 p51 subunit in chain B of HIV-1 RT. This could suggest that the p51 subunit also contribute as part of the NNRTI binding site.

Conclusion:

Ten potential inhibitors against HIV-1 RT obtained from the virtual screening of Indonesian herbal database such as mulberrin, plucheoside A, vitexilactone, brucine N-oxide, cyanidin 3-arabinoside, alpha-mangostin, guaijaverin,

erycris tagallin, morusin and sanggenol N provide an opportunity for further investigation as lead compounds.

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Supplementary material:

Table 1: Docking result of control compounds using AutoDock with HIV-1 RT as target

Rank based on ΔG	Name	ΔG (kcal/mol) (N = 3)	SD (kcal/mol)	CV (%)
1	Etravirine*	-8.82	0.030	0.340
2	Nevirapine*	-7.85	0.010	0.127
3	Rilpivirine*	-7.61	0.221	2.897
4	Efavirenz*	-7.33	0.026	0.361
5	Delavirdine*	-6.72	0.516	7.679
6	Raltegravir**	-4.49	2.328	51.887
7	Amprenavir**	-0.49	2.829	573.403
8	Tipranavir**	5.37	8.278	154.146
9	Darunavir**	6.55	7.410	113.135
10	Nelfinavir**	15.82	14.480	91.528
11	Lopinavir**	83.82	39.737	47.406
12	Saquinavir**	160.33	69.265	43.202
13	Ritonavir**	175.55	82.990	47.273
14	Atazanavir**	294.38	69.715	23.682

*Positive control used for this research was etravirine, nevirapine, rilpivirine, efavirenz, and delavirdine. These are member of HIV-1 NNRTIs (nonnucleoside reverse transcriptase inhibitors).

**Negative control used for this research were raltegravir (member of integrase inhibitor), amprenavir, tipranavir, darunavir, nelfinavir, lopinavir, saquinavir, ritonavir, and azatanavir (member of protease inhibitors)

Table 2: Top ten compounds docked by AutoDock based on its binding energy with HIV-1 RT

Rank	Name	ΔG (kcal/mol)	N	SD (kcal/mol)	CV (%)	Plant(s) source [13, 23]
1	Mulberrin	-11.28	2	0.3041	2.697	<i>Artocarpus fretessi</i> , <i>A. gomezianus</i> Wallich ex Trecul, <i>A. heterophyllus</i> , <i>Morus Alba</i> , <i>M. australis</i> , <i>M. mongolica</i>
2	Plucheoside A	-10.82	4	0.3293	3.044	<i>Pluchea indica</i>
3	Vitexilactone	-10.74	5	0.0438	0.408	<i>Vitex cannabifolia</i> , <i>V. cannabinifolia</i> , <i>V. trifolia</i> , <i>Tinospora rumphii</i>
4	Brucine N-oxide	-10.70	5	0.0614	0.574	<i>Strychnos atlantica</i> , <i>S. lucida</i> R. Br., <i>S. spinosa</i> , <i>S. wallichiana</i>
5	Cyanidin 3-arabinoside	-10.66	4	0.1800	1.689	<i>Mangifera indica</i> , <i>Acrotriche serrulata</i> , <i>Empetrum nigrum</i> , <i>Epacris gunnii</i> , <i>Gaylussacia</i> spp., <i>Leucopogon collinus</i> , <i>Rhododendron</i> spp., <i>Vaccinium padifolium</i> , <i>Sterculia parviflora</i> , <i>Theobroma cacao</i> , <i>Penstemon</i> spp, <i>Phalaris arundinacea</i> , <i>Polygonum</i> spp, <i>Aronia melanocarpa</i> , <i>Malus sylvestris</i> , <i>Cinchona ledgeriana</i> , <i>Saxifraga</i> spp, <i>Camelia japonica</i>
6	Alpha-mangostin	-10.51	4	0.2130	2.028	<i>Allanblackia monticola</i> STANER L.C., <i>Garcinia kowa</i> , <i>G. dulcis</i> , <i>G. echinocarpa</i> , <i>G. fusca</i> , <i>G. Mangostana</i> , <i>G. terpnophylla</i> , <i>Cratoxylum cochinchinense</i>
7	Guaijaverin	-10.49	3	0.1270	1.210	<i>Foeniculum vulgare</i> , <i>Arctostaphylos uva-ursi</i> , <i>Calluna vulgaris</i> , <i>Chamaedaphne calyculata</i> , <i>Richea angustifolia</i> , <i>R. scoparia</i> , <i>Hypericum erectum</i> Thunb., <i>Hibiscus mutabilis</i> , <i>Theobroma cacao</i> L., <i>Eucalyptus cypellocarpa</i> , <i>Psidium guajava</i> , <i>Securidaca diversifolia</i> , <i>Polygonium aviculare</i> , <i>Zanthoxylum bungeanum</i> , <i>Taxodium distichum</i>
8	Erycristagallin	-10.43	3	0.3402	3.261	<i>Erythrina abyssinica</i> , <i>E. crista-galli</i> , <i>E. orientalis</i> , <i>E. subumbrans</i> , <i>E. variegata</i>
9	Morusin	-10.43	5	0.3290	3.155	<i>Artocarpus fretessi</i> , <i>Morus alba</i> , <i>M. australis</i> , <i>M. mongolica</i>
10	Sanggenol N	-10.36	4	0.1668	1.611	<i>Morus australis</i>