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Molecular Docking of *Physalis angulata* and *Schleichera oleosa* as a Potential Inhibitor of NS5 Dengue Methyltransferase

Rahmawaty Hasan*, Diana Lady Yunita Handoyo, Laila Zukhruf

Department of Pharmacy, Faculty of Health Sciences, University of Ibrahimy, Jl. KHR. Syamsul Arifin No. 1-2 Sukorejo, Situbondo, East Java, Indonesia

*Corresponding Author: rahmahasan1234@gmail.com

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Abstract: *Oral infusion of Physalis angulata and Schleichera oleosa are potential plants that is traditionally used for Dengue Hemorrhagic Fever (DHF) treatment. Subsequently, it is essential to investigate P. angulata and S. oleosa for potential chemical compounds that could serve as alternative DHF therapies by molecular action targets. This study aimed to analyze the chemical compounds of Physalis angulata and Schleichera oleosa as an alternative therapy to DHF utilizing the molecular docking procedure. The methods are macromolecule and ligand preparation, validation as well as molecular docking, data analysis and visualization. The compounds were downloaded from the PubChem database and dengue methyltransferase protein was obtained from PDB (1L9K). Molecular docking has interacted with the Autodock and analyzed by Pymol, Discovery Studio Visualizer and Ligplot. The results showed that the test ligands had lower binding energies than the SAH as a native ligand, specifically 14-Hydroxyixocarpanolide -10.69 kcal/mol as a potential compound from Physalis angulata and Schleicherastatin 5 -10.25 kcal/mol as a potential compound from Schleichera oleosa. With hydrogen bonds and hydrophobic pockets, all of the test ligands bind the NS5 dengue methyltransferase active site. The results suggested that Physalis angulata and Schleichera oleosa possess offering compounds for inhibiting NS5 dengue methyltransferase as a DHF treatment.*

Keywords*: Physalis angulata, Schleichera oleosa, Molecular docking, DHF*

INTRODUCTION

Dengue is a vector-borne viral disease primarily transmitted by mosquitoes. It is commonly referred to as break-bone fever due to the severe pain it causes. The causative agents of dengue are the dengue viruses (DENV-1 to DENV-4) (WHO, 2016). Dengue is prevalent in tropical and subtropical regions. Since its initial identification in Indonesia in 1968, the incidence of dengue cases has steadily increased, positioning Indonesia as the country with the second-highest number of Dengue Hemorrhagic Fever (DHF) cases among 30 countries with endemic regions. In 2021, Indonesia reported 73.518 DHF cases, resulting 705 deaths. East Java reported an incidence rate of 16,8 per 100.000 population and a case fatality rate of 1.07 compared to the national rate of 0.96 (Ministry of Health RI, 2002).

The primary focus of antiviral structure has been on the essential and non-essential proteins of DENV. The non-structural DENV proteins play a crucial role in virion assembly and replication. DENV's pathogenicity and viral replication are associated with seven non-structural proteins (NS): NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5 (Tambunan et al.,2017). The flavivirus genome encodes NS5, the largest of these seven non-structural proteins (Lim et al., 2015). The N-terminal of NS5 encodes methyltransferase (Mtase), while the C-terminal encodes RNA-dependent RNA polymerase (RdRp) (Johansson et al.,

2011). NS5 methyltransferase is responsible for the two-step methylation of guanine N7 and nucleoside 2′-O-methylation. The three active sites of this protein are the guanosine triphosphate (GTP) binding site, RNA-binding site, and S-adenosylmethionine (SAM)/S-adenosyl-l-homocysteine (SAH) binding site. Inhibiting the SAM/SAH, GTP, or RNA-binding sites of NS5 methyltransferase is considered one of the most efficient way for combating DENV infection, as it prevents viral proliferation (Thisyakorn, 2014).

The treatment of Dengue Hemorrhagic Fever (DHF) involves the administration of colloidal fluid therapy and Non-Steroidal Anti-Inflammatory Drugs (NSAIDs). However, the use of synthetic colloid fluids, such as 3% gelatin, 10% dextran, and 6% hydroxyethyl starch (HES), has been associated with adverse side effects, including allergic reactions and kidney failure. Additionally, NSAIDs are generally avoided due to their potential to induce bleeding (Rajapakse et al., 2012). The treatment of In response, the World Health Organization (WHO) has established guidelines recommending the incorporation of herbal medicine as a supportive treatment option to enhance public health outcomes. Developing a potential herbal treatment model presents a viable alternative for managing DHF. Herbal medicines are often preferred due to their cost-effectiveness and lower risk of side effects (WHO, 2016).

The active compounds in *Physalis angulata* and *Scheichera oleosa* are traditionally used for the treatment of DENV infection by various tribes across different regions. Both *P. Angulata and S. oleosa* exhibit potential as anti-inflammatory agents. Oral infusions of these plants are commonly employed in the traditional treatment of dengue fever (Bradacs, 2011). *P. Angulata and S. oleosa* are attractive sources of bioactive secondary metabolites, such as sesquiterpenes and flavonoids. Flavonoids, known for their antiviral properties, have demonstrated several anti-DENV effects through multiple mechanisms. Non-neutralizing heterotypic antibodies can induce antibody-dependent enhancement (ADE) in secondary DENV infection, facilitating increased entry of viral particles into phagocytes, which subsequently produce proinflammatory cytokines implicated in severe dengue pathogenesis (Martina et al., 2019). Sesquiterpenes, characterized by their lower volatility compared to terpenes, greater potential for stereochemical diversity, and stronger odors, possess anti-inflammatory and bactericidal properties (Ishnava et al., 2013).

P. angulata and *S. oleosa* require screening for potential chemical compounds that could serve as alternative treatments for Dengue Hemorrhagic Fever (DHF) (Saleh and Kamisah, 2020). The purpose of screening these chemical compounds is to identify active substances with specific molecular action targets, thereby optimizing pharmacodynamic activity based on the interaction patterns between the compounds and their targets (Martinez and Franco, 2018). Utilizing molecular docking techniques, this study aims to analyze the active compounds of *P. angulata* and *S. oleosa* that exhibit inhibitory activity against the DENV virus, focusing on the non-structural NS5 protein involved in viral genome replication. The objective is to evaluate these chemical compounds as alternative therapies for DHF, specifically targeting the inhibition of NS5 dengue methyltransferase through molecular docking studies.

RESEARCH METHODS

Materials and Tools

The equipment used in this study included computer hardware with specifications Intel® Core™ I7-4770 CPU @ 3.40GHz, 8GB RAM, Intel® HD Graphics Family and Windows 8 operating system, 64-bit operating system. The materials used in this study included 3D structure of the ligands of *Physalis angulata* and *Schleichera oleosa*, 3D structure of the NS5 dengue methyltransferase protein (PDB ID: 1L9K), KnapSAck,

PubChem, PASS Way, PDB RSCB, SuperPrediction, VegaZZ 3.2.3, Autodock Tools 4.0, PyMol 2.5 Schrodinger, Discovery Visualizer 3.5, SwissADME, LigPlot^{+} 2.2 EMBL-EBI.

Procedures

Ligand Preparation

VegaZZ was used to construct 3D structures of the ligands in the form of potential chemical compounds of *P. angulata and S. oleosa* by adding Canonical SMILES from the PubChem database. The identified ligands are obtained through the identification of metabolite content from *P. angulata and S. oleosa* via the KnapSAck data source. The next phase is to incorporate the Gasteiger payload and the AutoDock force field, as well as to minimize energy consumption. The generated file will be in.pdb format. It was then prepared in AutoDock by calculating the amount of active torque and saved in .pdbqt format (Hasan 2021).

Macromolecule Preparation

AutoDock Tools were utilized for the preparation of macromolecule. The native ligand and unnecessary residues were eliminated during the optimization of the macromolecule. Subsequently, hydrogen was added and Gasteiger charges were applied. The grid box on the active site of the macromolecule was identified (Martinez and Franco, 2018). The research focused on a variety of active compounds hypothesized to have inhibitory action against the NS5 dengue methyltransferase protein (PDB ID: 1L9K). By excluding all sulfate ions, hydrogen ions, and water molecules, the structure of NS5 dengue methyltransferase protein is prepared for molecular docking. S-adenosyl-L-homocysteine (SAH) as native ligand were extracted and saved separately as two different pdb files for re-docking (Abdulbaqi, 2022).

Method Validation

The native ligand is redocked with the macromolecule during the validation procedure. The RMSD value of 2.0 Å is used as the parameter. Additionally, the overlap of the native crystallographic ligands (x-ray diffusion) against the redocking results validates the ligands. Redocking between native ligand from NS5 dengue methyltransferase protein was used to validate the molecular docking approach. The RMSD analysis was used to evaluate the validation outcomes (Funkhouser, 2017; Hasan, 2021).

Molecular Docking

The Autodock 4.0 program with ADT has been utilized to carry out the molecular docking approach. The parameters are initially determined using a macromolecular rigid format. The parameters used are a total number of GA Runs = 50 and population size = 150 determined by applying the Lamarckian GA. The docking results of all of the investigated ligands resulted in G binding (kcal/mol). Gasteiger charges were applied to reduced ligand structures to get the lowest binding energy. Grid maps representing the complete protein were created using grid spacing of 14.413 X -41.255 X 0.618 in the x, y, and z-dimensions of 40 points. The Lamarckian Genetic Algorithm (LGA) was used to search for the lowest binding energy by implementing local minimization of the genetic algorithm to enable population modification (Atilgan and Hu, 2011).

Analysis of Molecular Docking

The docked ligand models with the lowest binding energy and the most conformational clusters in each binding were chosen. The two and three-dimensional conformational structures of the ligand-protein complexes were visualized using the Discovery Studio Visualizer 4.5 to investigate the binding models as a diagram interaction. For analyzing hydrophobic interactions, the Ligplot^{+} program was used 2.2 (Laskowski

and
Swindells, 2011). A physical-chemical profile identification has been carried out on the ligand with the lowest binding value using a database. According to Lipinski's five rules,these physical-chemical parameters indicate good candidate parameters for oral drugs (Lipinski et al., 2001)

RESULTS AND DISCUSSION

Docking can identify a new compound of interest in the therapeutic field, make it possible to predict the interactions between target proteins and ligands at the molecular level, as well as describe the structure-activity relationship. Utilization of molecular docking studies makes it possible to reduce costs and increase opportunities to find the desired new drug candidates, so that new drug discovery can be carried out more efficiently (Funkhouser, 2017). The steps taken in this study started with selecting the protein as the target receptor and the ligand as the compound to be tethered to the protein.

The initial stage of protein preparation is carried out by removing water molecules which aims to leave amino acids in the target protein so that during the docking process only the test compound interacts with amino acids (Kirsten and Guido, 2008). Water molecules must be removed in order to maximize the interaction between the test compound and the target protein. In general, the interaction that occurs between the ligand and the receptor is in the form of a hydrogen bond, so it is necessary to add hydrogen to optimize the interaction that will occur. Energy minimization is also important when preparing proteins for alpha carbon, backbone atoms, and sidechain atoms. This is done to stabilize the bonds in proteins (Rachmania, 2019).

Based on potential compounds screening through PASS Way, table 1 suggests the active compounds from *P. angulata and S. oleosa* that have the potential antiviral activity. The activity probability value represents the number thought to have the desired biological activity. Only the structural formula is required to acquire the expected biological activity profile for your drug; accordingly, prediction is possible even for virtual structures generated in computer but not yet synthesized (Mervin et al., 2015).

Table 1. Theoretical antiviral active compounds and their predicted with probability activity

Active Compound	Canonical SMILES	Probability Activity
	Physaliis angulata	
14-Hydroxyixocarpanolide	CC1CC(OC(=O)C1C)C(C)(C2CCC3(C2(CCC4C3C5C(O5)C6(C4(C(=O)C=CC6)C)O)C)O)O	0.342
Wiithanolide	CC1=C(C(=O)OC(C1)C(C)(C2CCC3C2(CCC4C3CC5C6(C4(C(=O)C=CC6O)C)O5)C)O)C	0.345
Withaphysalin A	CC1=C(C(=O)OC(C1)C2(C3CCC4(C3(CCC5C4CC=C6C5(C(=O)C=CC6)C)C(=O)O2)O)C)C	0.299
	Schleichera oleosa	
Schleicherastatin 5	CCC(CC(C(C)C1CCC2C1(CCC3C2C(=O)C=C4C3(CCC(C4)O)C)C)O)C(C)C	0.488
Schleicherastatin 6	CC(C)C(C)CC(C(C)C1CCC2C1(CCC3C2C(=O)C=C4C3(CCC(C4)O)C)C)O	0.491

Schleicherastatin 7	CC(C)C(C)CC(C(C)C1CCC2C1(CCC3C2C(=O)C=C4C3(CCC(C4)O)C)C)O	0.491

Autodock is a collection of docking automation tools. It is intended to predict how small molecules, such as substrates or drug candidates, bind to a known 3D structure receptor. Grid maps with grid spacing of 0.41 Å in the x, y and z- dimensions of 126 × 126 × 126 points were set to cover the entire protein. The parameters used to analyze the docking results include the Root Mean Square Deviation (RMSD), the docking method is said to be valid if it has an RMSD value of ≤ 2 Å (Funkhouser, 2017). The RMSD value describes the deviation value from the error that occurs during docking. The smaller the RMSD value indicates that the error deviation in docking is small. Figure 1 shows the re-docking of the NS5 dengue methyltransferase protein with a RMSD value of 1.29 Å and a binding energy value of -7.15 kcal/mol. This shows that the native ligand conformation resulting from re-docking of the target protein is similar to that of the crystallographic result.

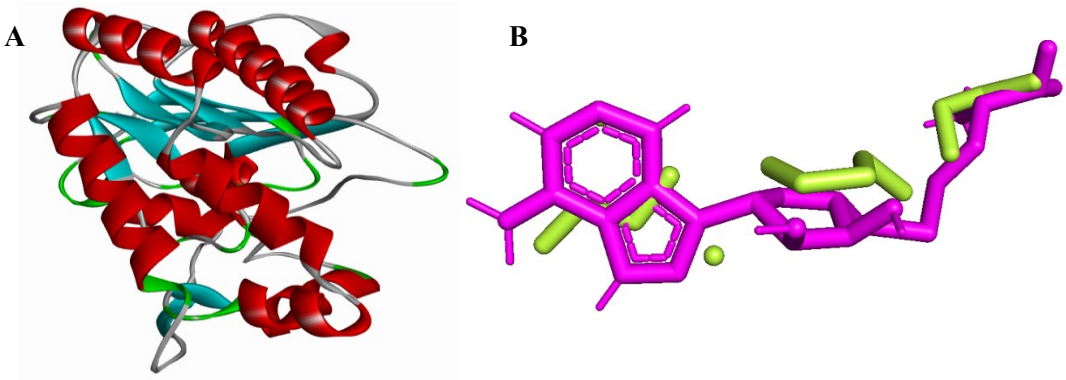

Figure 1. Macromolecule of NS5 dengue methyltransferase (PDB ID: 1L9K) (A), Overlays of redocking ligand (limon) and reference ligand of crystallography data (magenta) at 1L9K (b)

NS5 contains RNA methyltransferase (Mtase) at the N terminus and RNA-dependent RNA polymerase (RdRp) at the C terminus. The C-terminal portion of NS5 at residue positions 420-900 which is an RNA-dependent RNA polymerase (RdRp) is responsible for the synthesis of the template. Intermediate RNA for subsequent replication of the positive strand RNA genome. The NS5 methyltransferase located at the N-terminus functions in the newly formed RNA capping process. This enzyme plays a role in the mRNA capping process by transferring the methyl group from the cofactor Sadenosl-l-methionine (SAM/AdoMet) to the N7 atom of the guanine base of RNA and to the 2'OH group of ribose RNA (Podvinec et al., 2010). A Y-shaped cleft connects the two bond sides of the NS5 methyltransferase enzyme. The SAM bonding site (methyl group donor) is the first bonding site, and the RNA-cap binding site is the second, which is shallower and smaller in size. This enzyme has two methylation processes: first, a methyl group is submitted to the SAM bonding site, and then the methyl group is transferred to the guanine base of RNA (Lim et al., 2008).

Table2. Molecular docking results are sorted by lowest bonding energy value and amino acid residue interaction

Active Compound	Binding energy ΔG (kcal/mol)	KI (nM)	Amino Acid Residues
Physalis angulata			
14-Hydroxyixocarpanolide	-10.69	14.51	Gly A: 148, Lys A: 181, Asp A: 146, Gly A: 83, Val A: 55, Gly A: 58, Trp A: 87, Cys A: 82, Asp A: 79, Gly A: 81, Thr A: 104, His A: 110, Leu A: 103, Val A: 132, Ile A: 147, Val A: 130, Asp A: 131, Phe A: 133, Gly A: 106, Glu A: 111, Gly A: 85, Gly A: 86, Ser A: 56, Arg A: 84, Lys A: 105
Withanolide	-10.57	18.00	Asp A: 146, Lys A: 181, Gly A: 148, Ile A: 147, Val A: 132, Asp A: 131, Thr A: 104, Leu A: 103, Gly A: 106, Glu A: 111, Gly A: 81, Gly A: 85, Lys A: 105, Gly A; 83, Gly A: 86, Ser A: 56, Gly A: 58, Trp A: 87, Asp A: 79, Cys A: 82, Arg A: 84
Withaphysalin A	-9.77	69.22	Gly A: 106, Thr A: 104, Val A: 130, Asp A: 131, Val A: 132, Phe A: 133, Ile A: 147, Glu A: 149, Gly A: 81, Glu A: 111, Lys A: 181, Asp A: 146, Gly A: 83, Cys A: 82, Arg A: 84, Gly A: 85, Gly A: 86, Ser A: 56, Gly A: 58, Trp A: 87, Lys A: 105, Gly A: 148
Schleichera oleosa			
Schleicherastatin 5	-10.25	30.45	Arg A: 163, Phe A: 133, Val A: 132, Lys A: 105, Ile A: 147, Val A: 130, Gly A: 81, Gly A: 83, Arg A: 84, Asp A: 79, Trp A: 87, Ser A: 88, Cys A: 82, Gly A: 85, Gly A: 85, Ser A: 56, Gly A: 58, Asp A: 146, Thr A: 104, His A: 110, Gly A: 105, Glu A: 111, Asp A: 131
Schleicherastatin 6	-9.86	59.56	Asp A: 146, Trp A: 87, Gly A: 83, Thr A: 104, His A: 110, Gly A: 106, Val A: 132, Phe A: 133, Arg A: 163, Lys A: 105, Val A: 130, Ile A: 147, Gly A: 81, Gly A: 85, Gly A: 86, Ser A: 56, Cys A: 82, Arg A: 84, Gly A: 58, Asp A: 131, Glu A: 111

Schleicherastatin 7	-9.79	67.15	Lys A: 181, Glu A: 111, Gly A: 83, Thr A: 104, Gly A: 106, His A: 110, Arg: 163, Val A: 132, Phe A: 133, Lys A: 105, Val A: 130, Gly A: 58, Trp A: 87, Ile A: 147, Gly A: 81, Cys A: 82, Gly A: 86, Ser A: 56, Arg A: 84, Asp A: 146, Asp A: 131
Native ligand: S-Adenosyl-Homocysteine (SAH)	-7.15	5.71	Glu A: 112, Gly A: 83, His A: 110, Gly A: 106, Ile A: 147, Phe A: 133, Asp A: 131, Val A: 132, Leu A: 103, Val A: 130, Thr A: 104, Gly A: 81, Gly A: 85, Gly A: 58, Asp A: 146, Trp A: 87, Arg A: 84, Lys A: 105, Glu A: 111, Cys: 82, Gly A: 86, Ser A: 56

The active compounds from P. angulata and S. oleosa have lower bond energy values (kcal/mol) compared to the S-Adenosyl-Homocysteine (SAH) as the native ligand. The lower the bond energy required for ligand and protein interactions so that the ligand and protein bonds become more stable. Table 2 shows that 14-Hydroxyixocarpanolide is the most active compound of *Physalis angulata* and Schleicherastatin 5 is the most active compound of *Schleichera oleosa*. The active compound shows several matches of the same amino acid residue with SAH (S-Adenosyl-Homocysteine). SAH is a by-product of SAM as well as an analogue compound for binding site inhibitors. The residues on the bond side for SAM are Ser 56, Lys 61, Cys 82, Gly 86, Trp 87, Thr 104, Lys 105, Asp 131, Val 132, Phe 133, Asp 146, Ile 147, Lys 181 and Glu 217 while for the RNA-cap they are Lys 14, Leu 17, Asn 18, Leu 20, Lys 22, Phe 25, Lys 29, Ser 150 and Ser 151. Residues Lys 61, Asp 146, Lys 181 and Glu 217 are known to be the four residues has an important role in the active site of SAM, besides that the four residues are motifs found in other flaviviruses (Podvinec et al., 2010; Lim et al., 2013).

2D structures are represented as thick purple sticks, the binding site residues as brown sticks, and those involved in hydrophobic interactions as red eye-lashes. Figure 2 displays simple information on intermolecular interactions between protein complexes and ligands, including hydrogen bonds, hydrophobic interactions and atomic accessibility. Aside from hydrogen bond interaction, hydrophobic interaction is also relevant in the active site. Although not as strong as a hydrogen bond, hydrophobic interactions can cause significant changes in the docking free energy between ligand and macromolecule (Wei et al., 2006). The interaction shows hydrogen bonding in the form of a dotted line between the atoms involved and each residue of the hydrogen bond will be displayed in full. Unlike the case with hydrogen bonds, hydrophobic interactions are shown by displaying a red arc shape of each involved residue accompanied by radii radiating towards a ligand atom that participates in the interaction and vice versa, the atom of a ligand that also interacts is indicated by the presence radius toward a protein residue (Panigrahi and Desiraju, 2007).

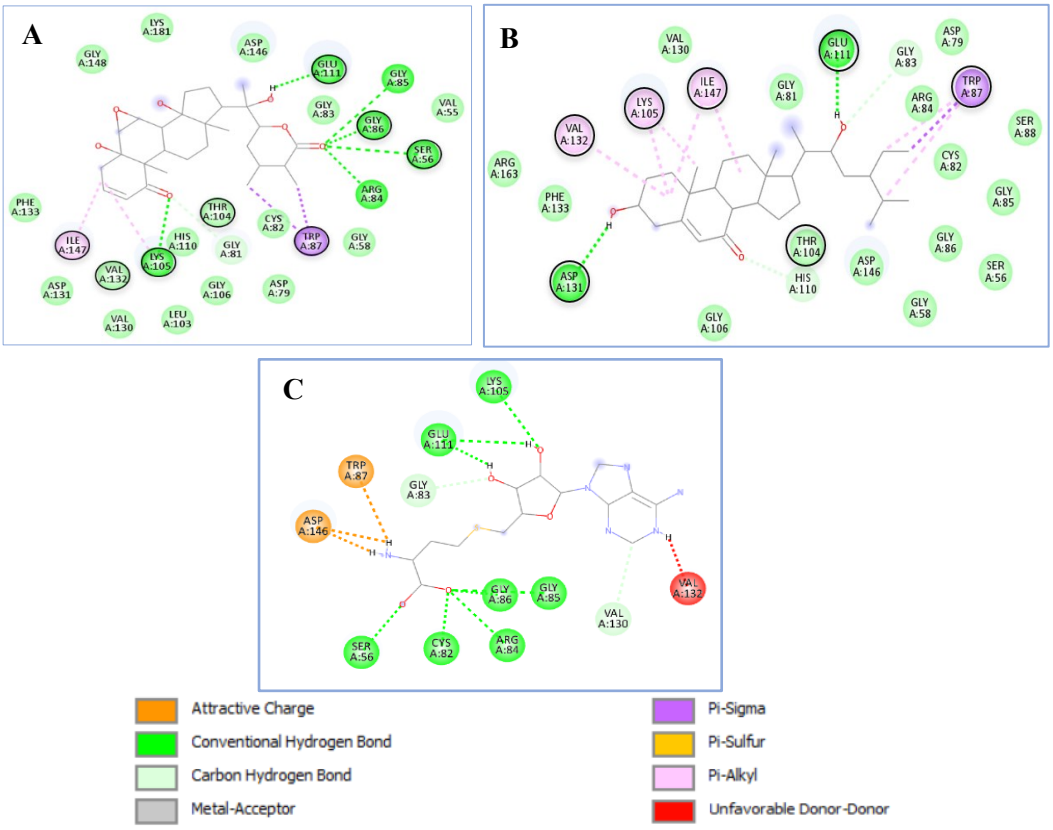

Figure 2. The similarity of bonding interactions between greatest potential ligands and native ligand: 14-Hydroxyxocarpanolide (A); Schlecherastatin 5 (B) S-Adenosyl-Homocysteine (SAH) as native ligand (C)

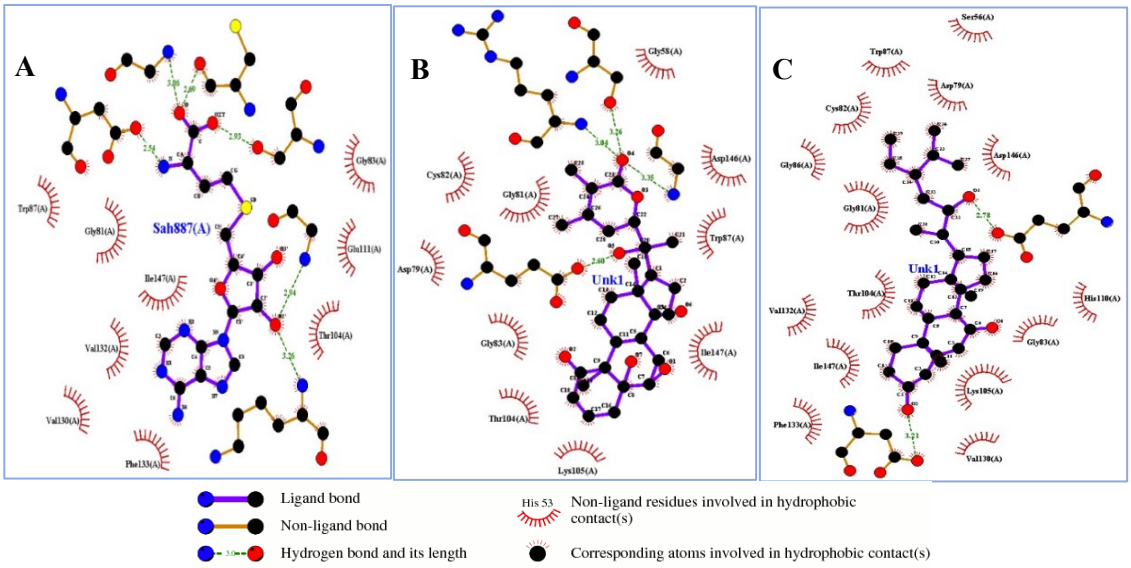

Figure 3. 1L9K receptor–ligand interaction diagram: SAH (A); 14-Hydroxyxocarpanolide (B); Schlecherastatin 5 (C)

Following the calculation of the binding affinity value, the LigPlot tool must be used to examine the interaction of amino acids between proteins and ligands. The use of ligplot for2Dvisualization makes more straightforward to observe amino acids that have been generated effectively via molecular bonding (Laskowski and Swindells, 2011). The interaction of diverse amino acids between greatest potential compounds and native ligand is the same, according to figure 3. 14-Hydroxyixocarpanolide and Schleicherastatin 5 have an interaction with 1L9K receptor. Bond lengths of hydrogen between 2.0 and 3.5 A were considered (Wallace et al., 1995). Overall, Figure 3 provides schematic information on the non-covalent interaction between the NS5 dengue methyltransferase protein and the greatest potential compounds.

Table 3. Physicochemical parameters

Chemical Compounds	MW	HBA	HBD	Log P	TPSA
Withanolide	414.35	2	0	7.856	26.300
Withaphysalin A	466.24	6	1	3.061	89.900
14-Hydroxyixocarpanolide	488.28	7	3	2.318	116.590
Schleicherastatin 5	444.36	3	2	5.452	57.530
Schleicherastatin 6	430.34	3	2	5.137	57.530
Schleicherastatin 7	430.34	3	2	5.111	57.530
Native Ligand (SAH)	384.12	11	7	-2.570	182.630

Determination of the physicochemical properties of a test ligand when it crosses the cell membrane can be identified by Lipinski's rule of five. Requirements that must be met by a potential compound based on the Lipinski rule starting from molecular weight <500 Da, log P value <5, number of donor hydrogen bonds <5 and number of acceptor hydrogen bonds <10, and molar reactivity in the range of 30 – 130. Ligands with weight Molecules <500 Da can easily penetrate cell membranes. The log P value indicates the polarity of the ligand in fat solvents or is non-polar if the log P value is > 5 because it can interact more easily through the lipid bilayer and is widely distributed in tissues. A low log P value indicates that the ligand tends to dissolve in water. The number of donor and acceptor hydrogen bonds is related to the chemical activity of drug molecules in the body (Lipinski, 2001). Table 3 demonstrates that all greatest potential compounds from *P. angulata and S. oleosa* fulfill the Lipinski rule criteria through determining physicochemical properties. Withanolide, on the other hand, deviates from the log P value (>5). The number is acceptable, and the active compounds likely to be a potential option for oral treatment. If the ligand meets the Lipinski rule criteria without any deviating values, then the ligand is drug-likeness or a potential compound as a drug candidate.. Drug-likeness refers to the similarity of a compound with an oral drug based on evaluation in the Lipinski rule.. The physicochemical parameters showed that all active compounds met the Lipinski rule criteria so that they could be candidates for oral drug for dengue hemorrhagic fever.

This study has potential limitations. The primary limitation of molecular docking is a lack of confidence in scoring functions' ability to provide correct binding energies. This is due to the fact that some intermolecular interaction parameters, such as solvation effect and entropy change, are difficult to anticipate precisely. Furthermore, several

intermolecular interactions that have been shown to be significant are rarely taken into account in scoring functions.

CONCLUSIONS

The results indicate that the test ligands have lower binding energies than the native ligand SAH, specifically 14-Hydroxyiixocarpanoliide -10.69 kcal/mol as the most potential compound from *Physalis angulata* and Schleicheirastatin 5 -10.25 kcal/mol as the most potential compound from *Schleicheira oleosa*. All test ligands bind the NS5 dengue methyltransferase active site with hydrogen bonds and hydrophobic pockets. The study suggests that *Physalis angulata* and *Schleicheira oleosa* have potential compounds for inhibiting NS5 dengue methyltransferase as therapy for DHF.

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REFERENCES

Abdulbaqi, J. 2022. Effect of Guggulsterone, A Sterol Identified in *Commiphora gileadensis* (Becham), on The Dengue Virus Enzymes: Pharmacokinetics, Molecular Docking and Molecular Dynamics Simulations Studies. *Journal of King Saud University - Science*, 34(6), 1-9. DOI: 10.1016/j.jksus.2022.102140.

Atilgan, E., Hu, J. 2011. Improving Protein Docking Using Sustainable Genetic Algorithms, *Journal of Computer Information Systems and Industrial Management Applications* 3, 248-255.

Bradacs G., Heilmann J., Weckerle C.S. 2011. Medicinal Plant Use in Vanuatu: A Comparative Ethnobotanical Study of Three Islands, *J. Ethnopharmacol*, 137(1), 434–448.

Funkhouser, T. 2017. Lecture: Protein-Ligand Docking Methods. Princeton University.

Hasan, R., 2021. Studi Bioinformatiika Kandungan Kimia Daun Kelor (*Moringa oleifera*) Terhadap Target Aksi Pemenuhan Zat Gizi Ibu Hamil, *Jurnal Ilmiah Pannmed*, 17 (2), 337-343.

Johansson, A. J. Brooks, D. A. Jans, and S. G. Vasudevan, J. 2001. A Small Region of The Dengue Virus-Encoded RNA-Dependent RNA Polymerase, NS5, Confers Interaction with Both The Nuclear Transport Receptor Importin-B and The Viral Helicase, NS3. *Gen. Virol*, 82(4), 735-745. DOI: 10.1099/0022-1317-82-4-735.

Kirstein and Guido. 2008. Concept of Pharmacophores and Their Application in Computer-Aided Drug Design. ICS UNIDO Workshop. Italy: Trieste.

Laskowski R A, Swindells M B. 2011. LigPlot+: Multiple Ligand-Protein Interaction Diagrams for Drug Discovery, *J. Chem. Inf. Model*, 51(10), 2778-2786.

Lim S.P. 2008. A Scintillation Proximity Assay for Dengue Virus NS5 2'-O-Methyltransferase-Kinetic and Inhibition Analyses, *Antiviral Research*, 80(3), 360-369.

LimS.P, Noble C.G, Shi P. 2015. The Dengue Virus NS5 Protein as a Target for Drug Discovery*, Antiviral Research* 119, 57–67. DOI: 10.1016/j.antiviral.2015.04.010

Lim,J.H.K. Koh, C.C. Seeh, C.W. Liew, A.D. Davidson. 2013. A Crystal Structure of The Dengue Virus Non-Structural Protein 5 (NS5) Polymerase Delineates Interdomain Amino Acid Residues That Enhance its Thermostability and De Novo Initiation Activities, *Journal of Biological Chemistry*, 288(43), 31105–31114.

Lipinski, C. A., Lombardo, F., Dominy, B. W., & Feeney, F. J. 2001. Experimental and Computational Approaches to Estimate Solubility and Permeability in Drug Discovery and Development Settings, *Advanced Drug Delivery Reviews* 23, 3-25.

Martinez, F.D., and Franco, J.L. 2018. Molecular Docking: Current Advances and Challenges, *Revista Especializada en Ciencias Químico-Biológicas*, 21(1), 65-87.

Mervin L. H., Afzal A. M., Drakakis G., Lewis R., Engkvist O., Bender A. 2015. Target Prediction Utilising Negative Bioactivity Data Covering Large Chemical Space, *J. Cheminform* 7, 1–16. DOI: 10.1186/s13321-015-0098.

Ministry of Health of Indonesia. 2022. Health Profile of Indonesia Year 2021. Jakarta: Ministry of Health Republic Indonesia.

Panigrahi S.K., Desiraju G.R. 2007. Strong and Weak Hydrogen Bonds in the Protein-Ligand Interface, *Proteins*, 67(1). DOI: 10.1002/prot.21253

Podvinec, M., Scarsi, M., Schmidt, T. 2010. Discovered Novel Inhibitors of Dengue Methyltransferase through in Vitro-Driven Virtual Screening on a Computer Desktop Grid, *J. Med. Chem*, 53(4), 1483-1495. DOI: 10.1021/jm900776m.

Rachmania, R.A. 2010. Modification of Oseltamivir as an Inhibitor of Neuraminidase of Influenza A subtype H1N1 Through Docking and Simulation of Molecular Dynamics, Tesis, Depok. Universitas Indonesia.

Saleh, M., and Kamisah Y. 2020. Potential Medicinal Plants for The Treatment of Dengue Fever and Severe Acute Respiratory Syndrome-Corona, *Biomolecules*, 11(42), 1-25.

S. Rajapakse, C. Rodrigo, and A. Rajapakse. 2012. Treatment of Dengue Fever: Infection and Drug Resistance, *Dove Medical Press,* 5(1), 103-112.

Tambunan, U., Nasution, M., Azhima, F., Kerami, D. 2017. Modification of *S*-Adenosyl-l-Homocysteine as Inhibitor of Nonstructural Protein 5 Methyltransferase Dengue Virus Through Molecular Docking and Molecular Dynamics Simulation Drug Target Insights, *SAGE Publications*, 11(1), 1-14

Thisyakorn, U. 2014. Latest Developments and Future Directions in Dengue Vaccine, *Ther Adv Vaccines*, 2(1), 3–9

Wallace AC, Laskowski RA, Thornton JM. 1995. Ligplot+: a Program to Generate Schematic Diagrams of Protein-Ligand Interactions, *Protein Eng*. 8, 127-134

Wei D. Q., Zhang R., Du Q. S., Gao W. N., Li Y., Gao H., Wang S. Q., Zhang X., Li A. X., Sirois S. and Chou K. C. 2006. Anti-SARS Drug Screening by Molecular Docking, *Amino Acids*, 31(1), 73-80.

World Health Organization. 2016. WHO Traditional Medicine Strategy. Hongkong: WHO.

Zhao,T. S. Soh, J. Zheng, K. W. K. Chan, W. W. Phoo. 2015. A Crystal Structure of The Dengue Virus NS5 Protein Reveals A Novel Inter-Domain Interface Essential for Protein Flexibility and Virus Replication, *PLoS Pathogens*, 11(3), 1-27.