Revealing Potency of Bioactive Compounds as Inhibitor of Dengue Virus (DENV) NS2B/NS3 Protease from Sweet Potato (*Ipomoea batatas* L.) Leaves

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Abstract

This study aims to identify the potency of bioactive compounds of sweet potato leaf as inhibitory agent to dengue virus (DENV) NS2B/NS3 protease by using computational study. The 3D structure of NS2B/ NS3 protease was collected from PDB and the 2D structure of several bioactive compounds of sweet potato leaf were obtained from PubChem. The visualization and data analysis were performed by using the PyMol software. According to the *in silico* analysis, result demonstrated that dehydroabietinol had the lowest free energy binding. However, based on the protein-ligand analysis, all the compounds showed the hydrogen bond and hydrophobic interaction. All the compounds with hydrogen bond could not be interacted with catalytic domain, but hydrophobic interaction could be interacted to the target domain via Ser135 by δ -Selinene and His51 by α -Caryophyllene. In summary, we conclude that δ -Selinene and α -Caryophyllene might have potencies as a therapeutically drug for dengue.

Keywords: Dengue virus, in silico, Ipomoea batatas, protease

Introduction

Indonesia is a tropical country and home of mosquito vector species of dengue virus (DENV), *Aedes aegypti* and *Aedes albopictus*^[1]. DENV is infectious agent dengue fever-causing epidemic diseases, current antiviral drug cannot succeed against this entity. Therefore, the development of the drug is required to treat DENV infection^[2]. DENV is member family from *Flaviviridae* and consist of four serotypes (DENV 1-4) ^[3].

Corresponding Authors: Yulanda Antonius yulandaantonius@staff.ubaya.ac.id Muhammad Badrut Tamam mh.badruttamam@gmail.com Indonesia is a large country in Southeast Asia and has a high plant diversity in the world. There are more than 5,000 medicinal plants that available all around us^[4]. Consequently, medicinal plants used by its population in curing many diseases^[5,6]. The medicinal plants generate a variant of chemical composition with the potency to prevent viral replication and probable resource for controlling viral infection^[7]. Plants have been described to have antiviral action and some have been accustomed to manage viral taints in humans and animals^[8]. Medicinal plants were found for antiviral compounds, such as Convolvulaceae^[9].

DENV is a single stranded and positive polarity RNA virus with a genome of about 11,000 bases in length. The genome encodes for three structural proteins and nonstructural proteins, its activated by trypsin-like NS2B/ NS3 protease. This enzyme has a catalytic triad domain, it contains a specific amino acid residual His51, Asp75, and Ser135 required for catalytic activity^[10]. Protease complex NS2B/NS3 have a key role in viral replication and make it's as a therapeutic target to development of protease inhibitor for DENV infection^[11]. In addition, sweet potato bioactive compounds reported as a potent inhibitor of the virus^[12]. Previous research by Pochapski *et al.* (2011) explained that chemical compound contained in sweet potato have potency as a drug but this mechanism action is unknown^[13]. Therefore, we identified the potency of bioactive compounds of sweet potato leaf as inhibitory agent to DENV NS2B/NS3 protease by using computational study.

Materials and Methods

Collection of NS2B/NS3 serine protease

The target protein in this research is DENV NS2B/ NS3 protease. Therefore, the 3D structure of NS2B/NS3 serine protease was obtained from protein database or RCSB with ID 2FOM. Furthermore, the protein was validated the model quality by using Ramachandran plot.

Collection of sweet potato leaves compounds

Various bioactive compounds from sweet potato leaves referred to previous research^[14] were retrieved from PubChem. The 3D structure of compound has collected in structure data format (SDF). Therefore, it must be converted by Open Babel software to produce flexibility 3D structure with protein data bank (PDB) format.

Molecular docking of NS2B/NS3 serine protease with sweet potato leaves compounds

Screening the potency of sweet potato leaves compounds by using virtual screening was conducted with molecular docking. There are several methods which could be applied by using molecular docking, such as specific docking and blind docking^[15,16,17]. This research was conducted by using blind docking by PyRx software to identify the potency of bioactive compound from sweet potato leaves as inhibitor NS2B/NS3.

Visualization of interaction between NS2B/NS3 serine protease with sweet potato leaves compounds

Then, we were using PyMol to visualize 3D liganprotein structure of NS2B/NS3 serine protease and various compounds of sweet potato. Furthermore, those interaction were analyzed by using LigPlot to compared chemical interaction^[18,19].

Results and Discussion

DENV is a single stranded and positive polarity RNA virus with a genome of about 11,000 bases in length. The genome encodes for three structural proteins and nonstructural proteins, its activated by trypsin-like NS2B/NS3 protease. This enzyme has a catalytic triad domain, it contains a specific amino acid residual His51, Asp75, and Ser135 required for catalytic activity^[10,11]. Protease complex NS2B/NS3 have a key role in viral replication and make it's as a therapeutic target to development of protease inhibitor for DENV infection.

The 3D structure has been obtained from PubChem, around thirty-six of a bioactive compound that's contained in the leaf essential oil of sweet potato, then its minimize by Open Babel because then its minimize by Open Babel because it's will making this ligand have lowest binding energy and generate structure flexibility NS2B/NS3 serine protease (2FOM) obtain from PDB, and then validating structure has been done using RAMPAGE, the result of structure validation showed by Ramachandran plot. Structure validation aims to evaluate the structure quality of targeted protein which is quantify by the number of favored amino acid that reach more than 90%^[19]. In this study, targeted protein was visualized based on surfaces structure. Furthermore, the NS2B/NS3 structure is arranged from two chain, A and B which contain α -helix, β -sheet, and coil.



Figure 1. Catalytic triad visualization on NS3/NS2B DENV. Targeted protein was shown in transparent surfaces structure (cyan) and cartoons (green), while the catalytic triad as drug target shown in stick structure colored in red to distinguish the drug target domain with the others.

Molecular docking is a part of in silico analysis that has main objective to identify the energy binding size and the interaction pattern between protein and its ligand according to the chemical interaction within the complex^[16]. Molecular docking has performed by PyRx software to know binding affinity level with grid positions x: -0,4637 y: -15,1662 z: 16,1087 and dimensions (Å) x: 52,1952 y: 56,6277 z: 46,8960 reffer to catalytic site domain on NS2B/NS3 serine protease with amino acid residual is His51, Asp75, and Ser135, that be visualized by PyMol software as stick in cartoon structure with surface on target protein (Figure 1). After we know the positions of the catalytic site, then docking grid was directed to its. Docking result indicated that compound has a lowest binding affinity is dehydroabietinol around -7,1 kcal/mol. We revealed another results, such as abietadiene (-6.9 kcal/mol), cembrene (-6.6 kcal/mol), δ -selinene (-6.5 kcal/mol), δ-cadinene (-6.4 kcal/mol), spathulenol (-6.2 kcal/mol), \beta-cuvebene (-6.0 kcal/ mol), γ -gurjunene (-5.9 kcal/mol), α -bergamotenol (-5.8 kcal/mol), β-caryophyllene (-5.8 kcal/mol),

β-elemene (-5.8 kcal/mol), allo-aromadendrene (-5.7 kcal/mol), β-chamigrene (-5.7 kcal/mol), β-panasinsene (-5.7 kcal/mol), trans- α -bergamotene (-5.7 kcal/mol), caryophyllene oxide (-5.6 kcal/mol), y-elemene (-5.6 kcal/mol), longifolene (-5.6 kcal/mol), α-caryophyllene (-5.5 kcal/mol), α-thujene (-5.5 kcal/mol), octadecanoic acid (-5.5 kcal/mol), bicyclogermacrene (-5.4 kcal/mol), limonene (-5.3 kcal/mol), terpinen-4-ol (-5.3 kcal/mol), eugenol (-5.2 kcal/mol), n-hexadecanoic acid (-5.2 kcal/ mol), α-farnesene (-5.1 kcal/mol), α-santalol (-5.1 kcal/ mol), y-terpinene (-5.1 kcal/mol), p-cymene (-5.1 kcal/ mol), p-menth-1-ene (-5.1 kcal/mol), bicycloelemene (-5.0 kcal/mol), α-pinene (-4.8 kcal/mol), β-pinene (-4.8 kcal/mol), cis-sabinene (-4.8 kcal/mol), and 1-octen-3-Ol (-4.5 kcal/mol). Binding affinity is energy bonding formed from the interactions some molecule with other, several parameters affect binding affinity such as amino acid residues and type of chemical interaction between ligand-protein such hydrogen, hydrophobic, and Van der Waals. Binding affinity commonly used as indicator of binding energy to determine the complex interaction which is considering as biological activity outcome^[16].



Figure 2. Targeted domain and δ-Selinene interaction. The ligand was shown as yellow stick structure, while the specific residual amino acid was shown as red color. The hydrophobic interaction was occurring in Ser135 area shown in black circle.

Based on results of molecular docking, a bioactive compound from sweet potato leaves have a potency to bind NS2B/NS3 with lowest binding affinity and possible formed stable complex of ligand-protein. The amount of free energy (ΔG) indicating as an ability of binding for a bioactive compound to the target protein. In this condition, the ligand bind to target protein and making of energy alteration such Gibbs free energy (ΔG), it has negative value when the system in equilibrium condition with constant pressure and temperature, because the widely of protein-ligand association can be determined by negative ΔG , so its determination of the proteinligand complex or ligand binding affinity^[20].



Figure 3. The α-Caryophyllene interaction to targeted domain. The ligand was shown as purple stick structure, while the red color is the residual amino acid in NS2B/NS3. The hydrophobic interaction was occurring in Ser135 which shown inside the black circle.

Potency as inhibitor NS2/NS3 serine protease in the oil essential of sweet potato leaves showing dehydroabietinol have lowest free energy binding, but refer to the result of analysis of protein-ligand domain interaction of the all compound showing, it's had two type of chemical interaction between ligand with protein domain as hydrogen bond and hydrophobic. All of the compounds have hydrogen bond cannot be interacted with catalytic domain, but hydrophobic interaction can be interacted to target domain, via Ser135 by δ -Selinene (Figure 2) and His51 by α -Caryophyllene (Figure 3). Catalytic triad (Ser135, His51, and Asp75) can be found in NS2B/NS3 and required to protease activity which functions as a mechanism for activation of DENV replication^[11]. So, it is predicted that the two compounds are very possible to interact with the catalytic site domain in NS2B/NS3. Therefore, it can be potentially as an inhibitor compare to dehydroabietinol which has lower binding affinity without chemical interaction site in targeted domain in NS2/NS3 serine protease.

Conclusion

In summary, the δ -Selinene and α -Caryophyllene are predicted to have potency as dengue disease medication through the inhibitory mechanism against DENV. Specifically, the ligands are binding to one of catalytic triad of residual amino acid. This interaction inhibits the targeted protein activation when the virus replication occurs.

Conflict of Interest: The author declare that they have no conflict of interest.

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Ethical Approval: No ethical approval needed.

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