In Silico Analysis of Active Compounds from *Allium tuberosum* as Drug Candidate for Inhibitor DENV-3 Envelope Protein

Moh Mirza Nuryady[1*)], Levina Imelda Juliana[1], Kiky Martha Ariesaka[2]
[1]*Biology Education Department, Faculty of Teacher and Training Education, University of Muhammadiyah Malang*
[2]*Department of Parasitology, Faculty of Medicine, State University of Malang*
*E-mail: mirzanuryady@umm.ac.id

ABSTRACT

Dengue fever has become a global health issue, the development of dengue vaccine has not yet been established. Medicinal plants are an ideal alternative for DENV infection drugs. The purpose of this study was to determine in silico the potential of active compounds from *Allium tuberosum* as envelope protein inhibitors of DENV-3. The method of this research is to do docking analysis of compounds with DENV-3 envelope protein and analysis of amino acid residues using MVD, pharmacokinetic analysis using SwissADME, toxicity analysis using ProTox-II. The best docking value for the potential activity to inhibit the receptor DENV-3 is the thymidine compound (RS: -81.1245 kcal/mol). The highest activity of thymidine is the most promising as a drug candidate, as evidenced by the toxicity analysis which is predicted to have non-carcinogenic, non-mutagenic, inactive properties against hepatotoxicity, cytotoxicity and immunotoxicity parameters, as well as pharmacokinetic analysis that fulfills 6 parameters of lipopolicity, molecular weight, polarity. , insolubility, insaturation, and flexibility which indicate the drug candidate of thymidine is safe for its bioavailability. The conclusion from the results of this study is that one compound has the ability as an antiviral, binding score with DENV-3 is good, and is safe in terms of pharmacokinetics and toxicity, namely thymidine compound.

Keywords: Dengue treatment, Tuberrosides, DENV-3, In silico.

INTRODUCTION

Dengue virus is transmitted by Aedes mosquitoes (Sukohar, 2014). The dengue disease is caused by the family Flaviviridae which has 4 different serotypes. Dengue virus serotypes are from DENV 1, DENV 2, DENV 3, DENV 4 (Costa *et al*., 2012). According to Samsono (Sasmono *et al*., 2019) The incidence of dengue virus infection in Indonesia is generally caused by dengue virus serotype 3 (DENV3). The DENV genome is 10.7 Kb in size with single-stranded positive-sense RNA, encoding three structural proteins namely Capsid (C), Membrane (M), Envelope (E), and seven non-structural proteins (NS1 - NS7) (Haryanto et al., 2016). Both structural proteins and non-structural proteins have an important role in the process of infection into human cells, so they are generally studied in the search for DENV vaccine candidates.

However, the development of the DENV vaccine has not yet been established, due to the variety of DENV serotypes (Messina et al., 2014). In addition to vaccine development research, currently research on anti-DENV drug discovery is also being developed. The development of the search for drug candidates was carried out by preliminary testing in the form of an in-silico test of active compounds with viral ligands by bioinformatics.

Allium tuberosum contains various bioactive compounds, including adenosine, thymidine and tuberosine (Jannat *et al*., 2019). *Allium tuberosum* has been identified as having anti-inflammatory, antiviral and immunomodulatory activities, its bioactive compounds have shown promising properties in various studies, further research which are promising for development as a DENV drug candidate (Nhut *et al*., 2020). So far, adenosine, thymidine and tuberosine have not been widely studied regarding their activity as candidate virus inhibitors, especially DENV. This study aims to analyze tuberoside as a DENV drug candidate in silico.

In this in silico study, the viral ligand that will be used is the DENV E glycoprotein complex. Glycoprotein E plays a role when the virus will enter the host cell by binding to the host receptor (Routhu *et al*., 2019). Furthermore, there will be a conformational change in the host membrane due to a decrease in the pH of the endosome, which will lead to fusion of the virus with the host cell membrane. The selection of the DENV E protein was based on the important fact that it is a key protein before the virus fuses. The hope is that with the

interaction of the active compound tuberoside with the glycoprotein E DENV complex, it will prevent viral infection to host cells.

METHODS

Ligand structure preparation

Compound IUPAC name information on *Allium tuberosum* obtained from the PubChem database (https://pubchem.ncbi.nlm.nih.gov). Next, prepare the ligand by drawing a 2D structure with ChemBioDraw Ultra 16 software. Each of the above structures is then converted into 3D using the ChemBio3D Ultra 16 program. Then energy is minimized using the Merck Molecular Force Field 94 (MMFF94) method on the ChemDraw application.

Preparation of protein structure E DENV-3

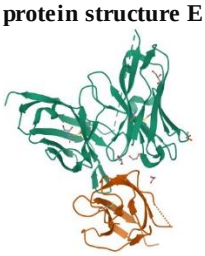

Figure 1. Crystal structure of the dengue virus serotype 3 envelope protein domain III

The structural protein structure of DENV-3 in .pdb document format was downloaded via the RCSB macromolecular biology structure information portal (https://www.rcsb.org). The downloaded protein is the DENV-3 envelope protein (PDB ID: 3UZE) (Hadi & Hamzah, 2013).

Molecular docking

The docking process begins by entering the compound preparation results and the DENV-3 E protein preparation results into the Molegro Virtual Docker (MVD) software (CLCbio, 2013). At this stage, the removal of water molecules (H_2O) around the protein structure is also carried out, it aims so that the water molecules do not interfere with the docking process, so that it can be ascertained that only ligands interact with proteins, then cavity detection, validation of the docking method and then docking of test compounds with receptors are carried out target (Zaidan et al., 2019).

Prediction of compound toxicity

Toxicity test was carried out using the ProTox-II website (https://toxnew.charite.de/-protox_II/), using canonical SMILES the compounds would be processed for their toxicity properties.

Pharmacokinethic analysis

The pharmacokinetic analysis was carried out through the SwissADME website which can be accessed via (http://www.swissadme.ch/). The first step was to copy the canonical SMILES of the three active ingredients, and paste them in the SwissADME instruction column. The pharmacokinetic/bioavailability analysis is processed when clicking the run button

RESULTS AND DISCUSSION

Cavity Determination and Receptor Validation

Cavity detection aims to predict areas that have the potential as active sites for proteins to bind to ligands (Pratoko, 2012). The active site protein is green, and the Cavity used is Cavity 5 Vol 33.28 Surface = 102.4 (Figure 2).

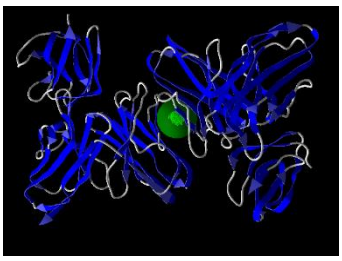

Figure 2. The blue band represents the Eprotein DENV-3, while the green circle represents the active site of the E protein

The validation of the docking method was carried out by re-docking the native ligand with the envelope protein DENV-3 PDB ID 3UZE (Table 1). The parameter used to provide an assessment of the validity is by looking at the RMSD (Root Mean Square Deviation) value. RMSD is a parameter that describes how big the change in the interaction of the ligand surface on the crystal structure of the protein before and after docking (Nursanti *et al*., 2022). The docking method is said to be valid if the RMSD value is < 2 so that it can be used for docking the test compound. The smaller the resulting RMSD value indicates that the predicted ligand position is getting better (Puspaningtyas, 2013).

Table 1. Validation of the docking method

Ligand EPE_254 [B]	Result
RMSD (Å)	1.5719
Rerank Score (kkal/mol)	-62.4358
Moldock Score (kkal/mol)	-72.543

Compound docking results and residual amino acid interactions

The results of the docking analysis of DENV-3 envelope protein (PDB ID: 3UZE) with compounds in *Allium tuberosum* (Table 2), the lowest RS value was found in thymidine compounds with a value of -81.1245 kcal/mol, meaning that thymidine compounds had the

highest activity compared to other compounds. other tests, because it has the lowest energy value so that the protein-ligand bonding ability is more stable (Wei & Cai, 2008). The results of the rerank score (RS) describe the binding of the ligand to the receptor, the rerank score can be used to evaluate the quality of docking, predict and measure the value of binding affinity, and can find the right ligand conformation by looking at the lowest value. Based on the RS, it is possible to predict the activity of the test compound when it has bound to a protein, the lower the RS the more stable the bond between the protein-ligand so that the activity of the compound can be said to be higher and vice versa. (Zaidan *et al*., 2019). 3D visualization of ligand-receptor interactions can be seen in (Figure 3).

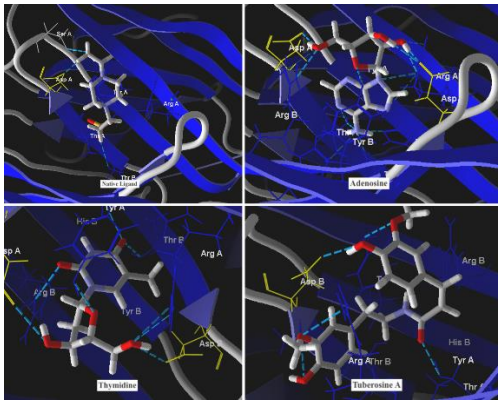

Figure 3. 3D visualization of ligand-receptor (3UZE) interactions

The results of molecular docking between the ligand and the receptor can also be seen in the interaction of the resulting amino acid residues so that we can find out more about the analysis of molecular docking and can determine whether the compound has the same inhibitory activity as the native ligand (Schaeffer, 2008). The thymidine test compound produced the same amino acid residues as the native ligand, namely Tyr 81, Arg 20, Thr 72, Thr 72 (Table 2). According to (Syahputra *et al*., 2014) The same amino acid residue as the native ligand indicates that the ligand is able to inhibit the activity of the target protein and has the potential to have the same function as the native ligand. Thymidine compounds also have more hydrogen bonds than the original protein ligands, this illustrates that the more hydrogen bonds between molecules, the more stable the bonds (Syahputra et al., 2014). 2D visualization of ligand-receptor interactions can be seen in (Figure 4).

Tabel 2. Result of molecular docking and amino acid residues

Compound	Rerank Score	Residual Amino Acid Interactions	
		Hydrogen bond	Steric Bond
Adenosine	-74.0006	Arg 20, Arg 20, His 83, Thr 72*, Tyr 81*, Asp 74, Arg 20	Arg 20*, Arg 20*
Thymidine	-81.1245	Asp 74, Asp 74, Arg 20, Tyr 81*, Arg 20, Thr 72*, Thr 72*	Thr 72, His 83
Tuberosine A	-0.17026	Tyr 81*, Asp 74, Thr 72*	Ala 73, Ala 73, Ala 73, His 83, His 83, Arg 20*, Arg 20*, Thr 72, Arg 2*, Tyr 81

Note: *same amino acid residue as the original ligand

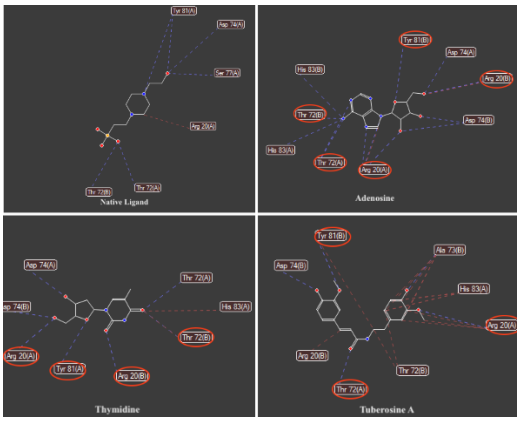

Figure 4. 2D visualization of ligand-receptor interactions (hydrogen bond: blue line; steric bond: red line; Red circle: same amino acid residue as native ligand)

Prediction of compound toxicity

Toxicity prediction aims to determine the nature of a candidate drug compound along with the possible risks that arise from these compounds and can have an impact on humans (Sqra, 2022).

Table 3. Toxicity prediction of thymidine compound, computed by ProTox-II

Thymidine	
Toxicity Class	6 (non-toxic)
Hepatotoxicity	Inactive
Carcinogenicity	Inactive
Immunotoxicity	Inactive
Mutagenicity	Inactive
Cytotoxicity	Inactive

The results of the toxicology prediction (Table 3) with the parameters of hepatotoxicity, carcinogenicity, immunotoxicity, mutagenicity, and cytotoxicity showed that the compounds studied were thought to be non-carcinogenic, non-mutagenic, meaning that the compound was predicted to have the property of not causing chromosomal changes (cannot change genetics). and does not trigger the growth of cancer cells (Hardjono, 2017). Classification of toxicity were classified based on *Globally Harmonized System* (GSH), which stated that toxicity was divided into six classes based on the LD_{50} value. Classes 1 - 3 had a high level of toxicity, making them extremely hazardous , while grades 4 - 6 had low toxicity, they were nonetheless potentially hazardous (Umboro *et al*., 2020). Thymidine compounds also showed inactive results on the parameters of hepatotoxicity, cytotoxicity and immunotoxicity. The thymidine compound showed good toxicity class results, namely class 6 (non-toxic), so based on the table it can be concluded that the compound that showed the best results was thymidine compound.

Analysis pharmacokinetic

Prediction of pharmacokinetic properties (Table 4) is carried out to see how effective a drug compound is in the body when consumed. Parameters observed included ADME (absorption, distribution, metabolism and excretion).

The results of the gastrointestinal (GI) absorption parameters of thymidine compounds showed high absorption. High absorption of a drug in the intestine can mean that the drug is good and can be distributed throughout the body (Lohidashan *et al*., 2018). Bioavailability score is a computational formulation to see the permeability of Caco-2 cells in the probability of a compound that can be well absorbed in the human body if it has a value of 0.55 and if it is 0.17 then it cannot be absorbed properly by the body (Martin, 2005). Based on the results of the bioavailability score of thymidine compounds obtained a value of 0.55. This shows that thymidine compounds can be well absorbed by the body in Caco-2 cells.

Table 4. ADME predictions of thymidine compound, computed by SwissADME

Thymidine	
Molecular weight (g/mol)	242.23
No. of hydrogen bond donors	3
No. of hydrogen bond acceptors	5
No. of rotatable bonds	2
Total polar surface area (A°)	104.55
Log P (iLOGP)	1.12
Log S (ESOL)	-0.73
GI Absorption	High
Lipinski's (Ro5)	Yes, 0 violation
Bioavability score	0.55

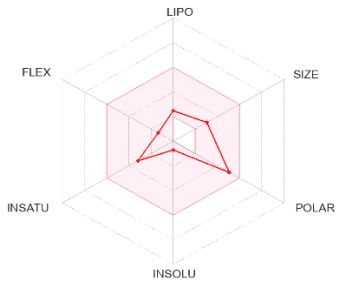

Figure 5. Radar bioavailability of thymidine Compound

The bioavailability radar can be seen in (Figure 5), the pink color represents the optimal range of the compound as an oral drug based on 6 parameters (lipophilicity: the ability of a chemical compound to dissolve into fat, size: 150-500 g/mol, polarity: TPSA value in the range of 20- 130Å), solubility: logS value < 6, flexibility: rotatable bond < 9 and saturation: carbon fraction on sp3 hybridization < 0.25) (Daina *et al*., 2017). Based on the picture, it can be seen that the thymidine compound is in the pink area so it can be predicted that the compound has good oral bioavailability. Other pharmacokinetic parameters showed that the thymidine compound was predicted to have a high gastrointestinal (GI) absorption with PGP- which indicates that once the compound enters the cell, it will not be pumped out again (Chelliah, 2008). This data is also reinforced by

the results of the drug-likeness prediction (table) which shows that the thymidine compound complies with Lipinski's rule (Ro5) with 0 errors (Table 4).

To determine whether or not these compounds can be used as drug candidates / to determine the activity of a compound, it is not enough just to look at the binding affinity value of the molecular docking alone, but also a compound that has low toxicity, is easily absorbed by the body, has good permeability and has good properties. Good oral bioavailability. Based on data that has been obtained from several in silico tests, compounds that show the best results and have the potential to be developed as drug candidates are found in thymidine compounds, these compounds show stable ligand-receptor binding affinity values, good amino acid interactions, low toxicity values, It has good oral permeability and bioavailability.

CONCLUSION

The conclusion from the results of this study was that thymidine compound has ability for antiviral, highest binding score with DENV-3, and safe in terms of pharmacokinetics and toxicity. Thus this study recommends to conduct further in vitro and clinical studies of the thymidine compound present in *Allium tuberosum* to confirm its potential as a DENV-3 drug candidate.

REFERENCES

Chelliah DA. 2008. Biological Activity Prediction of an Ethno Medicinal Plant Cinnamomum camphora Through Bio-informatics. *Ethnobotanical Leaflets*. **12**: 181-190.

CLCbio. 2013. *Molegro Virtual Docker User Manual, MVD 2013 6.0 for Windows, Linux, and Mac OS X*. A CLC Bio Company.

Costa RL, Voloch CM & Schrago CG. 2012. Comparative Evolutionary Epidemiology of Dengue Virus Serotypes. *Infection, Genetics and Evolution*. **12**(2): 309-314.

Daina A, Michielin O & Zoete V. 2017. SwissADME: A Free Web Tool To Evaluate Pharmacokinetics, Drug-Likeness and Medicinal Chemistry Friendliness of Small Molecules. *Scientific Reports*. **7**(March): 1-13.

Hadi D & Hamzah N. 2013. Studi Hubungan Kuantitatif Struktur-Aktivitas, Fitur Farmakofor, dan Docking Molekuler Senyawa Turunan Pirazolo-[3,4-d]-pirimidin sebagai Inhibitor Mer Tirosin Kinase. *Acta Pharmaceutica Indonesia*. **38**(1): 1-10.

Hardjono S. 2017. Prediksi Sifat Farmakokinetik, Toksisitas dan Aktivitas Sitotoksik Turunan N-Benzoil-N'-(4-fluorofenil)tiourea sebagai Calon Obat Antikanker melalui Pemodelan Molekul. *Jurnal Ilmu Kefarmasian Indonesia*. **14**(2): 246-255.

Haryanto S, Hayati RF, Yohan B, Sijabat L, Sihite IF, Fahri S, Meutiawati F, Halim JAN, Halim SN, Soebandrio A & Sasmono RT. 2016. The molecular and clinical features of dengue during outbreak in Jambi, Indonesia in 2015. *Pathogens and Global Health*. **110**(3): 119-129.

Jannat, K., Rahman, T., & Rahmatullah, M. 2019. Traditional uses, phytochemicals and pharmacological properties of Allium tuberosum Rottler ex spreng. *J Med Plants Stud*. **7**: 214-220.

Lohidashan K, Rajan M, Ganesh A, Paul M & Jerin J. 2018. Pass and Swiss ADME Collaborated in Silico Docking Approach to The Synthesis Of Certain Pyrazoline Spacer Compounds for Dihydrofolate Reductase Inhibition and Antimalarial Activity. *Bangladesh Journal of Pharmacology*. **13**(1): 23-29.

Martin YC. 2005. A Bioavailability Score. *Journal of Medicinal Chemistry*. **48**(9): 3164-3170.

Messina JP, Brady OJ, Scott TW, Zou C, Pigott DM, Duda KA, Bhatt S, Katzelnick L, Howes RE & Battle KE. 2014. Global Spread of Dengue Virus Types: Mapping The 70 Year History. *Trends in Microbiology*. **22**(3): 138-146.

Nhut PT, An TNT, Minh LV, Truc TT & Anh NHT. 2020. Phytochemical screening of Allium Tuberosum Rottler. ex Spreng as food spice. *IOP Conference Series: Materials Science and Engineering*. **991**(1): 12021.

Nursanti O, Aziz A & Hadisoebroto G. 2022. Docking dan Uji Toksisitas Secara Insilico untuk Mendapatkan Kandidat Obat Analgesik. *INPHARNMED Journal*. **6**(1): 35-46.

Pratoko DK. 2012. Molecular Docking Senyawa Fitokimia Piper Longum (L.) Terhadap Reseptor Siklooksigenase-2 (Cox-2) Sebagai Antiinflamasi. *Chemistry*

Progress. **5**(1): 31-36.

Puspaningtyas A. 2013. Docking Molekul dengan Metoda Molegro Virtual Docker dari Ekstrak Air Psidium Gujava, Linn dan Citrus Sinensus, Peels Sebagai Inhibitor Pada Tirosinase Untuk Pemutih Kulit. *Jurnal Kimia Terapan Indonesia*. **15**(1): 31-39.

Routhu NK, Lehoux SD, Rouse EA, Bidokhti MRM, Giron LB, Anzurez A, Reid SP, Abdel-Mohsen M, Cummings RD & Byrareddy SN. 2019. Glycosylation of Zika Virus is Important in Host–Virus Interaction and Pathogenic Potential. *International Journal of Molecular Sciences*. **20**(20): 5206.

Sasmono RT, Kalalo LP, Trismiasih S, Denis D, Yohan B, Hayati RF & Haryanto S. 2019. Multiple Introductions of Dengue Virus Strains Contribute to Dengue Outbreaks in East Kalimantan, Indonesia, in 2015-2016. *Virology Journal*. **16**(1): 93.

Schaeffer L. 2008. The Role of Functional Groups in Drug-Receptor Interactions. *The Practice of Medicinal Chemistry*. 464-480.

Sqra CD. 2022. Komparasi Penilaian Risiko Kesehatan Bahan Kimia : Metode CHRA dan SQRA. *IAKMI Jurnal Kesehatan Masyarakat Indonesia*. **3**(1): 1-12.

Sukohar A. 2014. Demam Berdarah Dengue (DBD). *Jurnal Medula*. **2**(02): 1-15.

Syahputra G, Ambarsari L & Sumaryada T. 2014. Simulasi Docking Kurkumin Enol, Bisdemetoksikurkumin dan Analognya Sebagai Inhibitor enzim12-lipoksigenase. *Biofisika*. **10**(1): 55-67.

Umboro RO, Bimmaharyanto E, Yanti NKW & Kesehatan F. 2020. Uji efektivitas antioksidan (IC50) dan toksisitas akut (LD50) fraksi etanol daun nangka (Artocarpus Heterophyllus Lam.). *JUPE (Jurnal Pendidikan Mandala)*, **5**(6): 187-196.

Wei H & Cai RG. 2008. Cosmological Constraints on New Agegraphic Dark Energy. *Physics Letters, Section B: Nuclear, Elementary Particle and High-Energy Physics*. **663**(1-2): 1-6.

Zaidan S, Rahmat D, Djamil R & Mumpuni E. 2019. Activity of Compounds in *Sargassum* sp. as Anti-atherosclerosis with Ligand-Receptor Comparison HMG-CoA Reductase- Simvastatin (1HW9) and In-Silico Toxicity Test. *Ilmu Kefarmasian Indonesia*. **17**(1): 120-125.