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Antiviral Potential of Eucalyptus Oil (*Melaleuca cajuputi*) as the Primary Protease Inhibitor of SARS-CoV-2 Revealed by Molecular Docking

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ABSTRACT

The disease caused by the Corona Virus has afflicted hundreds of millions of individuals. The virus that causes the disease is SARS-CoV-2. To treat a variety of ailments, the Indonesian population favours the usage of medicinal herbs. The 1,8- cineol, karyophyllene, limonene, α-terpineol, α-pinene, dan β-pinene chemicals are present in eucalyptus oil (*Melaleuca cajuputi*) used in traditional medicine. This research was undertaken in silico to investigate the efficacy of these drugs as SARS-CoV-2 Main Protease (M^{pro}) inhibitors. The 1,8- cineol, karyophyllene, limonene, α-terpineol, α-pinene, dan β-pinene chemicals were molecularly docked using AutoDock Vina. Berdasarkan hasil molecular docking diperoleh Gibbs free energy (ΔG) dari kariofilen (-5.6 Kkal/mol), 1,8-cineole (-5.2 Kkal/mol), α-terpineol (-5.0 Kkal/mol), α-pinen (-4.9 Kkal/mol), β-pinen (-4.9 Kkal/mol), dan limonen (-4.8 Kkal/mol). Based on the results, the molecular docking data on these compounds likewise demonstrated no RMSD value greater than 2 Å. It indicates that the connection between the receptor and ligand is stable and that the response can occur spontaneously. The active site of the M^{pro} enzyme is the amino acid residue, where the interaction between the receptor and ligand occurs. Based on the bioactivity test, it can be inferred that the 1,8- cineol, karyophyllene, limonene, α-terpineol, α-pinene, dan β-pinene chemicals have varying bioactivities on different pharmacological targets. Because they satisfy Lipinski's requirements, these chemicals can also be utilized as oral medications. In addition, the toxicity test demonstrated that the test ligands exhibited minimal toxicity.

Keywords: antiviral potential, eucalyptus oil (*Celaleuca cajuputi*), protase inhibitor, SARS-CoV-2, molecular docking.

Introduction

The SARS-CoV-2 infection-caused outbreak of coronavirus illness 2019 (COVID-19) has spread to numerous countries, including Indonesia. This virus travels from China to neighbouring nations primarily by droplet transmission (Adityo, Cleopas, Ceva, & et al., 2020: 7 (1)). SARS-CoV-2 spreads and infects children, adults, and senior citizens (Sutaryono, Andarsari, & Karjono, 2020). According to information acquired by WHO on December 12, 2020, there are more than 69.5 million confirmed cases of COVID-19 and more than 1.5 million deaths worldwide (WHO Coronavirus (Covid-19) Dashbroad, 2020). The Indonesian COVID-19 Task Force announced on December 12 2020, that there were over 661 thousand confirmed cases and over 18 thousand fatalities (Covid-19 Distribution Map, 2020). According to these statistics, the SARS-CoV-2 viral infection has claimed numerous lives. A variety of means must eradicate it.

Because the SARS-CoV-2 virus infection is still ongoing, all treatment options, including conventional medication, should be examined. The Chinese government has incorporated traditional medicine for the treatment and prevention of COVID-19 into its clinical recommendations or guidelines. The Chinese official clinical standards have incorporated traditional Chinese medicine therapy with conventional therapy. In contrast to China, traditional Indonesian medicine cannot yet be utilized in Indonesia due to the need for more study on its efficacy, safety, and potential as a candidate for COVID-19 treatment. In the first phases of in silico research, efforts to identify traditional medicine candidates with promise as COVID-19 therapies can be examined (Narkbede, Pise, Cheke, & Shinde, 2020). In drug development, computational or in silico research is becoming increasingly popular, and even several commercially accessible medications for t(Francomano, Caruso, Barbarossa, & et al., 2019)he treatment of various ailments were created using the in silico method (Phillips, Stewart, Woodling, & Xie, 2018). Among the approaches utilized in silico-based research is the molecular docking technique. By modelling and studying the interaction process between molecules in silico or computationally, the molecular

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docking approach is utilized to develop and discover new medications (Chaudhary & Mishra, 2016). For mass manufacturing and consumption, in silico research produced with molecular docking techniques or other approaches must be retested by in vitro or in vivo investigations or clinical trials (Phillips, Stewart, Woodling, & Xie, 2018).

The development of COVID-19 medicines utilizing the molecular docking approach necessitates a macromolecular target with a substantial effect on SARS-CoV-2. The Main Protease (M^{pro}), a macromolecule of SARS-CoV-2, is one of the most potent enzymes (macromolecules) in the life cycle of this virus. Mpro is the fifth non-structural protein (nsp5) that regulates SARS-CoV-2 replication and transcription. Due to its vital role, this enzyme is ideally suited as a macromolecular target for COVID-19 drug development (Jin, Du, Xu, & et al., 2020).

In addition to macromolecular targets, docking approaches require candidate molecules as test ligands for study. The major component of traditional Indonesian eucalyptus oil, 1,8-cineol, is recognized to possess numerous medicinal effects. Multiple in silico investigations indicate that 1,8-cineol possesses antiviral activity against Herpes simplex virus type-1 (HSV-1) and infectious bronchitis virus (IBV). This chemical possesses antibacterial activity against Staphylococcus aureus, Escherichia coli, Moraxella catarrhalis, and many other microorganisms. In addition to its antibacterial and antiviral activities, 1,8-cineol possesses additional therapeutic capabilities, including cancer medicine, sedative, hypertension medication, and neuropathy treatment. It contains several other chemicals which are known to have anti-inflammatory, antioxidant, and antibacterial properties, as well as other medicinal benefits . In vitro and in vivo research have demonstrated that the oil of Eucalyptus has several therapeutic benefits that are advantageous to health. However, human clinical trials are still required before the general public can exploit them (Sales, Felipe, & Bicas, 2020).

According to the previous description, researchers are interested in investigating the effectiveness of traditional Indonesian medicine, specifically eucalyptus oil, against SARS-CoV-2. The research will be conducted computationally or in silico via molecular docking with test 1,8- cineol, karyophyllene, limonene, α-terpineol, α-pinene, dan β-pinene ligands target Main Protease (M^{pro}) SARS CoV-2. Using the molecular docking approach, researchers will attempt to assess the effect of the test ligand in order to address numerous issues regarding eucalyptus oil's potential as a COVID-19 treatment (Salehi, Upadhyay, Erdogan, & et al., 2019). The researcher picked the in silico research approach using molecular docking technology because it saves time and money compared to commencing a direct in vivo, in vitro study with trial and error.

Method

1. Software and Hardware

In this study, a computer with RAM (Random Access Memory) of 8 GB DDR 4, 2400 MHz, CPU (Central Processing Unit) of Intel Core i5-7300HQ (4 Core), 2.5 GHz, GPU (Graphics Processing Unit) of 4 GB NVIDIA GeForce GTX 1050, and Microsoft Windows 11 operating system was utilized. Software such as Autodock Vina version 1.2.0, AutoDock Tools version 1.5.6, and Biovia Discovery Studio 2019 are free downloadable.

2. Ligand Selection

The selection of ligands was based on earlier studies undertaken by other researchers on the therapeutic effects of numerous ligands present in eucalyptus (*Melaleuca cajuputi*) plant oil. Based on our examination of the relevant literature, we chose a ligand with an overall therapeutic effect. Information on the therapeutic impact gleaned from digitally searchable study outcomes. Each ligand's three-dimensional structure was retrieved from the PubChem database. Ligan uji yang dipililh beserta *PubChem ID*-nya antara lain: (1) 1,8-cineole (CID 2758), (2) caryophyllene (CID 5281515), (3) α--terpineol (CID 442501), (4) α-pinene (CID 6654), (5) β-pinene (CID 14896), and (6) limonene were chosen as test ligands (CID 22311). The researchers also dock with remdesivir (CID 121304016), which has been recommended as a COVID-19 medication by the FDA and compare the results to those of the test ligand docking.

3. Receptor Selection

As with ligands, the choice receptor structure is chosen by analyzing some research. The researchers were interested in selecting M^{pro} as a target due to its crucial role in the SARS-CoV-2 life cycle. M^{pro} is the fifth non-structural protein (nsp5) that plays a critical role in the replication and transcription of SARS-CoV-2. The three-dimensional structure of the target was derived from the X-ray crystallography findings of the co-crystals, which may be viewed through the Protein Data Bank (PDB) database with the code 6W63. To check the structure of the target protein retrieved from the Protein Data Bank, the researcher used plot analysis or Ramachandran diagrams routinely utilized by other researchers to analyze protein conformation. The Ramachandran plot study revealed that 91.7% of amino acid residues were in the most preferred region, 8% were in the authorized region, and 0.4%

were in the not permitted region (disallowed).17 This protein structure may be deemed of high quality since its amino acid residues (apart from glycine) location is optimal and exceeds 90 %.

4. Ligand Preparation

The three-dimensional ligand structure file downloaded will first be converted from *SDF format to *PDB format. The conversion was performed using the 2019 edition of Biovia Discovery Studio. The ligands were then synthesized using Autodock Tools version 1.5.6 following their conversion. At this step of preparation, the ligand's torque will be modified, and the file will be stored in *PDB format. The structures of the test 1,8-Cineole (CID 2758), Caryophyllene (CID 5281515), Limonene (CID 22311), α-Terpineol (CID 442501), α-Pinene (CID 6654), β-Pinene (CID 14896), as well as the control ligand Remdesivir (CID 121304016), were prepared. The structure of our X77 built-in control ligand was retrieved from the Protein Data Bank database (PDB ID 6W63).

5. Receptor Preparation

At the receptor preparation stage, the H_2O group is removed, the X77 natural ligand is separated or deleted from the Mpro PDB ID 6W63 receptor, and the file is stored in *PDB format. Then, hydrogen is added by clicking, and the file is saved in *PDBQT format.

6. Docking Method Validation

Using AutoDock Tools version 1.5, the structure of the M^{pro} SARS-CoV-2 receptor and its 1,8-Cineole, Caryophyllene, Limonene, α-Terpineol, α-Pinene, and β-Pinene was converted from *PDB format to *PDBQT format. . The molecular docking technique is executed by affixing each ligand to the M^{pro} receptor using a legitimate technique. Using AutoDock Vina version 1.2.0, docking simulations between the test ligand and the receptor and the comparison ligand and the receptor were performed. The docking process is repeated three times. The output of the docking simulation will be displayed as the G value of each ligand and the best (smallest) G value. Using the Biovia Discovery Studio 2019 application, the interaction between each ligand and receptor may be visualized. Interactions can take the form of hydrogen bonds and hydrophobic interactions. Using Biovia Discovery Studio 2019, bond distances may also be represented graphically.

7. Docking Simulation

Using AutoDock Tools version 1.5, the structure of the Mpro SARS-CoV-2 receptor and its 1,8-Cineole, Caryophyllene, Limonene, α-Terpineol, α-Pinene, dan β-Pinene ligands diubah dari format *.pdb menjadi format *.pdbqt dengan bantuan program AutoDock Tools versi 1.5.6. was converted from *PDB to *PDBQT format. . The molecular docking technique is executed by affixing each ligand to the Mpro receptor using a legitimate technique. Using AutoDock Vina version 1.2.0, docking simulations between the test ligand and the receptor and the comparison ligand and the receptor were performed. The docking process is repeated three times. The output of the docking simulation will be displayed as the ΔG value of each ligand and the best (smallest) ΔG value. Using the Biovia Discovery Studio 2019 application, the interaction between each ligand and receptor may be visualized. Interactions can take the form of hydrogen bonds and hydrophobic interactions. Using Biovia Discovery Studio 2019, bond distances may also be represented graphically.

8. Ligand Bioactivity and Bioavailability Test

Using Molinspiration, the bioactivity and bioavailability of the ligands were investigated. The chemical structure and coding of the Simplified Molecular Input Line Entry System (SMILES) were collected from the PubChem database for each of the innate, control, and test ligands. The SMILES code of each ligand was then manually input into version 2018.10 of the Molinspiration program, which may be accessed at www.molinspiration.com/cgi-bin/properties.

9. Toxicity Test

Using the ProTox-II software, the toxicity of ligands was assessed in silico. Similarly to the bioavailability and bioactivity tests, the Toxicity test requires the SMILES code obtained from the PubChem database. The program is then performed when the code has been input. The findings of the toxicity test will be presented as the median lethal dose (LD50) value.

Results

1. Docking Validation

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Redocking the X77 ligand with the target protein M^{pro} produced an RMSD of 1.1 and affinity energy of -7.9 kcal/mol. It is the optimal posture value based on the affinity energy and RMSD values—the docking procedure results in nine different positions. The docking poses score selection outcome is the second pose, as this pose has the best (lowest) affinity energy value of all postures that satisfy the RMSD threshold of > 0. The validation outcomes are represented in Figure 1.

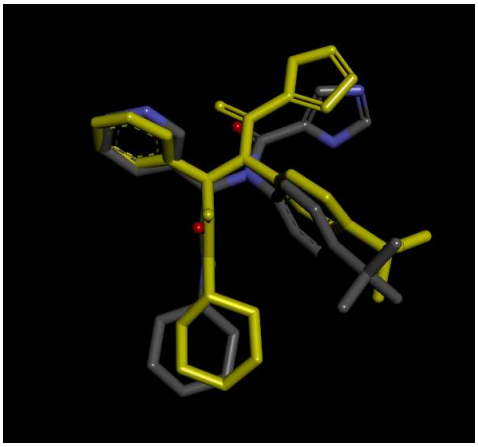

Figure 1. X-Ray crystallography results (Grey) and redocking results (Yellow)

2. Docking Simulation

Using the molecular docking approach, the free energy of the bond between the ligand and the receptor was estimated. Table 1 displays the outcomes of the docking simulation between all ligands and M^{pro}, whereas Figure 2 illustrates the interactions between ligands and the target protein. Caryophyllene molecules have the highest BSS value compared to other test ligands, with a value of 90 %; even this ligand has a more excellent BSS value than remdesivir, which reaches just 70 %. The docking findings also revealed that several investigated compounds derived from processed eucalyptus oil did not form a more robust connection (lower energy affinity) with M^{pro} than remdesivir.

Tabel 1. Binding site similarity and the results of the docking of the ligands.

Ligand	Energy Affinity (Kcal/mol)	RMSD (Å)	Hydrogen Bond	Hydrogen Bond Distance (Å)	π Bond & Hydrophobic Bond	%BSS
X77	-7.9	1.100	GLU A:166 GLY A:143	2.04 2.95	PRO A:168 CYS A:145 LEU A:27 THR A:25 HIS A:41 MET A:49 ARG A:188 ASP A:187 HIS A:164 HIS A:163 MET A:165 LEU A:141 SER A:144 GLN A:189 PHE A:140	100%
Remdesivir	-6.8	1.371	GLU A:166 ASP A:187 GLN A:18 GLY A:143	2.55 2.20 2.44 2.62	MET A:165 ARG A:188 PRO A:52 TYR A:54 HIS A:41 CYS A:44 MET A:49	70%

					CYS A:145 ASN A:142 LEU A:141 PHE A:140 HIS A:163 SER A:144 HIS A:164 LEU A:167 PRO A:168	
1,8-cineole	-5.2	0.899	-	-	MET A:165 CYS A:44 HIS A:41 MET A:49 PRO A:52 ASP A:187 ARG A:188 TYR A;54 GLN A:189	66%
Caryophyllene	-5.6	0.937	-	-	CYS A:145 HIS A:41 MET A:165 MET A:49 ASN A:142 HIS A:164 GLU A:166 GLN A:189 ARG A:188 ASP A:187	90%
Limonene	-4.8	1.129	-	-	MET A:49 HIS A:41 CYS A:44 ASP A:187 GLN A:189 ARG A:188 MET A:165 TYR A:54 PRO A:52	66%
α- Terpineol	-5.0	0.837	ASP A:187	2.30	MET A:165 MET A:49 GLN A:189 PRO A:52 ARG A:188 TYR A:54 CYS A:44 HIS A:41	66%
α-Pinene	-4.9	0.957	-	-	HIS A:41 MET A:165 MET A:49 CYS A:44 PRO A:52 ARG A:188 ASP A:187 GLN A:189	75%
β-Pinene	-4.9	0.931	-	-	MET A:165 MET A:49 CYS A:44	85,7%

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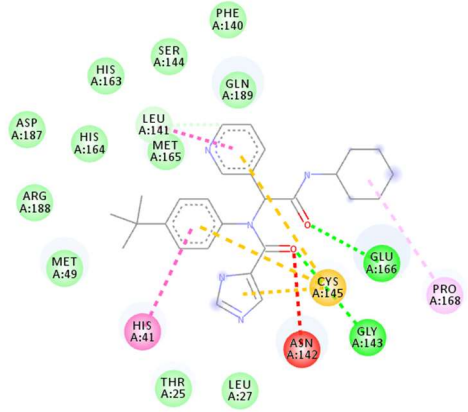
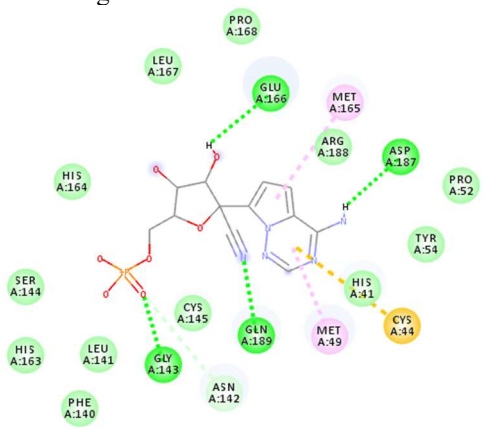
					HIS A:41 ARG A:188 ASP A:187 GLN A:189	

Description

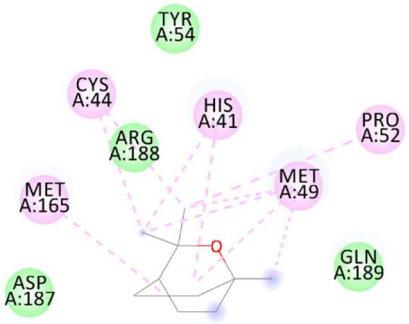
: Having residual bonding similarities with control or inherited ligands



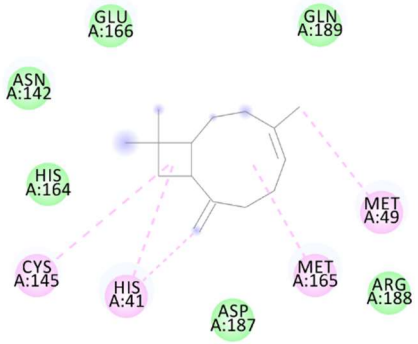
X77



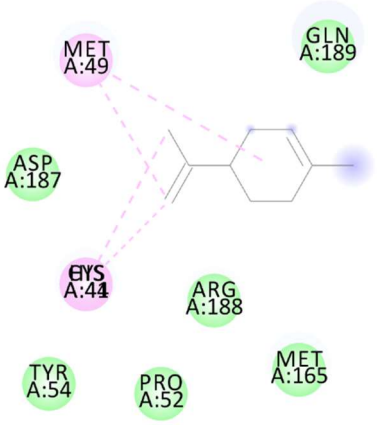
Remdesivir



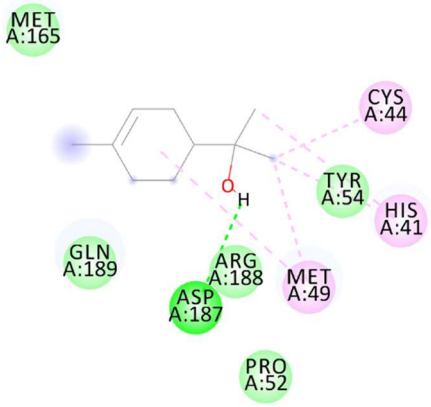
1,8-cineole



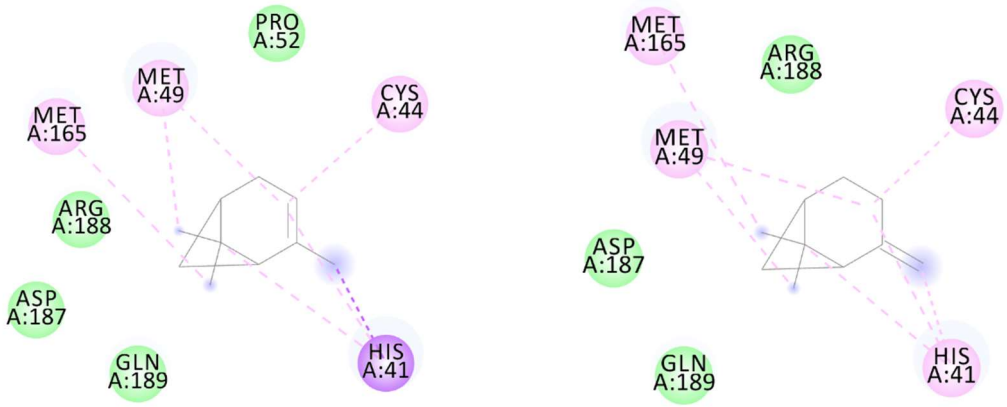
Caryophyllene



Limonene



α-Terpineol



α-Pinene **β-Pinene**

Figure 2. Interaksi ligan X77, remdesivir, 1,8-cineole, caryophyllene, limonene, α-terpineol, α-pinene, dan β-pinene.

3. Ligand Bio-activity and Bio-availability Test

Molinspiration software performs calculation and prediction of bioactivity scores for several drug targets such as GPCR ligands, ion channel modulators, kinase inhibitors, nuclear receptor ligands, protease inhibitors and other enzyme targets. The results of the bioactivity test for all ligands can be seen in table 2.

As for the bioavailability of various molecular properties such as Log P partition coefficient, Topological Polar Surface Area (TPSA), hydrogen bond donors and acceptors, rotating atomic bonds, atomic number, molecular weight can be predicted with the same program. Whether or not there is a violation of Lipinski's rules is also evaluated in this program. The results of the bioavailability test of all ligands can be seen in table 3.

Table 2. Prediction of the test ligand bioactivity score with the Molinspiration program.

Assessment	**Ligand**							
	1,8-cineole	**Caryophyllene**	**Limonene**	**α-Terpi**	**α-Pinen E**	**β-Pinen E**	**Remdes**	**X77**
GPCR ligand	-0,93	-0,34	-0,19	-0,51	-0,48	-0,53	0.27	0,16
Ion channel modulator	0,01	0,28	-0,27	0,15	-0,43	-0,32	-0.35	0,05
Kinase inhibitor	-1,60	-0,78	-2,01	-1,45	-1,15	-1,45	0.20	-0,25
Nuclear receptor ligand	-1,07	0,13	-0,34	-0,02	-0,62	-0,50	-0.48	0,46
Protease inhibitor	-0,90	-0,60	-1,38	-0,78	-0,85	-0,80	0.49	0,04
Enzyme inhibitor	-0,15	0,19	-0,21	0,14	-0,34	-0,34	0.38	0,02

Table 3. Evaluation of molecular properties and Lipinski rules of ligands with the Molinspiration program.

Ligand	**miLog P**	**TPSA**	***n* Atoms**	***n* ON**	***n* OHNH**	**n violation**	**n rotb**	**MW (g/mol)**
X77	4.36	90.98	34	7	2	0	7	459.59
Remdesivir	2.82	203.57	42	14	5	2	14	602,59
1,8-cineole	2,72	9,23	11	1	0	0	0	154,25

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Caryophyllene	5,17	0,00	15	0	0	1	0	204,36
Limonene	3,62	0,00	10	0	0	0	1	136,24
α-Terpineol	2,60	20,23	11	1	1	0	1	154,25
α-Pinene	3,54	0,00	10	0	0	0	0	136,24
β-Pinene	3,33	0,00	10	0	0	0	0	136,24

Description
miLog P : Partition coefficient of octanol-hydrogen
TPSA : Topological polar surface area
n Atoms : Atom total
n ON : Hydrogen bond acceptor total
n OHNH : Hydrogen bond donor total
n violation : violated Lipinski rule total
n rotb : Rotated tie total
MW : Molecular weight

4. Toxicity Test

Toxicity prediction results are displayed as LD50 (median lethal dose) values in mg/kg which can be classified into toxicity classes 1 (toxic) to 6 (non-toxic) according to the GHS (Globally Harmonized System) classification. seen in table 4.

Table 4. Prediction of the toxicity of the test ligands with the ProTox-II program.

Ligand	**LD_{50} (mg/kg)**
X77	650
Remdesivir	1000
1,8-Cineole	2480
Caryophyllene	5300
Limonene	4400
α-Terpineol	2830
α-Pinene	3700
β-Pinene	4700

Discussion

1. Docking Validation

The validation was carried out by redocking the X77 ligand and the Mpro target, showing the position of the default ligand before and after redocking which coincided as shown in Figure 1. The redocking results show the root mean square deviation (RMSD) value of 1.1 and the resulting affinity energy is of -7.9 Kcal/mol. The docking method that we do can be declared valid because the RMSD value of the best pose (2nd pose) predicted from redocking is 3Å (Jain & Nicholls, 2008).

2. Docking Simulation

After the results of the docking of various ligands with the Mpro protein target, the researchers analyzed many aspects to determine the potential of each ligand, especially the test ligand. These aspects include: (1) Binding site similarity (BSS), (2) types of ligand-protein interactions, (3) Root Mean Square Deviation (RMSD) (4) binding affinity energy.

The results of our analysis of the interaction between the ligand and the receptor succeeded in revealing the BSS percentage value of each ligand in order from the largest to the smallest as follows: caryophyllene (90%), β-

pinene (85,7%), α-pinene (75%), remdesivir (70%) and 1,8-cineole, limonene, α-terpineol ligands with the same value (66%).

Apart from the percentage of BSS, the type of interaction of each ligand with the receptor is also important to note. One of the intermolecular interactions such as hydrogen bonding is an important point in the development stage of medicines including herbal medicines. A hydrogen bond can be said to be stable if the bond length is less than 2,7Å. If the hydrogen bond length is more than 2,7 Å then the bond can be classified as a weak or unstable bond. 20

According to the examination of the docking data, only one test ligand forms hydrogen bonds with the amino acid residue of the receptor; specifically, the α-terpineol test ligand bonded to ASP A:187. The length of the hydrogen bond in -terpineol is smaller than 2,7Å, indicating that the hydrogen bond in this ligand is reasonably strong. The hydrogen bonding of α-terpineol is significantly stronger than remdesivir's four hydrogen bonds.

The bond is another weaker intermolecular interaction than hydrogen bonds. Due to the polarity of the atomic molecule, this bond is described as the attractive attraction between non-charged molecules or atoms that occupy neighboring positions. This was the most common form of link among the ligands we examined. 21.22

Same with bonding π, Hydrophobic bonds are weaker bonds than hydrogen bonds. However, this bond is an important strength because of its effect on helping to stabilize hydrogen bonds (Siswodihardjo, 2016). Hydrophobic bonds are bonds between water and non-polar molecules that do not contain ions and have a dipole moment or are hydrated because these molecules are insoluble or almost insoluble in water. Hydrophobic bonds are useful to help stabilize hydrogen bonds.

The results of docking ligands showed varying RMSD values. The RMSD value is declared effective if it is less than 2,00Å. 23 Based on the results of our docking simulation, we obtained the RMSD values of all ligand-protein interactions that did not exceed 2,00Å. This shows that all ligands including the ligands contained in eucalyptus oil are effective in inhibiting the main protease enzyme (M^{pro}).

Free energy Gibbs (ΔG) is the most important measure of the ability of a ligand to block the receptor. Free energy Gibbs (ΔG) is defined as the thermodynamic potential that is minimized when the system reaches equilibrium at constant pressure and temperature. ΔG provide information on the spontaneity of the bonding reaction. The reaction will proceed spontaneously if the value of ΔG value less than 0 Kcal/mol. Then the more negative the value of ΔG the more spontaneous the interaction between the ligand and protein.[24] The data from our study reveal the value of ΔG the largest test ligand in order from smallest to largest as follows; (1) remdesivir (-6.8 Kcal/mol), (2) caryophyllene (-5.6 Kcal/mol), (3) 1,8-cineole (-5.2 Kcal/mol), (4) α-terpineol (-5.0 Kcal/mol), (5) α-pinene (-4.9 Kcal/mol), (6) β-pinene (-4.9 Kcal/mol), and (7) limonene (-4.8 Kcal/mol).

3. Ligand Bio-activity and Bio-availability Test

Compounds or ligands with a bioactivity score more than 0.00 typically exhibit substantial biological activity, whereas values between -0.50 and 0.00 are regarded as moderately active molecules, and scores below -0.50 indicate that the molecule is inactive (Joshi, Kumar, & Sharma, 2018). The score results show that the test ligands with the best bioactivity in general are caryophyllene with the highest values for almost all types of drug targets when compared to other test ligands. However, the results of the bioactivity score on caryophyllene were still considered inactive as protease inhibitors (-0.60) and kinase inhibitors (-0.78). 1,8-cineole was the test ligand that had the lowest bioactivity of all the test ligands with four scores interpreted as insufficiently active (GPCR ligand (-0.93), kinase inhibitor (-1.60), nuclear receptor ligand (- 1.07), and a protease inhibitor (-0.90)).

When compared with the control ligand (X77) and the comparison ligand (remdesivir), the bioactivity of caryophyllene was still too poor. Ligand X77 and remdesivir both met the minimum criteria for being moderately active on each type of drug target. The control ligand X77 had a higher score when compared to remdesivir which only had one criterion of being moderately active.

Lipinski's rule is commonly used by pharmaceutical chemists in drug design and development to predict the oral bioavailability of drug molecules. According to Lipinski's rule, candidate molecules are likely to have good bioavailability when administered orally if they meet at least three of the four rules. The rules include that the molecular weight must be 500, octanol-hydrogen partition coefficient or Log P 5, have 5 hydrogen bond donors (OH and NH groups) and, have 10 hydrogen bond acceptors (mainly N and O) (Lipinski, Lombardo, & Feeney, 1997).

Based on the results of the study, it can be concluded that all the test ligands can be used as oral drugs because they meet at least three criteria. Caryophyllene is the only test ligand that violates Lipinski's rule because it has a Log P value > 5 (see table 3). However, because cariophyllene only violates one rule, it is possible that it can still be used as an oral drug. Molecular hydrophobicity or lipophilicity is indicated by Log P or partition coefficient. The Log P value of all test ligands except for caryophyllene has a value > 5 which means that it does not violate Lipinski's rule.

Table 3 shows that remdesivir does not meet Lipinski's criteria because it has a very high molecular weight (MW) (602.59 g/mol) and an excess of hydrogen bond acceptor (14). Remdesivir is a drug that is only available by injection. The availability of remdesivir as an injection drug is due to the fact that if taken orally, it will be

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easily broken down by the liver, which means that its bioavailability as an oral drug is very poor (Tiwari & Talreja, 2020).

Toxicity Test

In predicting ligand toxicity in silico, the researchers used the ProTox-II program which can be accessed online. Acute toxicity prediction results are displayed by the program in the form of LD50 (median lethal dose) in mg/kg units which can be classified into toxicity classes 1 (toxic) to 6 (non-toxic) according to the GHS (Globally Harmonized System) classification.

Class 1: fatal if swallowed (LD50 < 5 mg/kg)
Class 2: fatal if swallowed (LD50 5 - 50 mg/kg)
Class 3: toxic if swallowed (LD50 50 - 300 mg/kg)
Class 4: harmful if swallowed (LD50 300 - 2000 mg/kg)
Class 5: dangerous if swallowed (LD50 2000 - 5000 mg/kg)
Class 6: non-toxic (LD50 > 5000 mg/kg)

From the results of the study, it can be concluded that the control ligand X77 is the ligand with the worst toxicity and is classified as class 4 toxicity. All the test ligands had a toxicity score of class 5 except for caryophyllene which was in class 6 which was certainly better than the control and comparison ligands. In order, the safest to the least secure ligands, the median lethal dose is; caryophyllene (5300 mg/kg), β-pinene (4700 mg/kg), limonene (4400 mg/kg), α-pinene (3700 mg/kg), α-terpineol (2830 mg/kg), and 1,8-cineole (2480 mg/kg).

Conclusion

The molecular docking results revealed that there was no RMSD value > 2 . This shows that the interaction formed between the receptor and the ligand is stable and the reaction can take place spontaneously. The interaction formed between the receptor and the ligand also occurs at the active sites of the amino acid residues of the main protease (Mpro) enzyme. Percentage of Binding Site Similarity of caryophyllene, α-pinene, β-pinene, was still higher (good) when compared to remdesivir.

Docking simulations that we carried out on ligands with the Mpro SARS-CoV-2 protein also revealed that several natural compounds in eucalyptus oil such as: caryophyllene, 1,8-cineole, α-terpineol, α-pinene, β-pinene, and limonene have a poorer binding affinity when compared to remdesivir. It is based on the value of free energy Gibbs (ΔG) remdesivir lower than the other test ligands. Although the results of the docking of the caryophyllene molecule, 1,8-cineole, α-terpineol, α-pinene, β-pinene, and limonene not as good as remdesivir compounds, but having oral availability this herbal compound is still better because it meets the criteria *rule of five* (Ro5).

Compounds in eucalyptus oil have no more potential as an antiviral compared to remdesivir compounds. As a result, it is still necessary to search for other candidate compounds that have the potential as SARS-CoV-2 antivirals.

Our results are only preliminary screening to facilitate further studies both in vitro and in vivo (in animal models or clinical trials in humans).

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