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*Research Article*

# Molecular Docking Study: *Phyllanthus niruri L.*'s Active Compounds as Dengue Haemorrhagic Fever Therapy

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**Abstract**

Dengue Haemorrhagic Fever is a disease caused by the dengue virus through a mosquito vector Aedes aegypti. NS3 Helicase is known as one of nonstructural proteins consisting of some essential enzymes for virus replication. Nowadays ivermectin has been developed as an anti-dengue haemorrhagic fever with therapy target NS3 Helicase. The therapeutics drug for dengue haemorrhagic fever has not been found specifically. Methanol extract of meniran (*Phyllanthus niruri L.*) reported the activity to dengue virus with a concentration of 15,63 µg/mL. This research aimed to study the interactions and affinity of the active compound of meniran with the receptor (NS3 Helicase) and to know ADME and toxicity profile. From 56 active compounds of meniran, there was one best candidate as dengue haemorrhagic fever therapy which has energy binding (ΔG) and Inhibition Constanta (IC) lower than native ligand and ivermectin, it is nirurin with energy binding -4.87 kcal/mol. These candidate compounds have good absorption and distribution profiles so they are thought to be candidates for dengue fever therapy by targeting the NS3 Helicase receptor which is better than ivermectin and native ligands.

**Keywords:** Ivermectin, meniran, molecular docking, NS3 Helicase.

# Studi Docking Molekuler: Senyawa Aktif Phyllanthus niruri L. sebagai Terapi Demam Berdarah Dengue

**Abstrak**

Demam berdarah merupakan penyakit yang disebabkan oleh virus dengue melalui vektor nyamuk Aedes aegypti. NS3 Helicase diketahui merupakan salah satu protein non struktural yang terdiri dari beberapa enzim penting untuk proses replikasi virus dengue. Saat ini ivermektin tengah dikembangkan sebagai kandidat obat anti demam berdarah dengan menjadikan NS3 Helicase sebagai target terapinya. Pengobatan demam berdarah hingga saat ini belum ditemukan secara spesifik. Dilaporkan ekstrak metanol meniran memiliki aktivitas antivirus terhadap virus dengue dengan konsentrasi 15.63 µg/mL. Penelitian ini bertujuan untuk mengkaji interaksi serta afinitas senyawa aktif meniran (*Phyllanthus niruri L.*) dengan reseptor penyakit demam berdarah (NS3 Helicase) serta melihat profil ADME dan toksisitasnya. Dari ke-56 senyawa aktif meniran diambil satu senyawa kandidat terbaik yang memiliki energi ikatan (ΔG) dan konstanta inhibisi (KI) yang lebih rendah dari ligan alaminya yaitu nirurin dengan nilai energi sebesar -4.87 kkal/mol. Kandidat senyawa tersebut memiliki profil absorpsi dan distribusi yang baik sehingga diduga dapat dijadikan kandidat terapi demam berdarah dengan target reseptor NS3 Helicase yang lebih baik dari ivermektin dan ligan alaminya.

**Kata Kunci:** Ivermektin, meniran, molecular docking, NS3 Helicase.

## 1. Introduction

Dengue virus is the second virus known to cause disease in humans. This virus can cause dengue fever and dengue hemorrhagic Fever (DHF)[1]. Dengue Hemorrhagic Fever is a disease that is found mostly in tropical and subtropical regions, especially in Southeast Asia, Central America, America, and the Caribbean. The host of DHF is humans, and the agent is the dengue virus which belongs to the family Flaviviridae, and the genus flavivirus, consisting of 4 serotypes, namely DENV-1, and DENV-2. DENV-3 and DENV-4[2]. Transmitted to humans through the bite of infected mosquitoes, especially the Aedes aegypti and Aedes albopictus mosquitoes which are found in almost all corners of Indonesia[3].

Although a vaccine against dengue is now available, which is an important achievement, its long-term protective efficacy against each of the 4 dengue virus serotypes remains to be definitively determined. Therefore, drugs directed at viral targets or critical host mechanisms that can be safely used as prophylaxis or treatment to effectively ameliorate a disease or reduce disease severity and mortality are still needed to reduce the risk of dengue fever[4].

Ivermectin, a broad-spectrum antiparasitic drug, licensed for human use for >2 decades, has recently been shown to have antiviral action in DENV. This stems from the ability to inhibit three viral enzymes namely NS3 protease, NS3 helicase, and NS5 polymerase in DENV[5]. Two enzymes were previously identified as targets of ivermectin in silico. Experiments In vitro confirmed that NS3 helicase was indeed the target, with ivermectin EC50 inhibitory manifestations in the sub-micromolar range[6].

Based on the study of the identification of chemical content, green meniran (*Phyllanthus niruri L.*) contains tannins (catechols), saponins, and carbohydrates[7], while red meniran (*Phyllanthus niruri L.*) only contains tannins (catechols) and saponins[8]. From in vitro experiments, *Phyllanthus niruri* or meniran does have antiviral activity against the dengue virus which is further supported by differential regulation of various host and viral proteins[9]. The study indicates that the aqueous extract of Phyllanthus niruri has the potential to be a candidate for the development of an anti-dengue agent[10].

Based on this study was arranged to see the activity of the meniran compound as an antihemorrhagic fever with ivermectin as a comparison drug[5]. The research was conducted using computational chemistry (in silico application Autodock tools. The dengue fever receptor used as the molecular anchoring target is the NS3 Helicase enzyme on the Non-Structural Protein of the dengue virus, which plays a role in the replication process of the dengue virus. Information on physicochemical properties from the screening test of the interaction of the main compound with the active protein site is used to see the magnitude of the activation energy value of the anchorage. In addition, the ADMET test was carried out using a web-based application, namely PreADMET.

## 2. Materials and Methods

### 2.1. Tools

Asus Laptop with Windows 10 Home specifications 64-bit, Intel® CoreTM 2.0GHz CPU, 4.00 GB DDR3L RAM, NVIDIA GeForce 920MX graphics card, and 1TB HDD.

### 2.2. Materials

The ingredients used include Apigenin (Hefei Dielegance Biotechnology Co., Ltd), Sunflower Oil (Jan Dekker International), Kolliphor®RH40 (BASF), PEG 400 (Merck, Tbk), HPMC K15M (Dipa Prasada Husada), Na. CMC (Dai-Ichi Kogyo Seiyaku Co., LTD), Tween 80 (BASF), Aquadeion (Merck, Tbk).

Software: Operating Windows 10 64-bit MGL-Tools®, Discovery Studio Visualizer®[11], Notepad++®, as well as Protein Data Bank, PubChem, Pre-ADMET, and Toxtree sites.

### 2.3. Methods

#### 2.3.1. Preparation of Receptors

Three-dimensional structure of the


**Fig1**. (A) Three-dimensional (3D) structure of the NS3 receptor, (B) Innate native ligand of the NS3 Helicase receptor

protein of the NS3 Helicase obtained by X-ray crystallography method with a resolution of 2.04. The identity of the macromolecule is 2JLU format (.pdb). By using the Discovery Studio Visualizer® program, protein macromolecules are separated from residues such as water molecules and native ligands. The results of the separation are saved in .pdb format[12].

2.3.2. Preparation of Ligands

The three-dimensional structure of the ligands used compounds from Meniran plants downloaded from PubChem with the site http://pubchem.ncbi.nhl.nih.gov with format (.sdf) and then converted using Discovery Studio Visualizer® into .pdb form. and some compounds are not available on the PubChem drawn on the MarvinSketch format and then converted using Discovery Studio Visualizer® into .pdb format[13]. In total there are 56 compounds obtained from PubChem, and all of them are included in the molecular docking test.

2.3.3. Validation Method

Molecular docking method validation was carried out by re-docking between native ligands from target receptors using Autodock Tools® software. The analysis used to evaluate the validation results is the RMSD value[14]. Molecular docking begins with the preparation of the receptor and the test structure, setting the grid box, setting the docking parameters, running docking, then analysis and visualization of the molecular docking will be carried out.

2.3.4. Molecular Docking

The process begins with preparing receptors and test structures, setting up grid boxes, setting docking parameters, and running docking.

2.3.5. Pre-ADMET Test

The test aimed to analyze the initial pharmacokinetic parameters which include absorption and distribution and the toxicity test which includes the mutagenic and carcinogenic properties of the compound[15]. Tests were carried out using a special online on the http://preadme.bmdrc.kr/ site.

## 3. Result

The selection of receptors is based on their pharmacological activity and biochemical mechanism[15]. For the treatment of dengue fever, NS3 Helicase was chosen as the receptor. NS3 is a multifunctional protein in the dengue virus that has serine protease, helicase (DENV NS3H), RNA-stimulated triphosphatase (NTPase/ATPase/helicase), and RNA 5′-triphosphatase (RTPase) which are important in viral replication and translation. The selected receptor has a resolution factor value of 2.04 Å which is the requirement for a maximum resolution of 2.5 Å[16]. Before docking, the receptor was optimized by adding hydrogen atoms to provide high stability to the α-helix and β-sheet structures in the polypeptide chain, and then the receptor is ready to be used for the binding of the molecule[14].

The validation process is done by re-docking on pure receptors, the docking is said to be valid if it has an RMSD value <

**Table 1.** Molecular Docking Results Sorted by Lowest Bond Energy Value (ΔG)

| No | Active Compound/Ligand | ΔG (kcal/mol) | Hydrogen Bonds | Amino Acid Residue | KI (mM) |
|---|---|---|---|---|---|
| 1 | Native Ligand | -3.16 | 3 | ALA606; ASP609; LYS618 | 4.84 |
| 2 | Comparative Drugs (Ivermectin) | -0.36 | 1 | LYS357 | 118.86 |
| 3 | Nirurin | -4.87 | 4 | TYR354; GLN355; LYS380; THR400 | 161.34 |

2Å[14]. The results of re-docking obtained an RMSD value of 0.79 Å, which means that the receptor meets the requirements and can be used as a molecular anchoring target.

The total content of active compounds in the meniran plant is 56 compounds consisting of several groups of flavonoids, tannins, lignans, alkaloids, and terpenoids. As a comparison, a native ligand from the receptor was used and one of the drugs in several studies was said to have potential as an anti-dengue fever drug, namely ivermectin. Ligands that have been prepared in .pdb format need to be optimized because the docking program used needs to have ligands with the right molecular mechanism parameters and atomic types so that the results are not distorted and to produce a 3D structure that is clean from all ligands and has a position or the best conformation. Most ligand preparation programs will perform minimize energ[14]. The optimized ligands are different from the unoptimized ligands. This is due to its lower energy allowing the ligand to bind more spontaneously to the[15].

### 3.1. Analysis of Physicochemical Properties

Physicochemical properties of ligands based on Lipinski's rule were used to determine the hydrophobic/hydrophilic character of a compound to pass through cell membranes by passive diffusion[17] Of the 56 compounds tested by molecular docking, five compounds did not requirements of the Lipinski rule of five and the rest had the requirements, with an average molecular weight of less than 500 Da, and a fairly good hydrophobic character seen from the Log P value parameter which average is in the range of -0.4 to 5. It is included in the seven test compounds with the lowest bond energies, which means that this meniran plant can be made into oral preparations.

### 3.2. Molecular Docking of Test Compounds with NS3 Helicase Receptors

The coordinates used in molecular anchoring are the coordinate centers (X, Y, Z) with ranges 33,322, -32,717, and 28,133. The volume of the mooring grid used in this study was 40x40x 40 with a spacing of 0.375. In the tethering parameter, the number of GA Runs is changed to 100. Each number of GA Runs is used for the maximum number of evaluation mediums, which is 2,500,000. In the molecular docking process using AutoDock Tools, the receptors used are made

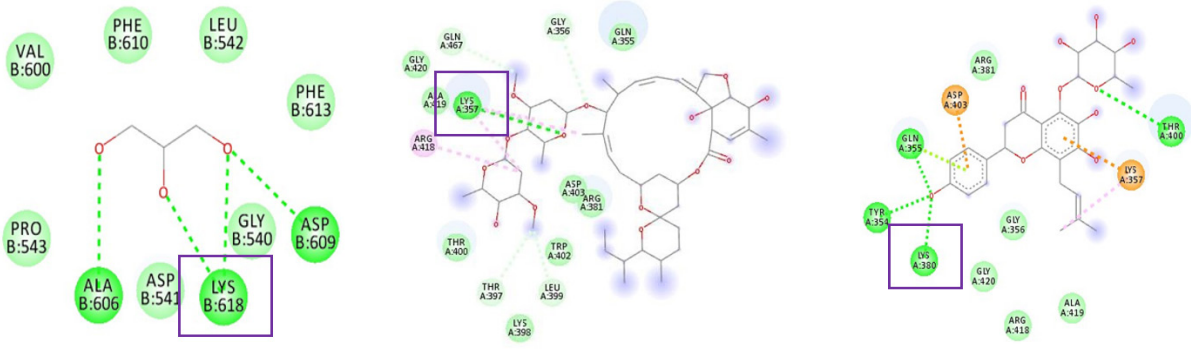


**Fig2.** Visualization of Amino Acid Residues with Hydrogen Bond on NS3 Helicase with (A) Native Ligand, (B) Ivermectin, and (C) Nirurin

rigid while the ligands are made flexible. This is done to adjust the structure of the most stable ligand to interact with the receptor[18].

3.3. Analysis of Molecular Docking Result

There are similarities in the amino acid residues produced from these three compounds with the NS3 Helicase receptor, namely Lysine. Based on the data generated, there is an interaction between the native ligand and the receptor which produces three amino acid residues, namely ALA606, ASP609, and LYS618, while for ivermectin as a comparison drug there is one hydrogen bond with the amino acid residue, namely LYS357. And the best compound with the lowest energy produced four amino acid residues is Nirurin.

3.4. Toxicity Test Analysis

Classification: In Vitro Caco-2 cell permeability (nm sec-1): >70 higher permeability (a), 4-70 medium permeability (b), <4 low permeability (c); %human intestinal absorption (%HIA): 70-100% well absorbed (a), 20-70% moderately absorbed (b), 0-20% poorly absorbed (c); %plasma protein binding: >90% strongly bound (a), <90% weakly bound (b).

## 4. Discussion

4.1. Molecular Docking Result

Analysis of the results of molecular docking can be seen from the free energy value Gibbs or bond energy (ΔG) and inhibition constant (KI). The lower the value of the bond energy, the bonding complex of the compound with the receptor will increase strongly because of the stability and strength of non-covalent interactions in the compound with receptors. Of the 56 Meniran compounds that were successfully anchored, there were 13 compounds whose energy was lower than their native ligands, and one compound with the lowest energy was taken, namely nirurine with an energy value of -4.87 kcal/mol. The energy value is

lower than the native ligand -3.16 kcal/mol and its comparator drug Ivermectin -0.36 kcal/mol. A low energy value will cause the more spontaneous bond between the ligand and the receptor because the ligand only requires low energy to bind or have an affinity for the receptor[15].

4.2. PreADMET Test Analysis

PreADMET is a test carried out to predict the pharmacokinetic profile of compounds whose scoring (ΔG and KI). In this study, the data taken as absorption parameters were the permeability of Human Colon Adenocarcinoma (Caco-2) and Human

**Table 2.** Results of PreADME and Toxicity Tests on Meniran Active Compounds

| No | Compound/Ligand Test | Caco-2 Cell (nm sec-1) | HIA (%) | PBB | Toxicity | Carcinoggenic |
|---|---|---|---|---|---|---|
| **1** | 2,3-Desmethoxy seco-isolintetralin | 27.80 | 93.94 | 86.26 | Non Mutagen | Negative |
| **2** | Astragalin | 6.13 | 27.49 | 73.14 | Non Mutagen | Negative |
| **3** | Beta-Sitosterol | 52.37 | 100 | 100 | Non Mutagen | Negative |
| **4** | Demethylenniranthin | 41.29 | 94.07 | 87.15 | Non Mutagen | Positive |
| **5** | Demethyl-secointetralin | 27.80 | 93.94 | 86.26 | Non Mutagen | Negative |
| **6** | Diosgenin | 56.62 | 95.80 | 97.65 | Non Mutagen | Negative |
| **7** | Epigalocatechin 3-O-error | 12.04 | 20.71 | 100 | Non Mutagen | Positive |
| **8** | Epicatechin 3-O-error | 13.21 | 40.58 | 100 | Non Mutagen | Positive |
| **9** | Fisetin 4'-O-glucoside | 6.24 | 11.78 | 58.75 | Non Mutagen | Negative |
| **10** | Nirurine | 9.86 | 62.22 | 89.10 | Non Mutagen | Negative |
| **11** | Quercetol | 28.88 | 93.81 | 87.55 | Non Mutagen | Negative |
| **12** | Routine | 8.49 | 3.47 | 42.23 | Non Mutagen | Negative |
| **13** | Secoisolariciresinol | 21.15 | 84.14 | 87.82 | Non Mutagen | Negative |

Intestinal Absorption (HIA) cells. Caco-2 cell is a representative parameter in vitro to be able to know how drugs transport through intestinal epithelium in human colon adenocarcinoma[16]. Another parameter HIA is represented in vivo, which is a value that calculates the bioavailability and absorption of the excretion ratio such as through urine, bile, and feces[16]. The average absorption value is at the medium level, which is in the range of 40-70%, meaning that the absorption rate of the test compound can be absorbed quite well in the intestines. The five compounds with the lowest energy also have medium or moderate absorption rates. The distribution value was predicted based on the binding to plasma proteins. In general, only drugs in the unbound form can diffuse across cell membranes and interact with pharmacological targets so plasma protein binding plays an important role in drug efficacy. Plasma protein binding (PPB) is the fraction of a drug that is available in free form for distribution to various tissues[18]. Of the 56 compounds, there were 13 had an affinity for plasma proteins above 90%, and 43 compounds with PPB values below 90%, which means that the compound content in this meniran plant can diffuse well through cell membranes. Specific bioavailability studies have not been found for all of these compounds, however, there are several studies that have tested the bioavailability of one of the meniran components is Lignans.

4.3. Toxicity Test Analysis

Toxicity prediction using computational methods is needed in the early stages of drug development[19]. Testing was carried out on the official PreADMET website at the address http://preadmet.bmdrc.org/. In silico toxicity testing is expected to be useful for helping toxicity tests on a laboratory scale to be more effective and efficient in the use of time, funding, and reducing the trial factor. Prediction of ligand toxicity aims to determine the character of the toxicity of each ligand. The toxicity test that was carried out this time was the Ames Toxicity test on the PreADMET site[20]. The Ames Test is a widely used method to assess the mutagenic potential of compounds using bacteria. Mutagens are agents that can cause mutations in chromosomes or DNA and cause changes in genetic information. While carcinogens are agents or anything that can trigger cancer or tumors. From the results of toxicity tests that have been carried out on 56 test compounds, almost all compounds are mutagens. There are only 13 compounds that are not mutagenic. One of the in vitro tests showed that Phyllanthus niruri was not toxic, and did not cause genotoxicity does not cause a mutagen[21].

## 5. Conclusion

Based on the research, it can be concluded that of the 56 active compounds of meniran, 13 test compounds have lower bond energy values than their native ligands. In this study, one compound with the lowest bond energy was taken as a candidate guide compound and had a good pharmacokinetic profile based on the results of the PreADME test with the permeability and absorption value of the medium, namely nirurine with a bond energy value of -4.87 kcal/mol. Thus the meniran plant has the potential as a therapy for dengue fever based on research by molecular docking on the NS3 Helicase receptor.

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