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Pharmacophore Analysis of Monoterpene *Melaleuca leucadendra* as an Inhibitor for 3CLPro of the SARS-CoV-2

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Abstract. *The monoterpene compound has been reported to have antiviral activities . This study aimed to test the monoterpene compound in Melaleuca leucadendra to inhibit the SARS-CoV-2 virus. The monoterpenes tested were α-Pinene, β-Pinene, Linalool, α-Terpineol, and Terpinene-4-Ol. The method used was computational through pharmacophore analysis. The indicator for the quality of the compound was the fit score. A fit score of more than 50% indicates a good-quality compound, while a fit score of less than that indicates a poor-quality compound. Based on the analysis results, the monoterpene compound in Melaleuca leucadendra can potentially inhibit the SARS-CoV-2 virus directly through the inhibition of 3C-like protease. The linalool showed a fit score of 55% with interactions of hydrophobic, electrostatic, and hydrogen bonds. All the compounds did not inhibit the metabolic process and were safe, possibly having no side effects based on ADMET analysis.*

Keywords: *Pharmacophore, monoterpene, Melaleuca Leucadendra, Sars CoV-2*

Citation
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INTRODUCTION

Although it has decreased, the pandemic caused by SARS-CoV-2 is not over yet. From a molecular perspective, the initial stage of this virus infection is linked to the human enzyme, namely ACE-2 (angiotensin 2 conversion enzyme) through a spike glycoprotein (Tai et al., 2020). Spike glycoprotein is part of the structural protein of coronavirus (Beyerstedt et al., 2021). Based on research by Wrap et al. (2020), the affinity of this spike glycoprotein to human ACE-2 is 10-20 times stronger than SARS-CoV, which is one of the reasons why Covid-19 is widely spread in all countries in the world.

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The SARS-CoV-2 virus has four major structures: a spike, a membrane, a nucleocapsid, and an envelope. In addition, SARS-CoV-2 also possesses 16 Non-Structural Proteins (NSP), including a 3C-like protease (3CLPro) (Chen et al., 2022). The protein 3C-like protease (3CLPro) is a SARS-CoV-2 protease that plays an essential role in virus genome replication. Furthermore, 3CLPro disrupts the immune system in the body by inhibiting the type 1 interferon pathway, decreasing p53 protein expression, activating TGF-β signaling, and inducing apoptosis (Kawarada et al, 2016). Based on this, the 3C-like protease protein is a potential protein that can be used as a target to inhibit the development of SARS-CoV-2. In addition, it has been widely known that there are many bioactive compounds that are used to inhibit virus replication proteins. One of the possible compounds is monoterpenes.

Monoterpenes are isoprene compounds characterized by a 10-carbon chain, either linear or cyclic. Themonoterpenoids are monoterpenes in which heteroatoms are added to the structure through biochemical reactions (Kabir et al., 2020). Monoterpenes are used to prevent and treat various diseases, offering biological effects of multiple entities such as antimicrobial, antiallergic, antioxidant, anti-inflammatory, and immunomodulatory (Vieira et al., 2017). *Melaleuca leucadendra* is a plant from the family of Myrtaceae that has been known to contain monoterpenes (Pujiarti et al., 2011), and is widely spread in Southeast Asia including Indonesia. This present study aimed to test the monoterpene compound of *Melaleuca leucadendra* to inhibit the SARS-CoV-2 virus in- silico. The result of this study can become a reference source for the compound *Melaleuca leucadendra* as an inhibitor of SARS-CoV-2.

MATERIALS AND METHODS

The tools used in this research consisted of software and hardware. The software was a LigandScout application, while the hardware used was a laptop computer with the following specifications; Processor: Intel(R) Celeron(R) CPU 4205U @ 1.80GHz, RAM: 4 GB, Operasi Sistem: 64-bit. The material used consisted of the chemical structure of the test compounds (α-Pinene, β-Pinene, Linalool, α-Terpineol, and Terpinene-4-Ol), which were downloaded from the website (https://pubchem.ncbi.nlm.nih.gov/) with the PubChem code as follows CID 6654 for α-Pinene, CID 14896 for β-Pinene, CID 6549 for Linalool, CID 17100 for α-Terpineol, and CID 11230 for Terpinene-4-Ol, and the chemical structure of the protein Angiotensin-Converting Enzyme 2 (ACE-2) and 3CLPro downloaded from http://www.pharmchem.uni-tuebingen.de/dekois/. The research procedure was based on Seidel et al. (2010) method.

Compound safety analysis was also carried out through the ADMET (Absorption, Distribution, Metabolism, Excretion, Toxicity) test via the link https://biosig.lab.uq.edu.au/pkcsm/prediction to analyze the safety profile and pharmacokinetics of the compounds.

RESULTS AND DISCUSSION

Pharmacophore Analysis of Monoterpene Compounds on Human ACE-2

Table 1 shows the pharmacophore results for monoterpene compounds in binding to ACE-2. In the table, the pharmacophore fit scores of each combination are shown. In addition, there are also features of the interaction between the test compound and the ACE-2 protein.

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Table 1. Results of pharmacophore analysis of Monotepene to ACE-2

Compound of Monoterpene	Feature	Pharmacophore Fit Score (%)
α-Pinene		38.73
β-Pinene		38.22
Linalool		48.80
α-Terpineol		49.19
Terpinen-4-Ol		49.07

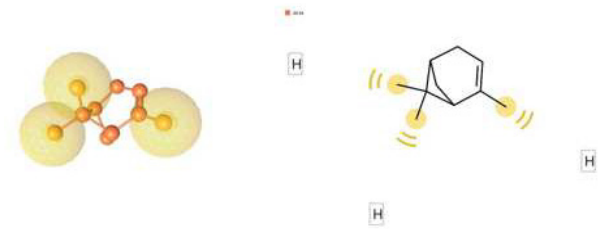

Figure 1. Features of α-Pinene pharmacophores, right – 3D structure of features, left – 2D structure of features

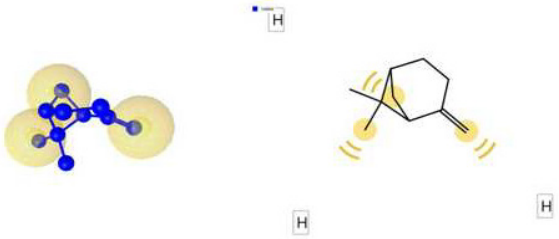

Figure 2. Features of β-Pinene pharmacophores, right – 3D structure of features, left – 2D structure of features

Figure 3. Features of Linalool pharmacophor, right – 3D structure of features, left – 2D structure of features

Figure 4. Features of α-Terpineol pharmacophores, Right – 3D structure of features, left – 2D structure of features

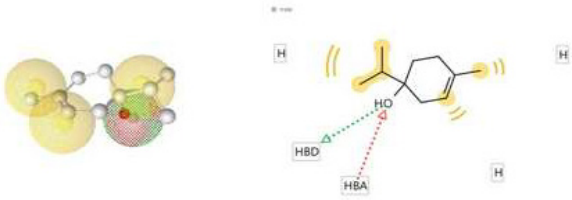

Figure 5. Features of Terpinen-4-Ol pharmacophores, right – 3D structure of features, left – 2D structure of features

The pharmacophore fit scores for each compound were α-Pinene (38.73%), β-Pinene (38.22%), Linalool (48.80%), α-Terpineol (49.1 9%), and Terpinene-4-Ol (49.07%). According to Muttaqin et al. (2019), a pharmacophore fit score of more than 50% indicates good pharmacophore features. However, the pharmacophore fit score range from 35-50%, indicating poor reactivity pharmacophore features of the compound. The poor reactivity was shown by α-Pinene, β -Pinene, Linalool, α -Terpineol, and Terpinene-4-Ol compounds, therefore those compounds cannot be used as competitors for the SARS-CoV-2 spike pro-

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tein in binding to human ACE-2.

Table 1 shows the interaction features between the test compounds to ACE-2 indicated by color, namely yellow, which indicates hydrophobic interaction, brick red color indicates electrostatic interaction, and green color indicates hydrogen interaction. Ummah et al. (2020) said that hydrogen bonding, as well as hydrophobic interactions, can affect the stability activity between the ligand and its target receptor. High number of hydrogen bonds, accompanied by hydrophobic interactions, indicates the ligand-receptor interaction is quite strong.

Result analysis shows that α-Pinene and β-Pinene compounds have a yellow color feature (Figures 1 and 2), which means that hydrophobic interactions are formed between these compounds with ACE-2. Meanwhile, linalool (Figure 3), α-Terpineol (Figure 4), and Terpinene-4-Ol compounds (Figure 5) have color combination features of yellow, brick red, and green which means that a combination of interactions formed between each compound with ACE-2. The variety of these interactions are hydrophobic, electrostatic, and hydrogen bonds. The display of each feature supports the pharmacophore fit score formed. The more interactions, the higher the pharmacophore fit score.

Pharmacophore Analysis of Monoterpene Compounds Against SARS CoV-2 3C-Like Protease

Based on table 2, linalool is a monoterpene group compound that has the potential to inhibit the SARS-CoV-2 virus. It is suspected that Linalool can bind to the 3CLPro protein with fairly good interaction (My et al., 2020). This is based on an analysis of the Linalool compound pharmacophore fit score of 55%. According to Muttaqin et al. (2019) the value of the pharmacophore fit score analysis above 50% indicates the reactivity of the compound to the test receptor. While the suitability value of the pharmacophore analysis results for α-Pinene, β-Pinene, Terpineol, and Terpinene-4-Ol was 43.95%, 43.99%, 48.36%, and 49.22% respectively, indicating less reactivity if these compounds to the test receptor.

Table 1. Results of pharmacophore analysis of Monotepene to ACE-2

Compound of Monoterpene	Feature	Pharmacophore Fit Score (%)
α-Pinene		43.95
β-Pinene		43.99
Linalool		55.03
α-Terpineol		48.36
Terpinen-4-Ol		49.22

The stability of the interaction of each compound as a result of pharmacophore analysis is shown in Figures 6-10. Based on the results, α-Pinene and β-Pinene compounds have a yellow color feature (Figures 6 and 7), indicating hydrophobic interactions are formed between these compounds with SARS-CoV-2 3CLPro. Meanwhile, linalool (Figure 8), α-Terpineol (Figure 9), and Terpinene-4-Ol (Figure 10) have color combination features of yellow, brick red, and green, indicating a combination of interactions is formed between each compound with SARS-CoV-2 3CLPro protein. The variety of these interactions are hydrophobic, electrostatic, and hydrogen bonds that may potentially inhibit

SARS-CoV-2. These results are supported by the report of Khomenko et al., (2021) that monoterpene compounds can inhibit syncytial respiratory virus replication.

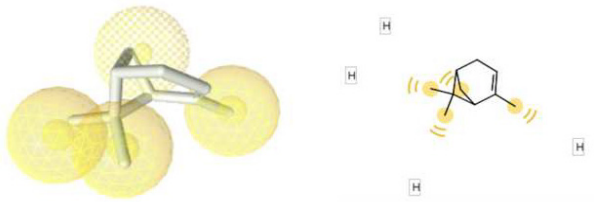

Figure 6. Features of pharmacophore Alpha-Pinene 3D (left) dan 2D (right)

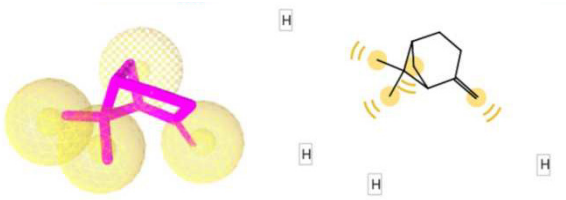

Figure 7. Features of pharmacophore Beta-Pinene 3D (left) and 2D (right)

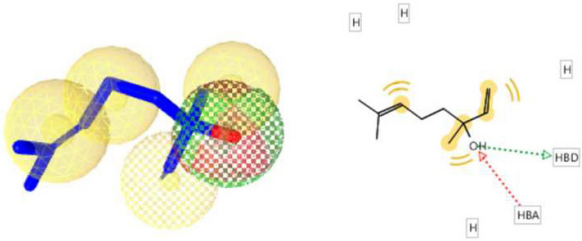

Figure 8. Features of pharmacophore Linalool 3D (left) and 2D (right)

Figure 9. Features of pharmacophore Alpha-Terpineol 3D (left) and 2D (right)

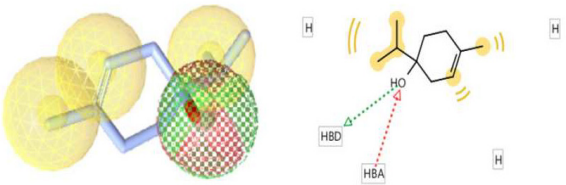

Figure 10. Features of pharmacophore Terpinene-4-Ol 3D (left) dan 2D (right)

ADMET Analysis of Monoterpene Compounds

Prediction of ADMET (absorption, distribution, metabolism, excretion, and toxicity) was carried out to predict the pharmacokinetics and toxicity of a compound. The result of ADMET prediction are presented in Table 3-4. A compound has good absorption if the absorption value exceeds 80%, while it will poorly absorb if the value is below 30% (Chander et al., 2017). Table 3 shows that the five compounds have good absorption, by the absorption value of more than 90%.

Permeability of CaCO2 is an in vitro model of the intestinal mucosa, and is often used to predict the absorption of oral drugs. The permeability of CaCO2 will be considered high if its value exceeds 0.90 (Hariz, 2017). Based on table 3, all compounds have high CaCO2 permeability because the value exceeds 0.90. In addition, the value of CaCO2, all compounds show a good value of distribution because they have a Volume Distribution in Steady State/VDss below 0.45. The VDss is a pharmacokinetic parameter used to determine the total dose of the drug that needs to be

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distributed evenly, so it is necessary that the administration concentration is the same as in blood plasma. According to Krihiryani et al. (2019), the VDss log value of less than -0.15 indicates low distribution, while the VDss log value of more than 0.45 indicates high distribution. Pires et al. (2015) said that the higher the VDss value, the more the drug distributed in the tissue exceeds the plasma, which can cause kidney failure or dehydration.

Parameter Blood Brain Barrier (BBB) is a parameter to assess a drug's ability to penetrate the blood-brain barrier. This parameter is carried out to increase the efficacy of drugs that have the purpose of treating the brain and also to reduce side effects and toxicity. The results showed that the five compounds had a BBB value of more than 0.3, meaning that the compounds are predicted to be able to penetrate the blood-brain barrier. This is in line with the statement of Pires et al. (2015) that a drug cannot penetrate the blood-brain barrier if the log BBB value is less than -1. According to Roncaglioni et al. (2013), a compound that can penetrate the blood-brain barrier is a good compound to be applied to treatments that target nerve cells.

Based on the results of this study, all the compounds did not inhibit the metabolic process and were safe, and potentially had no side effects. This can be seen in the results of CYP and OCT 2 substrates, where none of the compounds inhibited CYP34A, nor did they act as OCT 2 substrates (Table 3). CYP is an enzyme that catalyzes metabolic processes, and among the 57 types of CYP in mammals, two types of CYP are most widely used, one of which is CYP34A which plays in catalyzing drug metabolism reactions also as the synthesis of cholesterol, steroids, and other types of lipids (Kacevska et al., 2008; Fuhr et al., 2021). Meanwhile, OCT 2 substrate plays a role as renal transporter and clearance of endogenous drugs and compounds in the kidney (Pires et al., 2015). In addition, toxicity prediction was also carried out to see the level of toxicity of monoterpenes in *Melaleuca leucadendra.* The results showed that all compounds were not toxic (table 4). Ames toxicity indicates that the compound does not cause mutagenesis, while hepatotoxicity means that all compounds are not harmful to the liver (Sulistyaningrum et al., 2013). While the LD50 indicates the maximum dose that can cause 50% death. The LD50 values for each compound are1.77, 1.633, 1.704, 1.923, and 1.811 mol/kg for α-pinene, β -piene, linalool, α-terpinol, and terpiene-O1, respectively.

Table 3. Prediction results of ADME pharmacokinetic

Compound of Monoterpene	***Intestinal Absorption (human)* (%)**	**Caco2 *Permeability* (log pap pub 10^{-6} cm/s)**	**VDss (Human) (log L/ kg)**	**BBB *Permeability* (logBB)**	**CYP34A *Substrate***	**CYP34A *Inhibitor***	***Total Clearance* (log/ml/ min/kg)**	***Renal OCT2 Substrate***
Alpha-Pinene	96.041	1.38	0.667	0.791	no	no	0.043	no
Beta-Pinene	95.525	1.385	0.685	0.818	no	no	0.03	no
Linalool	93.163	1.493	0.152	0.598	no	no	0.446	no
Alpha-Terpineol	94.183	1.489	0.207	0.305	no	no	1.219	no
Terpinene-4-Ol	94.014	1.502	0.21	0.563	no	no	1.269	no

Table 4. Prediction results of ADME pharmacokinetic

Compound of Monoterpene	Ames Toxicity	Hepatotoxicity	LD50 (mol/kg)
Alpha-Pinene	no	no	1.77
Beta-Pinene	no	no	1.673
Linalool	no	no	1.704
Alpha-Terpineol	no	no	1.923
Terpinene-4-Ol	no	no	1.811

CONCLUSION

α-Pinene, β -Pinene, Linalool, α -Terpineol, and Terpinene-4-Ol are monoterpene in *Melaleuca Leucadendra* compounds that cannot be used as competitors for the SARS CoV-2 spike protein in binding to human ACE-2 but have the potential to inhibit the SARS CoV-2 virus directly. All the compounds did not inhibit the metabolic process and were safe, as well as potentially had no side effects based on ADMET analysis.

AUTHOR CONTRIBUTION

I.F.S. drafting manuscripts and correcting methods, Y.S. and I.D.K. adapt introduction, discussion, and conclusions based on the results of the review, O.T., G.G.A. and I.F.S. propose compounds be tested and adapted the idea to the method.

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CONFLICT OF INTEREST

The research was conducted without a conflict of interest.

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