In silico identification of potent inhibitors of heat shock protein 90 (Hsp90) from Indonesian natural product compounds as a novel approach to treat ebola virus disease

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\textbf{Abstract.} Heat shock protein 90 (Hsp90) is a 90-kDa molecular chaperone that has various biological functions, varying from cell cycle progression to the protein folding, that is crucial for the cancer cell development. Furthermore, the activity of Hsp90 is also essential for the replication of negative-stranded viruses as the host factor, including Ebola virus (EBOV), a virus from \textit{Filoviridae} family which is responsible for causing Ebola virus disease (EVD) outbreak in Africa in 2014. Thus, the inhibition of Hsp90 can be considered as the novel approach to combat EVD. In this research, we deployed an Indonesian natural products database to perform in silico ADME-Tox screening test and a series of molecular docking simulations against the Hsp90. A total of 3,429 ligands that have been docked, about thirteen ligands have outstanding pharmacological properties, and higher binding affinity in the binding site of Hsp90 than four referred standard ligands. In the end, we conclude that 1-O-galloyl-6-O-luteoyl-\alpha-D-glucose, euphorbianin and scutellarein 7-neohesperidoside as the best Indonesian natural product compound to inhibit Hsp90, suggesting a potential candidate to treat EVD effectively.

\textbf{Keywords:} Molecular docking simulation, Ebola virus (EBOV), Heat shock protein (Hsp90), Indonesian natural product, in silico ADME-Tox screening test

\section{1. Introduction}

Ebola Virus (EBOV) is categorized as ebolavirus genus in the \textit{Filoviridae} family. Ebola was identified in 1976 until now Ebola called as deadly hemorrhagic fever [1]. In West Africa on 2016 reported that Ebola virus caused more than 28,000 cases and more than 11,000 people who died because of this virus. Currently, no approved drug to cure EBOV infection, but there is still developing in the search for drugs to cure this disease which done by inhibiting Hsp90 as protein target that is showing promising results significantly reduced the replication of EBOV [2].

Heat shock protein 90 (Hsp90) is an ATP-dependent molecular chaperone which is essential in eukaryotes. It is required for the activation and stabilization of a wide variety of client protein, and many of them are involved in important cellular pathways. Since Hsp90 affects numerous physiological processes such as signal transduction, intracellular transport, and protein degradation, it became an appealing target for cancer therapy [3]. Recently, Hsp90 was shown to be an important host factor for the replication of negative-strand viruses [4]. In addition, the inhibition of Hsp90 has been shown to block vaccinia virus replication by interaction with the viral core protein 4a in the cytoplasm [5].
Computer-aided drug design and development (CADD) has become one of the most progressive research in many fields [6]. Recently, with CADD, we can find the drug candidate to cure several diseases by in silico method using a computer [7]. The advantage of this method for its reduced time consuming and cost-efficient technique [8]. Several studies have shown that there were new drug candidates which can be used. Natural products (NP) have the potential to be candidates that have interesting bioactivities [9]. As the second for biodiversity after Brazil, Indonesia have more than 30,000 plant species which is 9,600 from various plants species have pharmacological activities [10]. Thus, the research objective was to find the drug candidate to inhibit Hsp90 protein which can transform Ebola becomes Ebola virus (EBOV) using natural products compounds as the inhibitor for this protein.

2. Material and Methods
The methodology of this research was taken and modified based on the previously established research [11, 12]. This research used several offline software; DataWarrior v.4.6.1 [13], and Molecular Operating Environment (MOE) 2014.09 [14] software. For online access, the HerbalDB (http://herbaldb.farmasi.ui.ac.id), which contains the Indonesian natural product compounds was utilized [10], along with other references that cited several Indonesian natural product compounds [15-31]. Firstly, both standard and NP ligands, which collected from HerbalDB, were screened through a computational ADME-Tox scheme using DataWarrior v.4.6.1, before prepared and optimized further using MOE 2014.09 software. Then, the 3D structure of EBOV was done using MOE 2014.09 software as well. Finally, the molecular docking simulation was conducted to determine the best protein-ligand complexes based on the Gibbs free binding energy (AGbinding) value, Root-Mean-Square Deviance (RMSD) score and molecular interactions compared to the standard ligand.

3. Results and Discussions

3.1. Computational ADME-Tox Screening Test
About 3,429 Indonesian natural product compounds were collected from HerbalDB and several literature sources; all ligands must through ADME-Tox screening test using DataWarrior v.4.6.1 software to eliminate the unwanted ligands who have druglikeness score x<0. Moreover, the prediction of toxicity test was also performed to determine the potential drug candidates based on their mutagenic, tumorigenic, irritant and reproductive effective. in the end, about 527 Indonesian natural product ligands were retrieved and underwent the pre-docking simulation phase.

3.2. Pre-docking Simulation
3.2.1. Preparation of the Standard Ligand and the Indonesia Natural Product. In this study, the remaining Indonesian natural product ligands that passed the previous ADME-Tox screening test underwent the ligand preparation process, which was carried out using MOE 2014.09 by washing, optimizing, and minimizing energy from the 3D structure of all Indonesian natural product ligands. This preparation was performed by using MMFF94x forcefield with the Gas Phase as the solvation mode.

3.2.2. Preparation of Heat Shock Protein (Hsp90). In this study, 3D protein structure of Hsp90 was downloaded online from RCSB-PDB database (http://www.rcsb.org/pdb/home/home.do) with PDB ID: 1YET. The structure that has been downloaded was saved in a .pdb format. Next step is the removal of water solvent molecules (H2O), washing, optimizing, and minimizing energy from the 3D structure of Hsp90 protein using MOE 2014.09 software. This preparation using AMBER10:EHT force field with the Gas Phase as the solvation mode.

3.3. Molecular Docking Simulation
In molecular docking, simulation of this step is done through MOE 2014.09 software. Molecular docking described when the protein pose rigidly while the ligand is free to move to find a stable conformation [32]. In this study, the first screening of molecular docking about 527 best ligands was
performed with retain value of 30 without duplication. This retains the best number of ligand-protein complexes positions based on the Gibbs free binding energy ($\Delta G_{binding}$) value, Root-Mean-Square Deviance (RMSD) score and molecular interactions compared to the standard ligand result in 290 best ligands. Furthermore, the second screening was carried out to improve ligand conformation aberration with retain 100 result in 13 best ligands. The $\Delta G_{binding}$ generated in the molecular docking process is the value of affinity of the ligand for the protein in equilibrium when both attached as protein-ligand complexes. $\Delta G_{binding}$ also show the stability and strength of interactions possessed by ligands and proteins. The lower the Gibbs free energy value, the conformation and interaction created between ligands and proteins will be more stable and thermodynamically preferred. Other than that, RMSD value shows the difference in the distance of an atom in a protein to its rigid shape.

Out of 527 ligands that have been docked through this simulation, about thirteen ligands have outstanding pharmacological properties, and higher binding affinity in the binding site of Hsp90 than four referred standard ligands. In the end, we conclude that 1-O-galloyl-6-O-luteoyl-α-D-glucose, euphorbianin and scutellarein 7-neohesperidoside as the best Indonesian natural product compound to inhibit Hsp90 compared with Herbimycin as the standard ligand, suggesting a potential candidate to treat EVD effectively. This molecular docking simulation shows that the best 3 Indonesian natural product ligands were found lower binding energy than herbimycin but scutellarein 7-neohesperidoside have little bit higher value of RMSD than herbimycin. The result of the best thirteen Indonesian natural product ligands from this molecular docking simulation can be seen in Table 1.

**Table 1.** The Indonesian natural product to inhibit Hsp90 Protein based on the molecular docking simulation

| No | Molecules Name                                                                 | $\Delta G_{binding}$ (kcal/mol) | RMSD (Å) | Hydrogen Bonds |
|----|--------------------------------------------------------------------------------|---------------------------------|----------|----------------|
| 1  | Lissoclibadin 1                                                                | -12.8534                        | 1.8395   | 1              |
| 2  | 1-O-galloyl-6-O-luteoyl-α-D-glucose                                             | -12.3162                        | 0.8716   | 7              |
| 3  | Euphorbianin                                                                  | -12.2362                        | 1.7774   | 4              |
| 4  | 3′,4″-O-Diacetylaflzelin                                                        | -11.5048                        | 1.5239   | 3              |
| 5  | Nagelamide B                                                                   | -11.0759                        | 1.7596   | 1              |
| 6  | ax-4″-OH-3′-methoxymaysin                                                        | -10.9883                        | 1.5810   | 2              |
| 7  | Scutellarein 7-neohesperidoside                                                 | -10.9485                        | 1.8336   | 5              |
| 8  | Kaempferol-3-O-(2,3,4-tri-O-acetyl-α-L-rhamnopyranoside)                        | -10.7651                        | 1.2081   | 1              |
| 9  | Guajavin B                                                                     | -10.7174                        | 1.8186   | 4              |
| 10 | Gambiriin A3                                                                   | -10.6684                        | 1.8388   | 5              |
| 11 | Lissoclibadin 3                                                                | -10.6614                        | 1.8175   | 2              |
| 12 | Kaempferol-3-O-(2,3-di-O-acetyl-α-L-rhamnopyranoside)                           | -10.5603                        | 1.5618   | 1              |
| 13 | Lissoclinotoxin F                                                              | -10.4228                        | 1.9914   | 3              |
| S1 | Herbimycin                                                                     | -10.3121                        | 1.7426   | 3              |
| S2 | Gambogic Acid                                                                  | -10.3067                        | 1.9008   | 2              |
| S3 | Geldanamycin                                                                   | -9.5666                         | 0.0000   | 2              |
| S4 | Epigallocatechin gallate (EGCG)                                                 | -9.2348                         | 1.1238   | 4              |

In addition to free binding energy, molecular interaction between the ligands and binding pocket of Hsp90 protein in another important aspect for determining whether the ligand can be considered as an appropriate inhibitor or not. First, molecular interaction between standard ligand and Hsp90 protein was
observed as it can be seen from the Fig. 1. Herbamycin as the standard ligand has hydrogen bonds interaction in Lys58, Asn 51 and Asp93 with sidechain of their respective amino acids. Second, molecular interaction between 1-O-galloyl-6-O-luteoyl-α-D-glucose and Hsp90 protein was observed as well in Fig. 2. The hydrogen bond of 1-O-galloyl-6-O-luteoyl-α-D-glucose and Hsp90 protein is formed in Asn106 and Gly97 with the backbone of their amino acids, also hydrogen bonds formed in Asp93 and Lys112 with sidechain of their amino acids. Moreover, then, the interaction from Fig. 3 was observed too. The hydrogen bond was formed between euphorbianin and Hsp90 protein in Gly137, Gly135, Phe138 with the backbone of their amino acids, and in Asp93 with sidechain of their amino acids. Moreover, the last is Fig. 4. The hydrogen bond was formed too in Asn51, Asp54, Asn106, Asp93 with sidechain of their amino acids.

Figure 1. The 2D (a) and 3D (b) molecular interaction visualization of Hsp90 protein and Herbimycin ligand

Figure 2. The 2D (a) and 3D (b) molecular interaction visualization of Hsp90 protein and 1-O-galloyl-6-O-luteoyl-α-D-glucose ligand
4. Conclusions
Finding the best drug candidate using CADDD has been major research by today. Recently, with CADDD, we can find the drug candidate to cure several diseases by in silico method. The advantage of this method for its reduced time consuming and cost-efficient technique. Around 3,429 Indonesian natural product compounds underwent ADME-Tox screening prediction test and molecular docking simulations to find an alternative inhibitor for Hsp90 protein. Based on the results of this study, 1-O-galloyl-6-O-luteoyl-α-D-glucose, euphorbianin and scutellarein 7-neohesperidoside as the best Indonesian natural product compound to inhibit Hsp90 compared with herbimycin and other compounds as the standard ligands. In the end, these result have still needed to be tested further through molecular dynamics simulation to determine its ligand-Hsp90 complex stability under real biological environment before being tested into the wet experiment.
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