PharmacologyOnLine
Archives • 2021 • vol.3 • 1334-1351

IN SILICO STUDY OF THE TOXICITY AND ANTIVIRAL ACTIVITY PREDICTION OF JAMBLANG (Syzygium cumini) LEAVES ESSENTIAL OIL AS ACE2 INHIBITOR

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Abstract

ACE2 is a type 1 membrane integral glycoprotein which is highly expressed in the respiratory tract (lungs). The presence of excessive ACE2 becomes an opportunity for the entry of SARS-CoV-2 and the beginning of its pathogenesis. The mechanism of ACE2 inhibition or suppression of this enzyme is one of the targets for controlling SARS-CoV-2 infection. *Syzygium cumini* (jamblang) is an aromatic herbal plant that has broad-spectrum pharmacological properties, one of which is as an antiviral. This study aims to predict toxicity and predict compound activity against ACE2 receptors (PDB ID 1R4L). Previously, the predictions of physicochemical properties have been carried out using the SwissADME application. The physicochemical properties refer to Lipinski's Five Laws. The results showed that jamblang leaf essential oil complied with Lipinski's Five Laws with 0 errors each. Furthermore, the prediction of toxicity was carried out using the pkCSM Online Tools and Protox Online Tools applications. The results of the LD50 value and the classification of toxicity classes were classified according to GHS. Jamblang leaf essential oil compound had the highest LD50 of 5000 mg/kg and belongs to class 5. The prediction of compound activity was done using the Molegro Virtual Docker (MVD) application. The 1R4L receptor was said to be valid because it had an RMSD value <2. The best and lowest A. Rerank score (RS) of the comparison drugs was the essential oil compound of Jamblang leaf with RS of -73.338 kcal/mol. Jamblang leaf essential oil compounds interact with the ACE2 receptor (1R4L) in cavity 4. With this prediction, it had ACE2 inhibitory potential, so jamblang leaf essential oil compounds could be recommended for further development as candidates for antiviral drugs.

Keywords: Antivirus, ACE2, molecular docking, Syzygium cumini, in silico

Introduction

A series of medical interventions have been carried out to treat patients with medical indications of SARS-CoV-2 infection. However, most of these therapies are not specifically designed to treat COVID-19, but rather to treat previous illnesses that have COVID-19-like symptoms (1). Until now there are still no available therapeutic options to cure infections caused by SARS CoV-2. Therefore, a potential and specific therapeutic agent is needed to cure COVID-19 disease (1).

The potential therapeutic agents that can be used do not only come from synthetic drugs or chemicals. The purpose, these times, have emphasized on various medicinal plants that are potentially considered as part of a formulation or used safely in the prevention and management of infectious diseases such as COVID-19 (2). Medicinal plants have received more attention for their health benefits since ancient times (3. The content of active compounds in plants is proven to be utilized as a treatment for diseases caused by microbes. The various studies have stated that bioactive compounds in plants have many activities, one of which is antiviral. Based on this, medicinal plants are considered as good sources of SARS-CoV-2 antiviral (4).

Plants are a source of medicinal active compounds that have been widely used to treat diseases caused by microorganisms. There are many bioactive compounds reported to have antifungal, antibacterial, and antiviral activity. Natural products that are reported to have antiviral activity can be used as a starting point to look for the candidates of bioactive compounds that are likely to overcome SARS-CoV-2. Molecular docking can be used to predict how proteins (receptors) interact with bioactive compounds (ligands) (5).

Jamblang (Syzygium cumini) from the Myrtaceae family is a plant family that is rich in antioxidants and even members of the syzigium genus are considered as the very important essential oils. Prepared syzigium is mainly cultivated for essential oil, which is obtained by distillation from freshly ground leaves. Syzigium essential oil chemical composition may vary with plant maturity, geographic area and conditions (6).

Syzigium oil in the form of extract has various kinds of esters, especially menthylacetate and monoterpenes which produce aroma and taste (minty) beneficial for respiration. In-vitro studies and clinical studies demonstrate the therapeutic potential of the aromatic herbs in the treatment of respiratory diseases. Aromatic herbs have broad-spectrum pharmacological properties and are used in traditional medicine (7). Syzigium oil contains bioactive compounds and exhibits antibacterial, antiviral and antitussive properties (8). Syzigium essential oil has shown promise as an antiviral agent against several viral pathogens.

One of the steps carried out in the in silico study was the prediction of physicochemical properties based on Lipinski's 5th law. The analysis of physicochemical properties was an ideal introduction for orally available drug candidates. Currently, the general physicochemical properties analysis is carried out to analyze the properties of a compound before synthesizing new drug candidates (9). The three parameters of Lipinski's 5th Law are critical for the target structure and drug binding sites (10). This research has conducted a preliminary study to test the physicochemical properties of several jamblang leaf essential oil compounds using the SwissADME application. The results obtained were 4 bioactive compounds of jamblang leaf essential oil complying with Lipinski's 5th Law with 0 errors (11).

In this study, a comparison drug was used named nelfinavir. The selection of this drug was based on the drug's ability to inhibit the enzyme ACE2 or key cellular process required for the entry of SARS-CoV-2 into host cells (12). In-silico studies have shown that nelfinavir is a potent inhibitor that binds ACE2 through inhibition of viral replication (13, 14). So far, there have been no studies examining compounds in jamblang leaf essential oil against COVID-19 receptors. Therefore, this study aimed to find out the interaction of bioactive compounds of jamblang leaf essential oil with ACE2 receptors with in-silico and to determine the level of toxicity of jamblang leaf essential oil compounds with in-silico.

Methods

The type of research used was experimental laboratory research with in silico from jamblang leaf

essential oil compounds, 2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one; 1,2,4-Benzenetriol; Cyclononasiloxane; Cyclodecasiloxane; Octadecamethyloctasiloxane with the receptor of ACE2 PDB ID ID 1R4L using the application of Chem Bio Draw Ultra version 12, Chem Bio 3D Ultra version 12, Molegro Virtual Docker 6.0 (Molegro ApS), SwissADME, and pkCSM online tool.

The research was conducted in January - April 2021 at the Department of Biology, Faculty of Mathematics and Natural Sciences, Medan State University, Multifunction Laboratory, Faculty of Science and Technology, Ar-Raniry State Islamic University Banda Aceh and Biology Laboratory, Faculty of Mathematics and Natural Sciences Education. , University of Education Indonesia, Bandung.

The tools used in this research were hardware in the form of a set of Lenovo Yoga laptops with specifications of Intel Processor i7, 6 GB RAM, and 1 Tera of hard disk as well as operating system software of Windows 10, Chem Bio Draw Ultra Version 12 (Cambridge Soft), Chem Bio 3D Ultra Version 12 (Cambridge Soft), Molegro Virtual Docker 6.0 (Molegro ApS), pkCSM Online Tool, Protox Online Tool, SwissADME and SPSS Statistics 26.0 for Windows.

Compound Structure and Comparison of Nelfinavir

The ligands used in this study were the structure of jamblang leaf essential: oil 2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one; 1,2,4-Benzenetriol; Cyclononasiloxane; Cyclodecasiloxane; Octadecamethyloctasiloxane, and the comparison compound nelfinavir were structurally drawn using application of the Chem Bio Draw Ultra Version 12 (Cambridge Soft), Chem Bio 3D Ultra.

A B C

D E

Figure 1 Structure (A) 2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one; (B) 1,2,4-Benzenetriol; (C) Cyclononasiloxane; (D) Cyclodecasiloxane; (E) Octadecamethyloctasiloxane

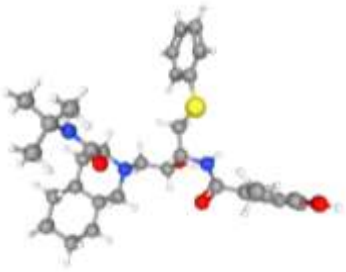


Figure 2 Structure of the comparison compound named nelfinavir

1R4L Receptor Structure

Three-dimensional structure of ACE2 with PDB ID 1R4L as the COVID-19 receptor protein downloaded from the protein data bank (PDB) website https://www.rcsb.org/structure/1R4L

Figure 3. ACE2 Receptor with PDB ID 1R4L

Research procedure

Ligand Preparation

The structure of ligands of jamblang leaf essential oil compounds was obtained from PubChem

(https://pubchem.ncbi.nlm.nih.gov) in Sdf format with Nelfinavir as a comparison. Next, the ligand preparation process was carried out by drawing a 2D molecular structure with software of Chem Bio Draw Ultra Version 12 and then copied to the software of Chem Bio 3D Ultra Version 12 to create a 3D structure. The next step was minimizing energy using the MMFF94 method to see the stereochemical forms of the compound and the most stable forms, and then they were replicated 3 times, averaged, and stored in SYBYL format (*mol2).

Protein Preparation

Receptor protein preparation was carried out using software of Molegro Virtual Docker 6.0. At this stage, the elimination of water molecules and the reference ligand as well as the addition of hydrogen atoms were carried out.

Molecular Docking Binding

1. Molegro docking binding is done using software of Molegro Virtual Docker 6.0 (Molegro ApS). There are several steps in the docking process as follows:
2. Downloading the receptor from the Protein Data Bank website. The downloaded receptor was ACE2 with PDB ID 1R4L.
3. Adding H atom to the receptor (because the downloaded receptor was deprived of its H atom) and fixing the downloaded receptor.
4. Detecting the site on the receptor where the drug would bind (interact). These places are in the form of holes (cavities) in the receptor structure.
5. Placing the 3D structure into the selected hole.
6. Seeing the view location of the compound in the receptor holes (cavities).
7. Doing compound docking at the receptor which is processed automatically by the software, Molegro Virtual Docker, and the results of each parameter were recorded.

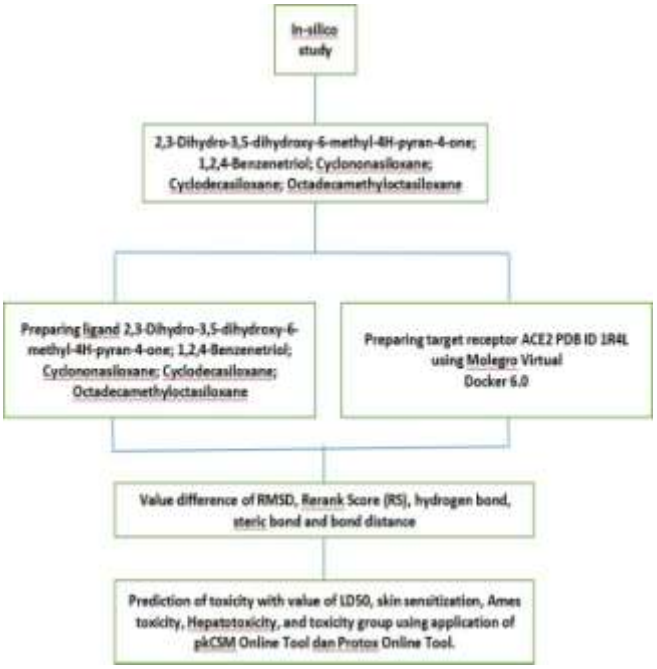


Figure 4. Research Work Scheme

Toxicity Prediction

Toxicity prediction was done by drawing a 2D molecular structure of the compound with software of Chem Bio Draw Ultra Version 12, and then copied to software of Chem Bio 3D Ultra Version 12 to create a 3D structure. After that, it was saved in the form of *.sdf or *.pdb files. Next stage was lookng for the SMILES code of the compound 2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one; 1,2,4-Benzenetriol; Cyclononasiloxane; Cyclodecasiloxane; Octadecamethyloctasiloxane by changing the 2D structure through the edit menu and copying it as SMILES code. Then SMILES code was copied to Microsoft Word or Microsoft Excel and saved for testing. In this SMILES format, the compounds were processed using the pkCSM Online Tool and the Protox Online Tool.

Data analysis.

The data analysis from the docking results was determined by the value of Root Mean Square Deviation (RMSD), Rerank Score (RS), hydrogen bonds, steric bonds and bond distances. The analysis of the toxicity data of 2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one; 1,2,4-Benzenetriol; Cyclononasiloxane; Cyclodecasiloxane; Octadecamethyloctasiloxanen was categorized its toxicity class using pkCSM

Online Tool and Protox Online Tool. In this test, validation was carried out 3 times so that there were more valid results obtained. The results obtained were presented in the form of statistical tables using application of SPSS Statistics 26.0 for Windows through Kruskall-Wallis method. This test could determine the test compound better than the comparison one by looking at the difference in the average value of the Rerank Score.

Results

Molecular Docking and Amino Acid Analysis

The study of in-silico (molecular docking) in this research was conducted on the essential oil compounds of jamblang leaves. Molecular docking is a method used in computer-aided structural molecular biology for drug/ligand design.

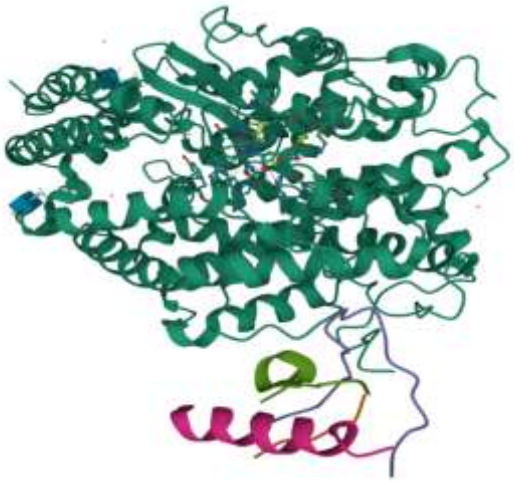


Figure 5. Download result of ACE2 target protein with PDB ligand ID 1R4L (ACE2 PDB, 2021)

Ligand and Receptor Preparation

Prior to the in-silico test, the ligand and receptor preparation were carried out first. Ligand preparation was carried out by first drawing using software of Chem Bio Draw Ultra 12.0. 2D structure images of ligands/compounds were used to obtain 3D structure using softwere of Chem Bio 3D Ultra 12.0. This was because all docking stages took 3D structure model. The results of the 2D and 3D structure of the ligand/compound were shown in Figure 6.

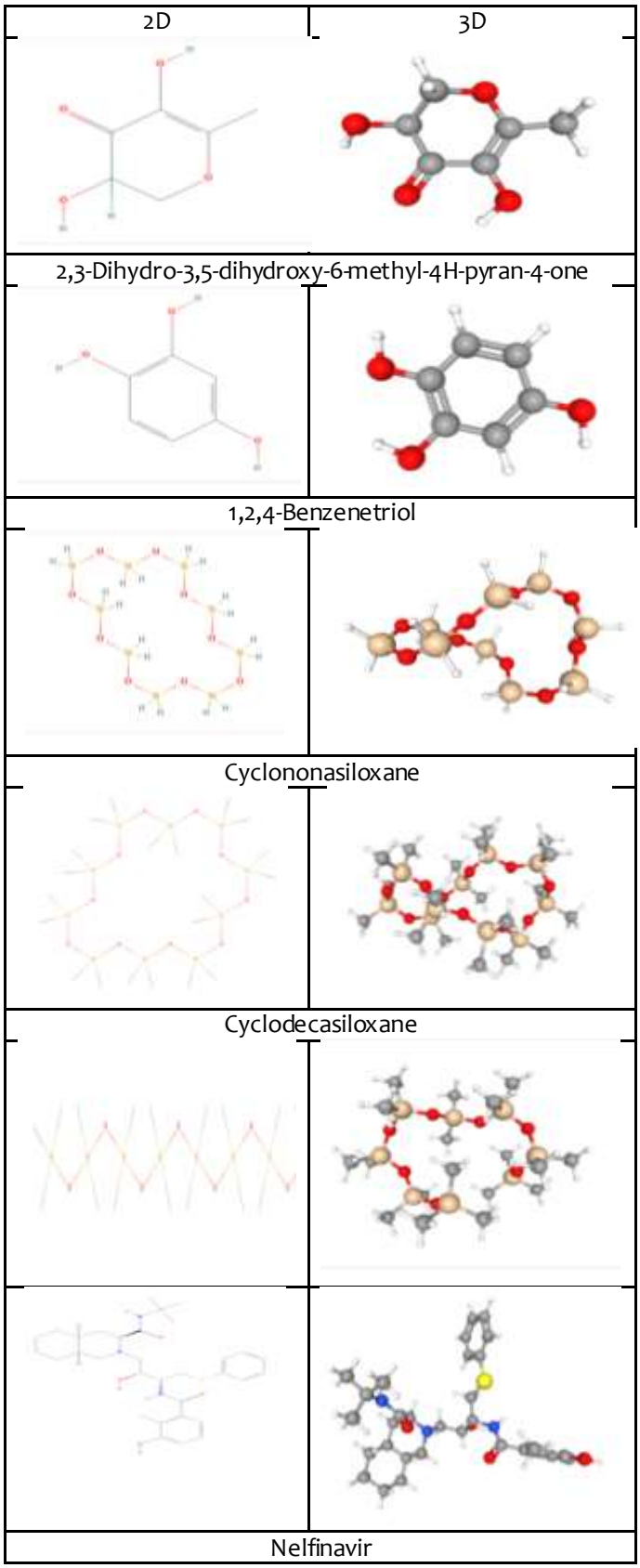
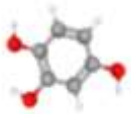
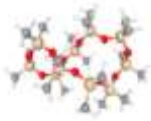
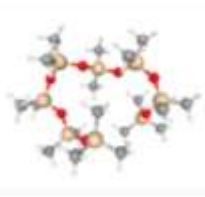


Figure 6. Visualization of 2D and 3D by applying Discovery Studio Visualizer

Table1. The results of determining the minimum energy (kcal/mol) of the Jamblang leaf essential oil in the Chem 3D Pro 12.0 application

Compound	Structure 3D	Minimum Energy (kcal/mol) Replication I	II	III	Average (kkal/mol) ±SD
2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one	 3,5-dihydroxy-6-methyl-2,3-dihydropyran-4-one	18.049	18.049	18.049	18.049 ±0,000
1,2,4-Benzenetriol	 benzene-1,2,4-triol	7.551	7.551	7.551	7.551 ±0,000
Cyclononasiloxane	 1,3,5,7,9,11,13,15,17-nonaoxa-2,4,6,8,10,12,14,16,18-nonasilacyclooctadecane	14.654	14.654	14.654	14.654 ±0,000
Cyclodecasiloxane	 2,2,4,4,6,6,8,8,10,10,12,12,14,14,16,16,18,18,20,20-icosamethyl-1,3,5,7,9,11,13,15,17,19-decaoxa-2,4,6,8,10,12,14,16,18,20-decasilacycloicosane	16.212	16.212	16.212	16.212 ±0,000
Octadecamethyloctasiloxane	 [dimethyl(trimethylsilyloxy)silyl]oxy-[[[[dimethyl(trimethylsilyloxy)silyl]oxy-dimethylsilyl]oxy-dimethylsilyl]oxy-dimethylsilyl]oxy-dimethylsilane	5.439	5.439	5.439	5.439 ±0,000
Nelfinavir	 (3S,4aS,8aS)-N-tert-butyl-2-[(2R,3R)-2-hydroxy-3-[(3-hydroxy-2-methylbenzoyl)amino]-4-phenylsulfanylbutyl]-3,4,4a,5,6,7,8,8a-octahydro-1H-isoquinoline-3-carboxamide	55.608	55.608	55.608	55.608 ±0,000

The protein was reviewed using software of Molegro Virtual Docker (MVD) shown in figure 7.

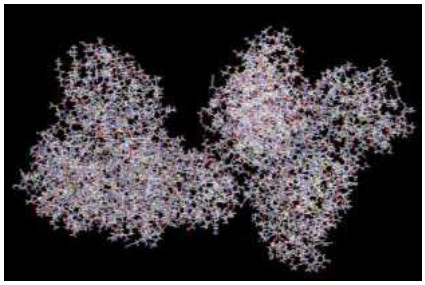


Figure 7. ACE2 Receptor

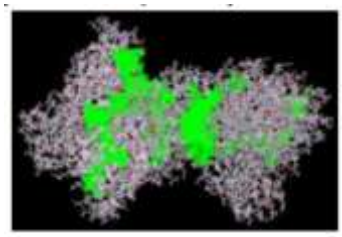
The results of making SMILES code for the jamblang leaf essential oil compound (nelfinavir) were shown in table 2.

Table 2. SMILES code results of jamblang leaf essential oil compounds and nelfinavir with ChemDraw Ultra 12.0

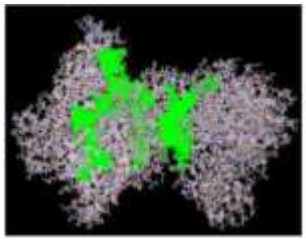
Compound name	SMILES Code
2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one	CC1=C(C(=O)C(CO1)O)O
1,2,4-Benzenetriol;	C1=CC(=C(C=C1O)O)O
Cyclononasiloxane	O1[SiH2]O[SiH2]O[SiH2]O[SiH2]O[SiH2]O[SiH2]O[SiH2]O[SiH2]1
Cyclodecasiloxane	C[Si]1(O[Si](O[Si](O[Si](O[Si](O[Si](O[Si](O[Si](O[Si](O1)(C)C)(C)C)(C)C)(C)C)(C)C)(C)C)(C)C)(C)C)C
Octadecamethyloctasiloxane	C[Si](C)(C)O[Si](C)(C)O[Si](C)(C)O[Si](C)(C)O[Si](C)(C)O[Si](C)(C)O[Si](C)(C)O[Si](C)(C)C
Nelfinavir	CC1=C(C=CC=C1O)C(=O)NC(CSC2=CC=CC=C2)C(CN3CC4CCCCC4CC3C(=O)NC(C)(C)C)O

Cavity Determination

The results of determining the cavity were shown in Figure 8.

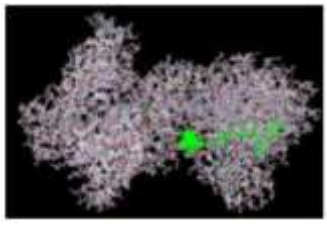


A



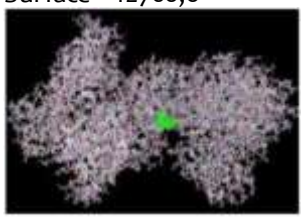
B

Cavity 1 Vol = 4692,99 Surface = 12706,6



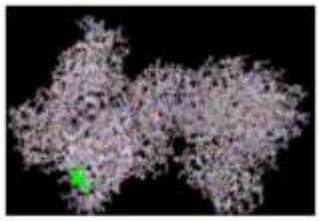
C

Cavity 2 Vol = 932,864 Surface = 2443,52



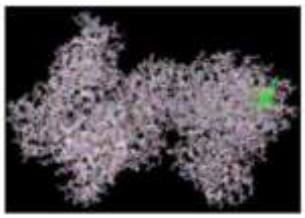
D

Cavity 3 Vol = 260,068 Surface = 871,68



E

Cavity 4 Vol = 140,288 Surface = 387,84



F

Cavity 5 Vol = 132,196 Surface = 433,92

Figure 8. The results of the detection of holes (cavity) on the 1R4L receptor (A) cavity as a whole, (B) cavity 1, (C) cavity 2, (D) cavity 3, (E) cavity 4, (F) cavity 5

Molegro Virtual Docking Settings

Before carrying out the validation and docking stages using the software of Molegro Virtual Docker (MVD), the parameters in the program must first be set. Here are the settings made on the software of Molegro Virtual Docker.

a. Scoring Function :

Score : MolDock Score [GRID]

Grid resolution (Å) : 0,30 Å

Ligand evaluation : Internal ES, Internal HBond, Sp2-Sp2 Torsions

b. Search Algorithm

Algorithm : MolDock Optimizer

Number of runs : 10

After docking : Energy minimization, Optimize H-Bonds

c. Parameter Setting

Max iterations : 1500

Max population size : 50

Max steps : 300

d. Multiple Poses : Enable energy threshold

1R4L Receptor Validation

The results of receptor validation were shown in table 3.

Table 3. the validation results of 1R4L receptors

Ligand	3WL_401 [A] Replication			Average ±SD
	I	II	III	
Value RMSD (Å)	0.33	0.33	0.32	0.327 ±0.006
Rerank Score (kkal/mol)	-85.509	-85.454	-85.568	-85.511 ±0.057
Moldock Score (kkal/mol)	-104.08	-104.06	-104.08	-104.072 ±0.009

The Docking results of jamblang leaf essential oil and Nelfinavir on 1R4L receptors

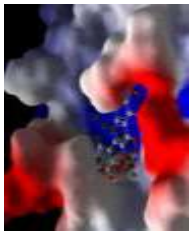
The results of the docking score obtained through docking between the ligands of the jamblang leaf essential oil compound and the comparison compound (nelfinavir) with 1R4L receptor using the software of Molegro Virtual Docker 6.0 were shown in table 4.

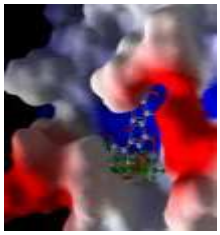
Table 4. The docking results of jamblang leaf essential oil compound and comparison compound (nelfinavir) with 1R4L receptors

Compound	Score parameter	Replication (kkal/mol) I	II	III	Average ±SD
2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one	MolDock Score	-85.927	-85.826	-85.743	-85.832 ±0.0921
	Rerank Score	-73.240	-73.540	-73.234	-73.338 ±0.1750
	H-Bond	-4.044	-4.180	-4.187	-4.137 ±0,0806
1,2,4-Benzenetriol	MolDock Score	-73.580	-72.405	-73.590	-1.340 ±0.0955
	Rerank Score	-62.660	-61.827	-62.671	-62.386 ±0.4841
	H-Bond	-1.393	-1.230	-1.398	-1.340 ±0.0956
Cyclononasiloxane	MolDock Score	-78.367	-78.353	-78.378	-78.366 ±0.0125
	Rerank Score	64.995	64.869	64.998	±0.0736
	H-Bond	-2.908	-2.930	-2.916	-2.918 ±0.01114
Cyclodecasiloxane	MolDock Score	-63.603	-63.593	-63.513	-63.570 ±0.0493
	Rerank Score	-57.890	-57.885	-56.417	-57.397 ±0.8490
	H-Bond	0.000	0.000	0.000	0,000
Octadecamethyloctasiloxane	MolDock Score	-74.384	-74.323	-74.333	74.347 ±0.0327
	Rerank Score	-64.454	64.305	-64.254	-64.338 ±0.1039
	H-Bond	0.000	0.000	0.000	0.000
Nelfinavir	MolDock Score	-154.243	-152.087	-159.767	-155.366 ±3.9612
	Rerank Score	-72.034	-66.480	-68.121	-68.878 ±2.853
	H-Bond	-5.520	-5.086	-5.412	-5.339 ±0.2259

Results of Interaction of Ligands with Amino Acids

The results of the interaction between ligands and amino acids at the GDP ID 1R4L target receptor were shown in Figures 9 and 10 as well as Tables 5 and 6.


2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one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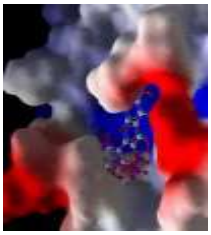
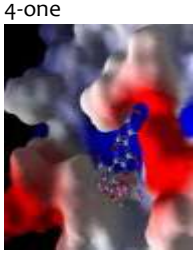

Cyclononasiloxane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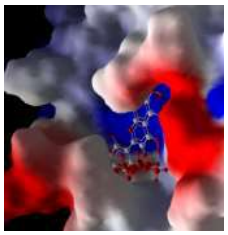

Octadecamethyloctasiloxane

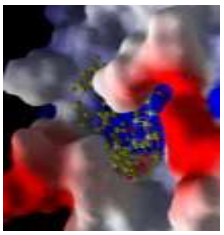

Nelfinavir

Figure 9. Three-dimensional form of interaction between jamblang leaf essential oil compound and comparison compound (nelfinavir) with amino acid receptor 1R4L

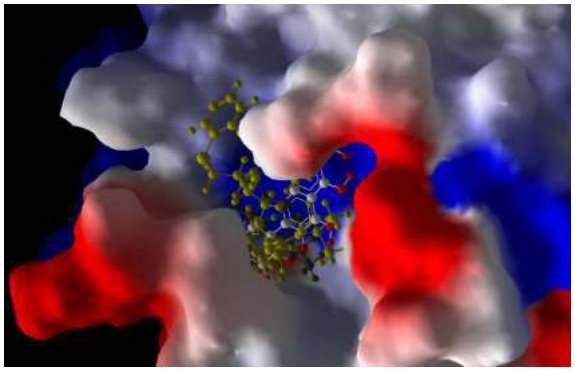


Figure 10. The Best compound binding pose (2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one) with comparison drug (nelfinavir)

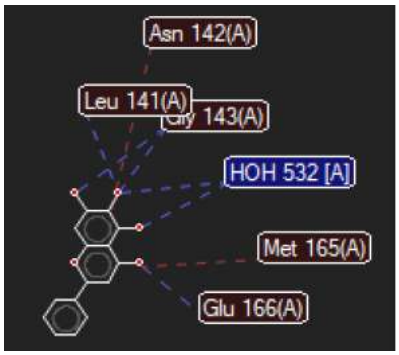
Table 5 Amino acids involved in hydrogen bonding in the 1R4L enzyme

Compound	Amino Acid	DIstance (Å)	Ligand Group
2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one	Tyr 54	2.64	O No.2
	Cys 44	2.97	O No.2
1,2,4-Benzenetriol	Gln 189	3.30	O No.10
Cyclononasiloxane	Asp 187	3.01	O No.10
Cyclodecasiloxane	-	-	-
Octadecamethyloctasiloxane	-	-	-
Nelfinavir	Cys 145	3.13	O No.34
	Gly 143	3.12	O No.34
	Asn 142	2.60	O No.35
	His 41	2.69	O No.32

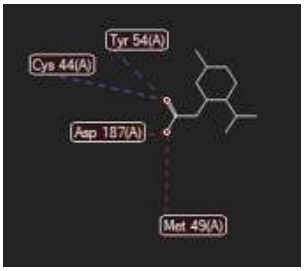
Table 6 Amino acids involved in the steric bonding of the 1R4L enzyme

Compound	Amino Acid	DIstance (Å)	Ligand Group
2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one	Asp 187	3.20	O No.3
	Met 49**	2.56	C No.3
1,2,4-Benzenetriol	Met 49**	2.29	O No.10
	Met 165*	3.15	O No.3
Cyclononasiloxane	Cys 44	3.13	C No.6
	Cys 44	2.96	C No.4
Cyclodecasiloxane	Met 186	2.76	O No.4
Octadecamethyloctasiloxane	Met 165*	3.22	C No.6
	Asp 48	3.21	C No.10
Nelfinavir	Thr 26	3.10	C No.22
	Thr 26	3.13	C No.21
	Thr 26	2.89	C No.30
	Thr 25	3.05	C No.31
	Asn 142	2.74	C No.19
	Met 49	2.78	N No.1

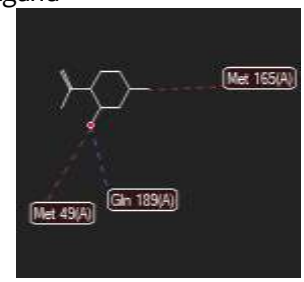
Details :
*: the same amino acid as the native ligand
** : the same amino acid as the comparison drug



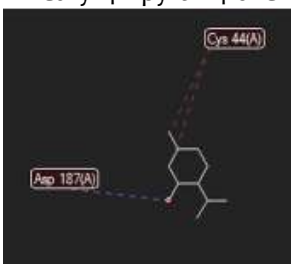
Native Ligand



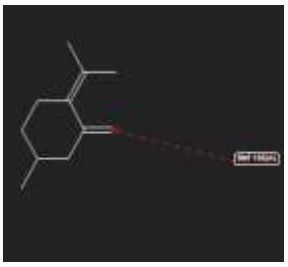
'2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one



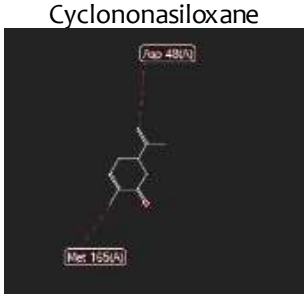
1,2,4-Benzenetriol



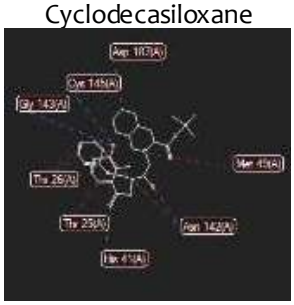
Cyclononasiloxane



Cyclodecasiloxane



Octadecamethyloctasiloxane



Nelfinavir

Figure 11. The results of amino acid interactions between 1R4L receptors (blue line: hydrogen bond; red line: steric bond)

Toxicity Prediction

The toxicity prediction results of jamblang leaf essential oil compounds and the comparison compound (Nelfinavir) were shown in table 7.

Table 7 Prediction of toxicity by using Protox Online Tool and pkCSM Online Tool

Compound	Toxicity				
	LD50 (mg/kg)*	Mutagenic test AMES**	Toxic to liver**	Skin Sensitivity**	Toxicity class*
2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one	3265	-	-	+	5
1,2,4-Benzenetriol	5000	-	-	+	5
Cyclononasiloxane	940	-	-	+	4
Cyclodecasiloxane	470	-	-	+	4
Octadecamethyloctasiloxane	1640	-	-	+	4
Nelfinavir	600	-	+	-	4

Details:
* applying Protox Online Tool
** applying pkCSM Online Tool

Mann-Whitney test

The results of the Mann-Whitney rerank score of jamblang leaf essential oil compounds on the 6M2N receptor can be seen in table 10.

Table 10.the test results of Mann-Whitney rerank score of jamblang leaf essential oil compounds on 1R4L receptors

	2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one	1,2,4-Benzenetriol	Cyclononasiloxane	Cyclodecasiloxane	Octadecamethyloctasiloxane
1,2,4-Benzenetriol	.100				
Cyclononasiloxane	.612	.254			
Cyclodecasiloxane	.002*	.164	.011*		
Octadecamethyloctasiloxane	.375	.447	.704	.031*	
Nelfinavir	.205	.704	.447	.076	.704

Discussion

Molecular Docking and Amino Acid Analysis

They have the ability to stop the SARS-CoV infection process and can be used as SARS-CoV-2 therapy (15). The essential oil component of jamblang leaves allows it to inhibit the work of SARS-CoV-2. In addition, it can act synergistically or potentiate other antiviral agents and relieve symptoms of COVID-19 (1). Molecular docking is a method used in computer-aided structural molecular biology for drug/ligand design. The purpose of protein and ligand docking is to predict the interaction between the ligand and the target protein in a three-dimensional structure (16). After the docking process, the next step was to analyze the amino acids by looking at the interactions that occur. This interaction is used to determine the presence of ligand interactions with amino acid residues on proteins (receptors) (17). Amino acid residues are amino acids that are present in a protein. The protein used in this study was ACE2 with PDB ID 1R4L which played a role in viral translation and replication.

The optimization results in table 1 above show that the average value and standard deviation value (±SD) of the smallest minimal energy was Octadecamethyloctasiloxane compound of 5.439 kcal/mol, while the largest minimum energy was nelfinavir with a value of 55.6076 kcal/mol. This energy was the most minimum energy in the stereochemical form and the most stable form for docking.

After the preparation of the ligand/compound was done, the receptor preparation was then carried out. Preparation of the protein that would be used was an important step to reach the optimal docking results (18). Receptors have been downloaded through the PDB data file (Protein Data Bank) with PDB ID 1R4L with the native ligand 2-acetamido-2-deoxy-beta-D-glucopyranose. The protein was reviewed using software of Molegro Virtual Docker (MVD) shown in figure 7. The MVD program would automatically correct the added protein to the workspace and directly add the H atom as well as correct if there are some amino acid residues that are wrong, either valence or charge. The program will display a warning if there is an error protein structure.

The next step was, before the toxicity prediction is made, that SMILES code was first made. SMILES code generation was done using the software of Chem Bio Draw Ultra 12.0. This step was done after drawing the jamblang leaf essential oil compound and the comparison drug (nelfinavir) in 2D form. Next, 2D structure was changed through the edit menu and copied as SMILES code. Then, SMILES code is copied to Microsoft Word or Microsoft Excel and saved for further testing. The results of making SMILES code for the jamblang leaf essential oil compound (nelfinavir) were shown in table 2. SMILE code of the jamblang leaf essential oil compound and the comparison compound (nelfinavir) were used for the toxicity prediction process using the pkCSM Online Tool and the Protox Online Tool.

Cavity Determination

Determination of the hole (cavity) served to detect the interaction between the ligand and the 1R4L receptor. The cavities at the receptors were shown in green patterns. Figure 8 showed 5 holes (cavities) that are possible to interact with the 1R4L receptor. Of the five holes detected, the selection was made by looking at the native ligand that interacts with the 1R4L receptor. The hole used was cavity 4 with a volume of 140.288 and a surface area of 387.84.

1R4L Receptor Validation

Receptor validation was carried out by re-docking the native ligand with the 1R4L target receptor using the software of Molegro Virtual Docker 6.0. The validation parameter used was by looking at the RMSD (Root Mean Square Deviation) value. The RMSD value was used to measure the similarity of the coordinates (pose) between two atoms (19). The receptor was declared valid and could be continued with the docking process of the test compound if it has an RMSD value ≤ 2 (19).

The receptor validation was replicated as many as three times by re-docking the native ligand with the hole (cavity 4) of the 1R4L target protein receptor. The 1R4L receptor had 4 different proteins including 3WL-_401 [A], 3WL_401 [B], 3WL_401 [C] and 3WL_401 [D]. The protein used was protein 3WL_401 [A] which bound to cavity 4. Based on the results of receptor validation in table 3, the average RMSD value was 0.327 .

RMSD describes the value of the atomic distance in one conformation with the nearest atom that had the same category as the atom in another conformation. The smaller the RMSD value was, the better the predicted ligand position was. This is because it got closer to the original conformation (20). These results indicated that the receptor validation criteria have been satisfied, because if the standard ligand binding results had RMSD value of 2, then the binding parameters could be accepted or declared valid (21).

Docking of Jamblang Leaf Essential Oil Compound and Nelfinavir at 1R4L Receptors

Docking of jamblang leaf essential oil compound and nelfinavir was carried out applying the software of Molegro Virtual Docker 6.0 with the coordinates of the native ligand site at 1R4L receptor. There are 3 parameters used, named MolDock Score, Rerank Score and H-bond (hydrogen bonds). These three parameters are scores that could measure the strength of drug binding to receptors. The Rerank Score value reflects the binding energy (total calculation of all existing bonds) required to form a bond between the ligand and the receptor, so it can be used to predict the activity of a compound (22).

The Docking results of jamblang leaf essential oil and Nelfinavir on 1R4L receptors

Based on table 4 of the docking score results above, there were three parameters, which were moldock score, rerank score and H-bond. Moldock score only aims to evaluate the geometry of the hydrogen bond angle where the hydrogen position is permanent (not rotated) (22). Therefore, to measure the affinity value, the rerank score was implemented. The Rerank score could be used to evaluate the docking quality, predict its affinity, and find the right ligand conformation by looking at the lowest value (20).

From the results of the receptor docking analysis of the Main protease (Mpro) PDB ID 1R4L with jamblang leaf essential oil compounds and a comparison compound (nelfinavir) as listed in Table 4, it was found that the lowest average Rerank Score was -73,338 kcal/mol for the test compound 2,3-Dihydro- 3,5-dihydroxy-6-methyl-4H-pyran-4-one. Meanwhile, the highest average Rerank Score was -57.397 kcal/mol in the cineole test compound. To determine whether a compound was predicted to have better activity, a comparison drug compound was used as a control. The drug nelfinavir as a comparison compound had a average Rerank Score of -68,878 kcal/mol. Based on these results, it can be said that the compound 2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one had the highest activity compared to native ligands, comparison drugs and other test compounds. The test compound that had a lower affinity value than the comparison one was predicted to have a more stable binding ability than the comparison compound (23).

The prediction of the test compound activity when it had bound to the target receptor was seen from its Rerank Score value. The lower the RS value was, the more stable the bond was between the ligand and the receptor. If the ligand bond with the receptor was more stable, it can be said that the activity of the compound was getting greater, so this means that the test compound had a higher activity than the compound which had a high RS value (22). The test compound that had the most potential as an ACE2 receptor inhibitor (GDP ID : 1R4L) was indicated by the most negative rerank score compared to other compounds. Thus, menthylacetate compounds can be recommended for further testing.

Results of Interaction of Ligands with Amino Acids

Amino acids are organic compounds characterized having a carboxyl group, an amino group, and a side chain attached to a central carbon atom. Amino acids are used as precursors for other molecules in the body. Linking amino acids together to form polypeptides can become proteins (24).

Binding site protein is area on molecules and ions (ligands) that can affect the conformation or function of the protein. Amino acid residues are also involved in the binding site area. The function of amino acids at the binding site is to bind the ligand. The interaction between the ligand and the macromolecular amino acid residues will form hydrophobic interactions, hydrogen bonds, van der Waals bonds and electrostatic bonds (23).

From the results of the amino acids interaction between the ligand and the receptor using software of Molegro Virtual Docker , there are three bonds obtained such as hydrogen bonds, electrostatic bonds, and steric bonds. On the other side, from the results of the interaction between jamblang leaf essential oil compounds and the comparison drug nelfinavir with the receptor, there are hydrogen bonds and steric bonds obtained. Figure 11 shows the results of two-dimensional interactions between ligands and amino acids. In the figure, hydrogen bonds are indicated by the blue dotted lines, while the steric bonds are marked with a red dotted line.

Tables 5 and 6 shows the results of compound bonding with amino acids, as well as bond distances and ligand groups. Hydrogen bonds had an important role in the docking mechanism and the resulting bond affinity. The majority of the ligands show low binding affinity and thus, can choose the target number associated with hydrogen bonding because they have a greater energy than electrostatic bonding and steric bonding (21). Hydrogen bonding occurs between the attachment of H atom to the electronegative atom. The electronegative atom is at a bond distance of 1.72-2.85 (24). There were some compounds that did not form hydrogen bonds. This was related to several parameters of physicochemical properties, namely HBA and HBD.

Cyclodecasiloxane and Octadecamethyloctasiloxane compounds did not have HBD. HBA and HBD affect the hydrogen bonds formed between the ligand and the receptor (23). In the six compounds, the amount is not met, so no hydrogen bonds are formed.

The interaction of amino acid residues determines whether the compound has the same biological activity as the comparison or native ligand (25). According to the results in the table above, it shows that the native ligand (3WL_A) of the 1R4L receptor binds to 6 amino acid residues, namely Leu 141, Gly 143, Gly 143, Glu 166, Asn 142, and Met 165. In the research of Pintilie et al (2020),The native ligand 3WL_A forms residues Gly 143, Asn 142, and Glu 166. The compound 2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one had amino acid residues of Met 49 and Asp 187 which had similarities with the amino acid residues of the comparison drug, namely Met 49. The 1,2,4-Benzenetriol compound has amino acid residues of Gln 189, Met 49, and Met 165 which had similarities to the comparison drug, named residues of Met 49 and to the native ligand, named residues Met 165. Cyclononacyloxane compounds had amino acid residues Asp 187 and Cys 44. They had amino acid residues Met 186. Octadecamethyloctasiloxane compound had amino acid residues Asp 48 and Met 165 which have similarities with the residues of the native ligand, namely Met 165.

There are similarities between compound residues with native ligand indicating the possibility of these compounds have the same activity. The

ACE2 receptor has 38 amino acid residues from the active site (26). Of the 38 amino acid residues, there are several amino acid residues that have the potential to form hydrogen bonds with ligands, including Thr 26, His 41, Asn 142, Gly 143, Cys 145, His 163, Glu 166, Gln 189, Thr 190 and Gln 192 (Ismail et al., 2021). In the native ligand 1R4L binds to the amino acid residues Asn 142, Gly 143, Glu 166.

Jamblang leaf essential oil compounds which have the same amino acid residue as the native ligands are named 1,2,4-Benzenetriol and Octadecamethyloctasiloxane. Other compounds do not have amino acid residues like native ligands, but have active amino acid residues on ACE2 such as His 41, Cys 44, Tyr 54, Asp 48, Asp 187, and Gln 189. This indicates that these compounds could bind with the receptor, had a stable binding, and was predicted to have the same activity as the native ligand. The hydrogen bonds between the test ligands with the same amino acid residues as the native ligands showed a similar type of interaction, especially describing similar activities in this case (26). Amino acid residues that are similar to native ligands indicate 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 (27).

The comparison drug, nelfinavir, binds to amino acid residues Thr 25, Thr 26, His 41, Met 49, Asn 142, Gly 143, and Cys 145. There were 3 compounds of jamblang leaf essential oil that have the same amino acid residues as nelfinavir, such as 2, 3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one and 1,2,4-Benzenetriol. The similarity between the residues of the compound with the comparison drug, nelfinavir indicated the possibility that these compounds have the same activity.

Based on tables 5 and 6, the native steric bond of the 1R4L receptor ligand bound to two amino acid residues, namely Asn 142 and Met 165. The compounds that bound to amino acids with the native ligand were 1,2,4-Benzenetriol and Octadecamethyloctasiloxane. The steric bond was also known as the Van der Waals bond. It was the attractive force between molecules and atoms that are not charged, and located close together with a distance of about 4-6 . Van der Waals bonds were involved in the interaction of the benzene ring with the plane of the receptor, as well as in the interaction of the hydrocarbon chain with protein macromolecules (receptors) (18). The steric bonds can provide a place for active amino acids with hydrogen bonds, so they can stabilize a bond (18).

Toxicity Prediction

The next in-silico study was to predict the toxicity of jamblang leaf essential oil compounds (2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one; 1,2,4-Benzenetriol; Cyclononasiloxane; Cyclodecasiloxane; Octadecamethyloctasiloxane) and the comparison compound (Nelfinavir) using the LD50 parameter with the Protox II Online Tools application which could be accessed freely via the https://tox-new.charite.de/protox_II/ website. In addition, the classification of toxicity classes was based on Globally Harmonized System (GHS) which was divided into 6 classes. The six toxicity classes were that class I (LD50≤ 5 mg/kg) was fatal if swallowed, class II (5<LD50≤50 mg/kg) fatal was if swallowed, class III (50<LD50≤300 mg/kg) was toxic if swallowed, class IV (300<LD50≤2000 mg/kg) was harmful if swallowed, class V (2000<LD50≤5000 mg/kg) might be harmful if swallowed and class VI (LD50>5000 mg/kg) was non-toxic.

Meanwhile, the prediction of compound toxicity using Ames toxicity, Skin sensitization and Hepatotoxicity were obtained from pkCSM Online Tools which are freely accessed through the website https://biosig.unimelb.edu.au/pkcsm/prediction. Toxicity prediction was carried out with the aim of knowing the toxicity and risks that arose from the compound and could have an impact on humans. The rediction of toxicity was important because the activity of a compound was not enough to be used as a drug candidate, but compounds that had low toxicity were also needed. LD stands for Lethal Dose. LD is the amount of material given and can cause the death of 50% of the test animal group. LD50 is one way to measure the potential for short-term poisoning (acute toxicity) of a material (28). Based on the table above, it shows four compounds were part of class 4 and two compounds were part of class 5. The compounds that were become part of class 4 were Cyclononasiloxane, Cyclodecasiloxane, Octadecamethyloctasiloxane, Nelfinavir. Meanwhile, the compounds classified in class 5 were 2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one, 1,2,4-Benzenetriol compounds.

According to Globally Harmonized System (GHS), the toxicity class based on the LD50 value is divided into 6 classes. Classes 1 to 3 had high toxicity, so they are quite dangerous. Meanwhile, grades 4 to 6 have low toxicity, so it is slightly dangerous. In the table of toxicity prediction results above, kamblang leaf essential oil compounss and comparison compounds (nelfinavir) fell into classes 4 and 5. This showed that these compounds had low toxicity, so they are slightly dangerous. The smaller the LD50 value is, the more toxic the compound is and vice versa. The greater the LD50 value is, the lower the toxicity is (29).

The next parameter, namely Ames Toxicity or mutagenic test, is a method used to assess the mutagenic potential of compounds using bacteria. A positive test result is indicated by the mutagenic compound, which is able to be responsible as a carcinogen (28). Based on the table above, all the compounds tested were not toxic to bacteria. The third parameter of toxicity prediction was hepatotoxicity test which was a method used to determine whether the compounds used were not toxic to the liver. Based on the table above, there was 1 compound that was toxic toward the liver, named the nelfinavir comparison compound. Meanwhile, other compounds are predicted to be non-toxic to the liver.

The main metabolite responsible for the hepatotoxic effect of menthofuran compounds is γ-ketoenal and/or epoxide formed by oxidation of the furan ring (12). The predicted results of nelfinavir toxicity were toxic to the liver. This was in accordance with the journal that nelfinavir had a toxic effect on the liver (13). The fourth parameter of toxicity prediction was the skin sensitization test, which was a hypersensitivity response mediated by T cells. It occurs when susceptible individuals are exposed to a sufficient number of contact allergens to induce activation, proliferation and clonal expansion of allergen-responsive T cells.

The skin sensitization test is a method used to determine whether the compounds used can irritate the skin (14). Based on the table above, only the comparison compound (nelfinavir) did not irritate the skin. The predicted results of skin sensitivity showed that jamblang leaf essential oil compounds could irritate the skin. Therefore, the use of jamblang leaf essential oils is not recommended for use on the face and skin area, especially in the nose (7). Jamblang leaf essential oil can be irritating in levels >4% in a formulation, but for those who have sensitivity, level of 0.1% is already irritating.

Mann-Whitney test

The tool to perform post hoc analysis for Kruskall-Wallis test was Mann-Whitney test (30). Mann-Whitney test is one form of testing in non-parametric statistical analysis, where the test is used to test the similarity of the distribution of two independent populations with the assumption that the distribution of the two populations is continuous and the measurement scale of the data is minimally ordinal (29). Mann-Whitney test in this study was to find out the rank scores of which compounds had significant differences. The criteria for the Mann-Whitney test is that there is a significant difference if sig. <0.05 and there is no significant difference if sig. 0.05 (30). From the results of the Mann-Whitney test of jamblang leaf essential oil compound with a comparison drug (nelfinavir), there were 3 compounds that had a significance of <0.05. These were Cyclodecasiloxane-2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one; Cyclodecasiloxane-Cyclononasiloxane; Octadecamethyloctasiloxane-Cyclodecasiloxane. From the significance value of the three compounds, it shows that the 3 compounds above had significant differences.

Based on the research that has been done, it can be concluded that:

1. Jamblang (Syzigium cumini) leaves essential oil compounds interact with ACE2 (1R4L) receptors in cavity 4. Thus, It is predicted to have the potential to inhibit ACE2 receptors. In addition, It is known that the compound with the best rerank score is 2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one.
2. Four jamblang (Syzigium cumini) leaves essential oil compounds according to the Globally Harmonized System (GHS) were classified in class 5 (2000 <LD50 5000 mg/kg) namely 2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran- 4-one, 1,2,4-Benzenetriol. Meanwhile,

Cyclononasiloxane, Cyclodecasiloxane, Octadecamethyloctasiloxane and the comparison drug (nelfinavir) were classified in class 4 (300<LD50≤2000 mg/kg).

Suggestion

Based on the research that has been done, the researchers provide suggestions to next researchers as follows:

1. Trying to do a docking between jamblang leaf essential oil compounds with COVID-19 receptors with different activities, so that information related to monoterpene antivirals in the jamblang plant can be added.
2. in silico Research is predictive, and thus, it is necessary to conduct in vitro and in vivo testing of jamblang leaf essential oil to find out its antiviral potential.

Acknowledgments

The authors thank to the DRPM Kemendikbudristekdikti which has funded this research with the 2021 multi-year basic research scheme numbered DIPA SP DIPA-023.17. The author also expresses appreciation to the research team members from the Department of Biology, Faculty of Mathematics and Natural Sciences, Medan State University; Biology Study Program, Faculty of Science and Technology, UIN Ar-Raniry Banda Aceh; Department of Sports Science, Faculty of Sports Science, State University of Medan; Department of Biology, Faculty of Mathematics and Natural Sciences Education, Indonesian Education University, Bandung.

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