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Molecular Docking and Pharmacophore Analysis of Compounds from Ginger (*Zingiber officinale)* as Inhibitor for Dengue DEN2 NS2B/NS3 Serine Protease

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serine protease.

ABSTRACT. Dengue hemorrhagic fever (DHF) is a disease caused by the dengue virus (DENV). Dengue virus can enter the human body through the *Aedes aegypti* and *Aedes albopictus* mosquitoes. According to the Indonesian Ministry of Health, dengue hemorrhagic fever (DHF) is still a serious health problem in Indonesia. The type of dengue virus serotype most commonly found to cause infection in the human body is the DENV-2 serotype. This study aims to determine whether Ginger (*Zingiber officinale*) isolate compounds have potential as dengue DEN-2 NS2B/NS3 inhibitors. Samples used are compounds with IUPAC names (S)-5-hydroxy-1-(4-hydroxy-3-methoxyphenyl) octan-3-one (4-gingerol) and (S)-5-hydroxy-1-(3-methoxy-4-methylphenyl) decan-3-one. The method used is molecular docking and Pharmacophore using the MOE (Molecular Operating Environment) 2022.0901 software package. The results obtained based on the observed parameters of the two compounds isolated from ginger (*Zingiber officinale*) could be estimated as potential dengue DEN2 NS2B/NS3 inhibitors.

INTRODUCTION

Dengue is a virus that belongs to the *Flaviviridae* family and the *Flavivirus* genus. Dengue virus has five serotypes; they are DENV-1, DENV-2, DENV-3, DENV-4, and DENV-5 (Wang *et al.*, 2016). The most common type of dengue virus serotype found as a cause of infection in the human body is the DENV-2 serotype. To replicate, the DENV-2 virus requires a protein complex protease (NS3) and its cofactor (NS2B), namely the NS2B/NS3 serine protease. The NS3 protein (which is assisted by NS2B as a cofactor) is a protein that has a vital role in the process of proteolytic and viral replication (Habibi *et al.*, 2020).

Protein NS3 protease is a serine protease that functions to cut the DENV polyprotein at several sites. NS3 protease activity is highly dependent on the presence of the NS2B protein. The NS2B protein is an integral protein which structure is always present in flaviviruses. The ability of the NS3 protein to act as the virus needs a protease to replicate; therefore, the protease domain has excellent potential as a target for developing dengue antiviral (Rachmayanti, 2015).

Ginger is a natural product that can be used as a medical plant. It contains some chemicals, like volatile oil and non-volatile oil components, capable of providing a practical toxic effect to kill mosquito larvae (Bitari *et al.*, 2023). Suadyani (2016) conducted a study on the effect of concentrations of ethanol extract of red ginger rhizome (*Zingiber officinale*) on the death of *Aedes aegypti* mosquito larvae. The results showed that various concentrations of red ginger rhizome ethanol extract administered to each treatment apparently affected *Aedes aegypti* larvae death (Suadyani *et al.*, 2016).

Two compounds, Panduratin A and 4-hydroxy panduratin, were isolated from *Boesenbergia rotunda*, and both of these compounds exhibited potent inhibitory effects on the dengue virus-2 NS3 protease (Chee *et al.*, 2010). In the drug design process, in silico studies are helpful for screening and predicting new compounds' mode of action and determining the optimal conformation with the lowest binding free energy. Furthermore, by disclosing critical details, in silico research can foresee how a potential drug would bind to its intended target (a protein). Some in silico research has been done on possible anti-dengue NS2B/NS3 (Fathima *et al.*, 2018) substances. However,

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regrettably, natural product-derived compounds have not yet been thoroughly researched for their potential as anti-dengue actions.

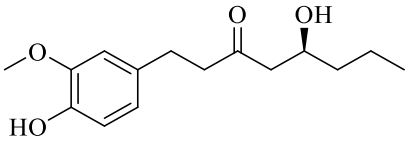
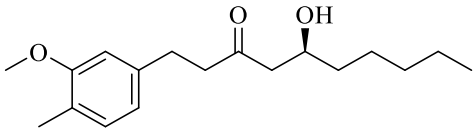
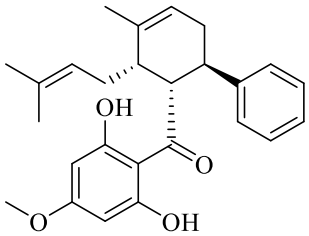
In this study, chemicals for this investigation were sourced from the NADI database, a collection of chemicals derived from natural products. There are 29 *Zingiber officinale* compounds reported, and only two compounds i.e. (S)-5-hydroxy-1-(4-hydroxy-3-methoxyphenyl)octan-3-one and (S)-5-hydroxy-1-(3-methoxy-4methylphenyl) decan-3one were docked since both of these compounds have similar structure hence, it can be employed to carry out the pharmacophore characteristics as well as the pharmacophore alignment hypothesis (Frimayanti *et al.*, 2020). Therefore, the primary goal of this study is to investigate the potentiality of a new inhibitor for dengue virus from *Zingiber officinale* using NS2B/NS3 serine protease as the target.

RESEARCH METHODS

Molecular Docking

The chemical makeup of the ligands was depicted using Chemdraw Professional 15.0 (Table 1). The molecular operating environment (MOE) 2022.0901 software package (Chemical Computing Group) was used to refine the 3D structure using the MMFF94x force field and 0.0001 gradient. The Protein Data Bank (i.e., www.rcsb.org) with PDB ID 2FOM was used to download the molecular structure of the protein. MOE 2022.0901 software package and Discovery Studio Visualizer (DSV, Biovia) were used to create the crystal structure of this protein. The energy of this protein was reduced using the CHARMM27 force field and an RMS gradient of 0.01 kcal/mol/A. Additionally, MOE 2022.0901 was used to minimize the number of H atoms, alpha carbon atoms, and backbone atoms. This protein could then be used as a receptor because it was saved in PDB format.

Table 1. Ligand molecular structure.

No	Structure
Compound **1**	 (S)-5-hydroxy-1-(4-hydroxy-3-methoxyphenyl)octan-3-one Chemical Formula: $C_{15}H_{22}O_4$ Molecular Weight: 266.34
Compound **2**	 (S)-5-hydroxy-1-(3-methoxy-4-methylphenyl)decan-3-one Chemical Formula: $C_{18}H_{28}O_3$ Molecular Weight: 292.42
Positive control (Panduratin A)	 (2,6-dihydroxy-4-methoxyphenyl)((1R,2R,3S)-4-methyl-3-(3-methylbut-2-en-1-yl)-1,2,3,6-tetrahydro-(1,1'-biphenyl)-2-yl)methanone Chemical Formula: $C_{26}H_{30}O_4$ Molecular Weight: 406.52

A site finder was used to identify the protein active site. The target sites for the docking process were Site 3 with amino acid residues (Leu128, Asp129, Phe130, Ser131, Pro132, Ser135, Tyr150, Gly151, and Gly153) and

Site 13 with amino acid residues (His51, Lys74, Asp75, Gly151, Asn152, Gly153, and Val154). The site was then set to become the dummy atom on the dock menu, and the MDB file with the ready-made ligand structure was chosen as the ligand. Next, to carry out the docking processes, the refinement was set to rigid, the posture was set to 50 and 10, and the placement was set as a triangle (Qi *et al*., 2007).

Pharmacophore Analysis

The Pharmacophore Query Editor and fingerprint of protein-ligand interaction were used to prepare and set up the Pharmacophore in the MOE program. Determination of pharmacophore features begins with aligning (superimposing) all proteins downloaded from the RCSB site. This alignment aims to discover the structural similarities of the ligands that have the potential as Dengue NS2B/NS3 inhibitors. Furthermore, all receptors and solvents that are one unit of the previous protein macromolecule are removed so that only the aligned ligands appear in the MOE window.

RESULTS AND DISCUSSION

The chemicals were docked as ligands into protein targets to clarify the ligand interactions with protein binding sites. Based on docking results, Panduratin A, as a positive control, has a binding free energy value of -6.08 kcal/mol and an RMSD value of 1.299. Positive control (Panduratin A) can bind with 11 amino acid residues such as His51, Asp75, Tyr161, Pro132, Gly151, Ser135, Ser131, Phe130, Tyr150, Leu128, and Gly153. The docking results (Table 2) showed that Panduratin A can interact with the His51 amino acid residue through hydrogen bonds in the phenyl group. In this case, the phenyl group acts as a hydrogen bond donor and is marked with a green dotted line. The spatial arrangement of Panduratin A is depicted in Figure 1.

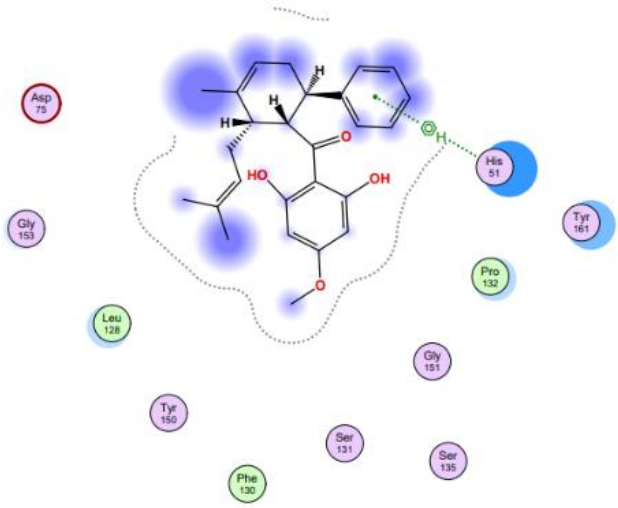


Figure 1. Spatial arrangement of Panduratin A.

Compound **1** has a binding free energy value of -6.08 kcal/mol and an RMSD value of 1.14. This compound has nine amino acids in common with the positive control (Panduratin A), His51, Tyr161, Gly153, Leu128, Ser135, Pro132, Gly151, Ser131, and Tyr150. The phenyl group from compound **1** has binding interaction with His5, Asp75 (catalytic triad), and Phe130 through hydrogen bonds. This compound also interacts via Van der Waals interaction with Asp129. It presumably causes compound **1** was estimated as an active agent for inhibiting DEN2 NS2B/NS3 serine protease. Figure 2 depicts the spatial arrangement of compound **1**.

Based on the docking results, compound **2** has a binding free energy value of -6.47 kcal/mol and an RMSD value of 1.10. This compound has ten amino acids in common with the positive control (Panduratin A), namely the amino acids Asp75, Gly151, Tyr150, Pro132, Ser131, Leu128, Phe130, Gly153, Ser135, Tyr161. The interaction of Van der Waals and hydrophobic bond was performed with Asp75 and Arg54, respectively. These interactions may cause compound **2** to become active to inhibit DEN2 NS2B/NS3 serine protease. The spatial arrangement of compound **2** is presented in Figure 3.

Table 2. Docking results of ligands and positive control.

Compound	Parameter						
	S (kcal/mol)	**RMSD**	**H Bond**	**Hydrophobic**	**Van der Waals**	**Other Interactions**	**Factor of Binding**
Panduratin A (positive control)	-6.08	1.29	**His51**	-	**Asp75**	**Tyr 161, Pro132, Gly151, Ser135, Ser131, Phe130, Tyr150, Leu128, Gly153**	11
Compound **1** (Pose 5)	-6.08	1.14	Asp75, **His51,** Phe130	-	Asp129	**Tyr161, Gly153,** Asn152, **Leu128, Ser135**, **Pro132, Gly151, Ser131, Tyr150**	9
Compound **2** (Pose 20)	-6.47	1.10	-	Arg54	**Asp75**	Val72, Trp50, **Gly151, Tyr150,** His51, **Pro132, Ser131, Leu128, Phe130,** Asn152, **Gly153, Ser135, Tyr161**	10

The binding free energies of compounds **1** and **2** were compared with the binding free energy of positive control (Panduratin A) (Table 2). Indeed, both of these compounds have low binding free energy values, but compound **1** is more dominant with a lower binding free energy value and RMSD value obtained less than 2. Hence, the docking method is valid (Prieto-Martinez *et al.*, 2018). RMSD value indicates the deviation or error value that occurs when docking. A smaller RMSD value indicates a smaller deviation or error (Kausar *et al.*, 2019; Fatriansyah *et al.*, 2022) that occurs when docking.

Furthermore, based on the amino acid compatibility between the test ligand and the positive control, which has 11 amino acids, compound **1** has nine amino acids, and compound **2** has ten amino acids. More amino acid matches between the test ligand and the positive control will give better results. Then, based on the chemical bonds formed, compound **1** is more bound to the active site than compound **2**. Based on this, compound **1** and compound **2** can be categorized as compounds with potential as DENV-2 NS2B/NS3 inhibitors.

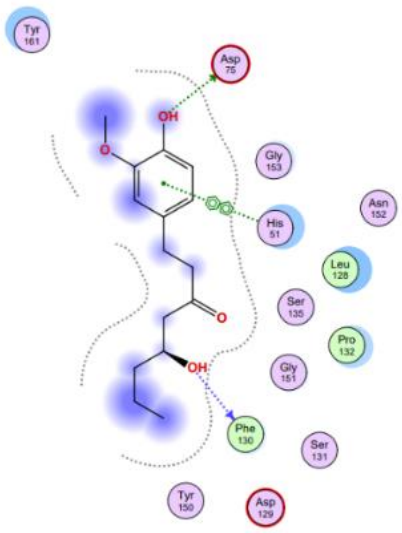


Figure 2. The spatial arrangement of compound **1**.

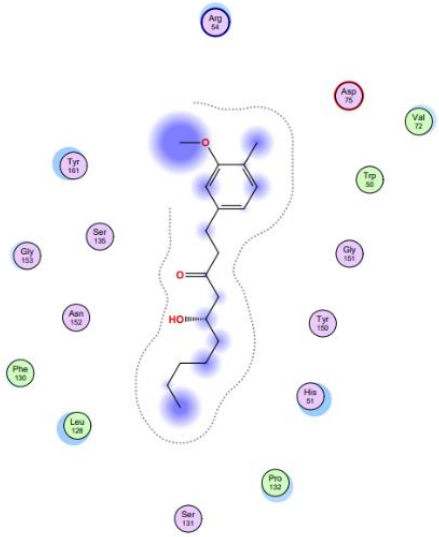
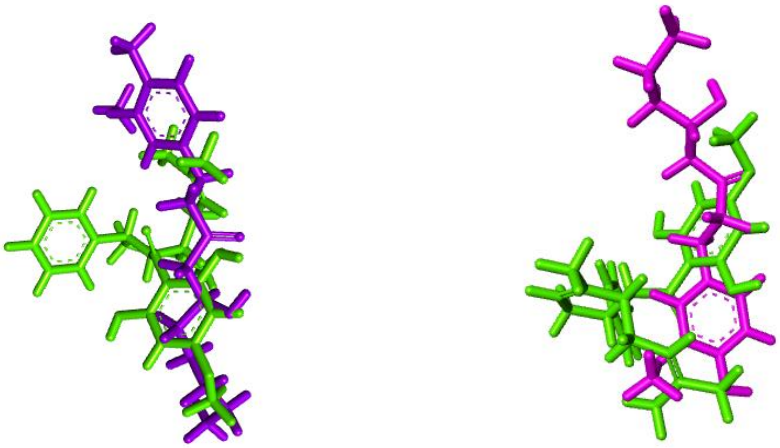


Figure 3. The spatial arrangement of compound **2**.

Superimposition is a binding method that shows several residues play an essential role in determining binding interactions for all ligands (Frimayanti *et al.*, 2023). Superimposition aimed to observe whether the ligand poses were similar to the orientation or binding method of Panduratin A around the active site of NS2B/NS3 serine protease (Frimayanti *et al.*, 2011). The results of superimposition visualization, as presented in Figure 4, were carried out on compounds **1** and **2,** with Panduratin A as the positive control. The results showed that compounds **1** and **2** had a conformation that matched Panduratin A. Hence, compounds **1** and **2** were estimated to be inhibitors of the dengue virus DEN2 NS2B/NS3 serine protease.



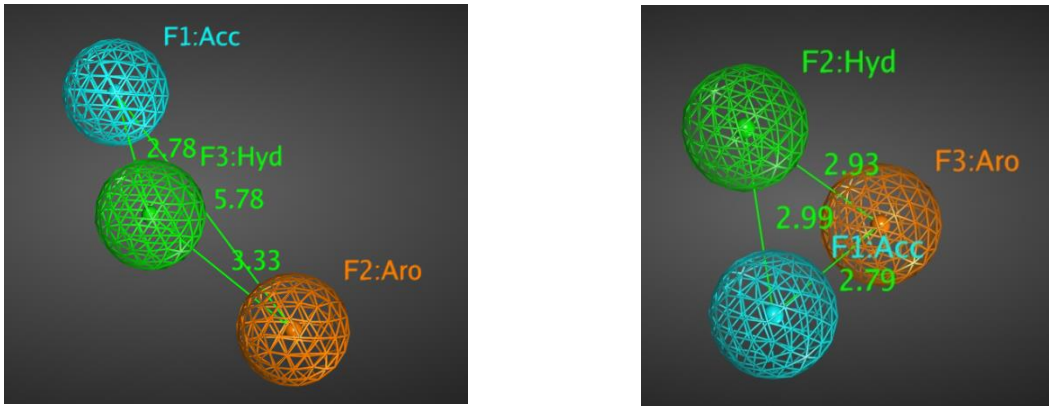
(a) **(b)**

Figure 4. Superimposition of positive control (green) with (a) compound **1** and (b) compound **2**.

Pharmacophore is a molecular framework that carries essential features responsible for the biological activity of drugs. Pharmacophore also can be used to describe a collection or several features. Pharmacophore elements are commonly referred to as features. Features can be defined as a group of atoms that have donor hydrogen bonds or aromatic rings. Generally, features can activate a compound by responding to target proteins, which is also essential for the activity of a compound (Jung *et al.*, 2018).

Based on pharmacophore results that have been carried out, compound **1** has features of a hydrogen-bond acceptor group (F1:Acc), an aromatic ring (F2:Aro), and a hydrophobic group (F3:Hyd), which play an essential role in ligand-receptor interaction. The distance between the proton-withdrawn group (F1:Acc) and the hydrophobic group (F3:Hyd) is 2.78 Å. The distance between the proton-withdrawn group (F1:Acc) and the aromatic group (F2:Aro) is 5.78 Å, and the distance between the hydrophobic group (F3:Hyd) and the aromatic

group (F2:Aro) is 3.33 Å. The smaller distance gives better results (Sindhu *and* Srinivasan, 2014). Pharmacophore features for compound **1** is depicted in Figure 5.



(a) (b)

Figure 5. Pharmacophore query for (a) compound **1** and (b) compound **2**.

Compound **2** has the same features as compound **1,** namely the hydrogen-bond acceptor group (F1:Acc), the aromatic ring (F3:Aro), and the hydrophobic group (F2:Hyd). The distance between the proton-withdrawn group (F1:Acc) and the hydrophobic group (F2:Hyd) is 2.99 Å, distances of the proton-withdrawn group (F1:Acc) and the aromatic group (F3:Aro) is 2.79 Å, and distance between the hydrophobic group (F2:Hyd) and the aromatic group (F3:Aro) is 2.93 Å. The presence of a hydrophobic group in a ligand had a major influence on the activity of a compound (Kristam, 2013). Figure 4 presents the pharmacophore query for compound **2.**

The pharmacophore query that has been obtained is then used as a reference template in the virtual screening process. The distance between each pharmacophore feature must be consistent when designing new drugs because changes in the conformation of these bonds will affect the activity of the designed compounds.

CONCLUSION

Molecular docking and pharmacophore studies have been carried out; it was found that molecular docking of two compounds isolated from ginger (*Zingiber officinale*) estimated that compound **1** and compound **2** have potentiality against dengue DEN2 NS2B/NS3. Pharmacophore query showed that compounds **1** and **2** have hydrogen bond acceptor bonds, hydrophobic bonds, and aromatic rings. Based on the attributes of compounds **1** and **2,** both of these compounds (compound **1** and **2)** can be used as promising agents against dengue.

CONFLICT OF INTEREST

There is no conflict of interest in this article.

AUTHOR CONTRIBUTION

NF: Supervision, Conceptualization, Methodology, manuscript review and editing; RR: collecting data, Data Analysis, Manuscript Drafting; EM: Supervision; Manuscript Review and Editing.

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