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Potential antiviral activity of eleutherine, isoeleutherine, eleuthinone and elecanacine compounds in *Eleutherine palmifolia* (L.) Merr against NSP3 SARS-COV-2: In silico study

Belia Bima Nafisa [1,*], Sanarto Santoso [2], Zainabur Rahmah [3] and Roihatul Muti'ah [4]

[1] *Master Program in Biomedical Sciences, Faculty of Medicine, Brawijaya University, Malang, East Java, Indonesia.*
[2] *Department of Microbiology, Faculty of Medicine, Brawijaya University, Malang, East Java, Indonesia.*
[3] *Department of Parasitology, Faculty of Medicine and Health Sciences, Maulana Malik Ibrahim State Islamic University, Malang, East Java, Indonesia.*
[4] *Department of Pharmacy, Faculty of Medicine and Health Sciences, Maulana Malik Ibrahim State Islamic University, Malang, East Java, Indonesia.*

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Abstract

Eleutherine, isoeleutherine, eleuthinone and elecanacine are secondary metabolites of Dayak onion (*Eleutherine palmifolia* (L.) Merr) belonging to the naphtoquinone group and considered to have antiviral activity by inhibiting HIV replication. These considerations were used in this study using the Non-structural protein 3 (NSP3) in SARS-CoV-2 which plays a role in virus replication. This study aims to predict the antiviral activity, physicochemical properties and toxicity. Antiviral activity predictions were reviewed based on the interaction of the ligand with the NSP3 receptor (PDB ID: 7JIT) using Molegro Virtual Docker 6.0. Prediction of physicochemical properties refers to the Lipinski Ro5 and parameters of TPSA using swissADME. Toxicity prediction based on LD_{50} classification and AMES toxicity, hapatotoxicity, skin sensitization parameters using ProTox-II and pkCSM. The results showed that all the compounds in the study had inhibitory activity in terms of Rerank Score (RS) which represents the form of bound energy and were compared with Ribavirin as the comparator drug. The RS (Kcal/mol) for eleuthinone is -102.853, isoeleutherine -108.493, eleuthinone -108.005 and elecanacine -104.640. The RS of all compounds was lower than Ribavirin (-94.195) indicating higher affinity than Ribavirin. All the compounds in this study matched the Lipinski's Ro5 and TPSA parameters with LD_{50} in toxicity class IV and were predicted to be safe, except for elecanacine which was predicted to be mutagenic and eleuthinone which was predicted to be hepatotoxic. So that, overall eleutherine and isoeleutherine compounds can be recommended as candidates for SARS-CoV-2 antiviral compounds.

Keywords: *Eleutherine palmifolia* (L.) Merr; Non-structural protein 3 (NSP3); SARS-CoV-2; In Silico; Molegro Virtual Docker

1. Introduction

Corona virus disease 2019, also known as COVID-19, has spread throughout the world. Corona virus disease 2019 is a highly contagious and pathogenic disease caused by infection with SARS-CoV-2 (Severe Acute Respiratory Syndrome Coronavirus 2) [1]. Clinical manifestations in COVID-19 patients range from asymptomatic to symptomatic, mild pneumonia, severe pneumonia, ARDS (acute respiratory distress syndrome), sepsis, and septic shock [2]. Human-to-human transmission is how SARS-CoV-2 spreads. SARS-CoV-2 transmission from symptomatic patients via droplets expelled when coughing or sneezing [3].

* Corresponding author: Belia Bima Nafisa

SARS-CoV-2 is a betacoronavirus with several proteins, including a spike (S), membrane (M), envelope (E), and nucleocapsid (N) [4]. Aside from that, SARS-CoV-2 has a non-structural protein known as NSP [5]. NSPs are single-stranded DNA binding proteins that transport the replicated viral genome from the nucleus to the cytoplasm [6]. The NSP3, also known as PLpro, plays an important role in viral replication by inhibiting host protein translation [7]. The NSP3 contains 5'RNA-triphosphatase (RTP), nucleoside triphophatase (NTPase), and helicase at its C-terminus. The NSP3 is essential for virus survival, making it an attractive drug target [8]. The strategy used in developing antiviral therapy is by utilizing herbal plants as an alternative curative and preventive treatment that is effective and safe in the spread of COVID-19 [9].

Dayak onion (*Eleutherine palmifolia* (L.) Merr) is one of the herbal plants that has the potential to be developed as an antiviral. This plants tuber has antiviral activity by inhibiting HIV replication. The IC_{50} of 8.5 g/mL indicates the value of replication inhibition [10]. The naphtoquinone group and its derivatives, such as eleutherine, isoeleutherine, eleuthinone and elecanacine are abundant in secondary metabolites in *Eleutherine palmifolia* (L.) Merr [11]. The naphtoquinone group of compounds and their derivatives have antiviral properties [12].

As a result, a previous research in the discovery of herbal medicines using computational means, namely in silico, is required [13]. The in silico research method used is molecular docking, with the goal of understanding the interaction between protein and ligand, which can be calculated based on biophysical concepts to produce a score function (binding affinity) and the best binding position, indicating the possibility of developing a specific ligand into a new ligand [14]. In this research, the ligands tested included eleutherine, isoeleutherine, eleuthinone, elecanacine, and the antiviral drug Ribavirin. This research aims to predict the compounds eleutherine, isoeleutherine, eleuthinone and elecanacine, which can interact with the NSP3 receptor (PDB ID: 7JIT) in the potential for inhibition of the SARS-CoV-2 virus, as well as predict the physicochemical properties and toxicity of these compounds.

Based on this description, a preliminary study is needed by focusing on the main target of virus replication activity and considering the antiviral activity of the compounds isoeleutherine, eleutherine, elecanacine, and eleuthinone in *Eleutherine palmifolia* (L.) Merr on NSP3 in SARS-CoV-2. This research is very important because it can be the first step in finding a candidate for an antiviral drug made from herbs with the hope of becoming a therapeutic solution for COVID-19 patients that causes fewer side effects.

2. Material and methods

2.1. Software

ChemDraw Ultra v.12.0.2.1076, SwissADME Online Tool, pkCSM Online Tool, ProTox-II Online Tool, Avogadro, and Molegro Virtual Docker (MVD) version 6.0 were used.

2.2. Target and template selection

The NSP3 receptor with PDB ID code 7JIT 1.95 Å resolution was chosen. The test compounds were eleutherine, isoeleutherine, eleuthinone, elecanacine, and the antiviral drug Ribavirin in 3D and the SMILES code from ChemDraw Ultra version 12.0.

2.3. Preparation compound

Eleutherine, isoeleutherine, eleuthinone, and elecanacine compounds were created using the ChemDraw Ultra version 12.0 application, while Ribavirin as the comparator drug was obtained from the PubChem website. The 2D molecular structure was saved as SMILES code and then converted into a 3D structure by pressing the Structure button and Convert Name to Structure in the *pdb storage format using the ChemDraw Ultra version 12.0 application. Furthermore, energy minimization was performed for three replications using the Avogadro application and the MMFF94 force field of the Steepest Descent algorithm, and the results were saved in the form *mol2 (SYBYL2.*Mol2).

2.4. Molecular docking

To determine the interaction between the test compound and the receptor, molecular docking was performed using the Molegro Virtual Docker version 6.0 application. Root mean square deviation (RMSD) parameter value of less than 2Å was used for molecular docking validation. The parameters of molecular docking results were determined using the Rerank Score, Moldock Score, and H-Bond, and were replicated three times. Furthermore, it was based on the interaction of amino acid residues via hydrogen bonds and steric bonds.

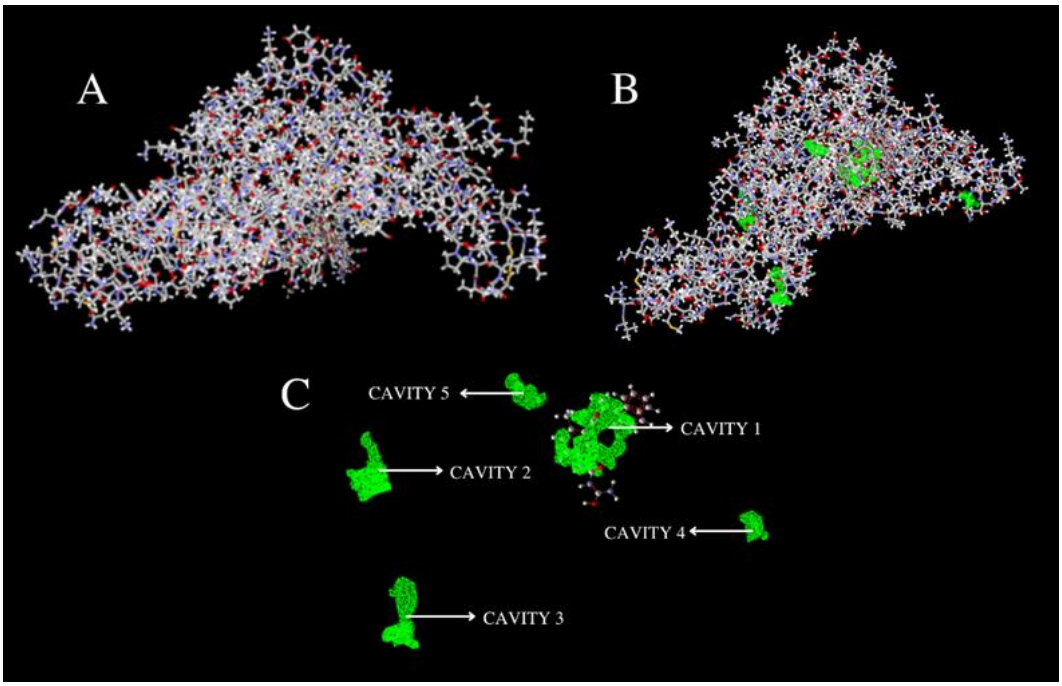
2.5. Prediction of physicochemical properties and toxicity

Physicochemical and toxicity predictions were carried out by entering the SMILES code for each compound obtained from the ChemDraw Ultra version 12.0 application into the online application, namely SwissADME, ProTox-II and pkCSM. Physicochemical prediction of test compounds and reference drugs using the SwissADME program which can be accessed online. Physicochemical prediction based on The Lipinski's rule of five (Ro5) consisting of molecular weight (BM), number of hydrogen bond donor (HBD), number of hydrogen bond acceptor (HBA), oil to water partition coefficient (Log P) and number of bonds that can be rotated (Torsion) [15]. Other studies have added another descriptor of physicochemical properties, namely the topological polar surface area (TPSA) [16]. Toxicity prediction in this study used 2 programs that can be accessed online, namely ProTox-II and pkCSM. ProTox-II to determine oral toxicity based on the value of 50% lethal dose (LD50) and its toxicity class with reference to the GHS (Globally Harmonized System) standard. pkCSM is used to determine toxicity based on ames toxicity, hepatotoxicity and skin sensitization.

3. Results

3.1. Receptor preparation and validation

To remove water molecules, ligands, and ions, the Molegro Virtual Docker version 6.0 application was used to validate the receptor [17]. Because most macromolecular structure data in PDB files lacks hydrogen atoms, optimization was accomplished by adding hydrogen atoms. Water molecules was also removed because they can disrupt the belay process. For the docking process, the cavity with the greatest volume and surface area was chosen, namely cavity 1 with a volume of 68,608 and a surface of 240.64. After determining the cavity, the receptor must be validated by re-docking the native ligand on the chosen receptor. To obtain the RMSD value, the process was repeated three times. The 7JIT receptor had an RMSD of 1,090 Å. According to another research, there were four RMSD thresholds: a value less than one was considered very good, a value of 1-2 Å was considered good, a value of 2-3 Å was considered moderate, and a value greater than 3 Å was considered wrong/incorrect [18]. Figure 1 depicted the results of receptor preparation and validation.



7JIT receptors (A), Cavity (green cavity) on the 7JIT Receptor (B) and Cavity (green cavity) accompanied by native ligand (C).

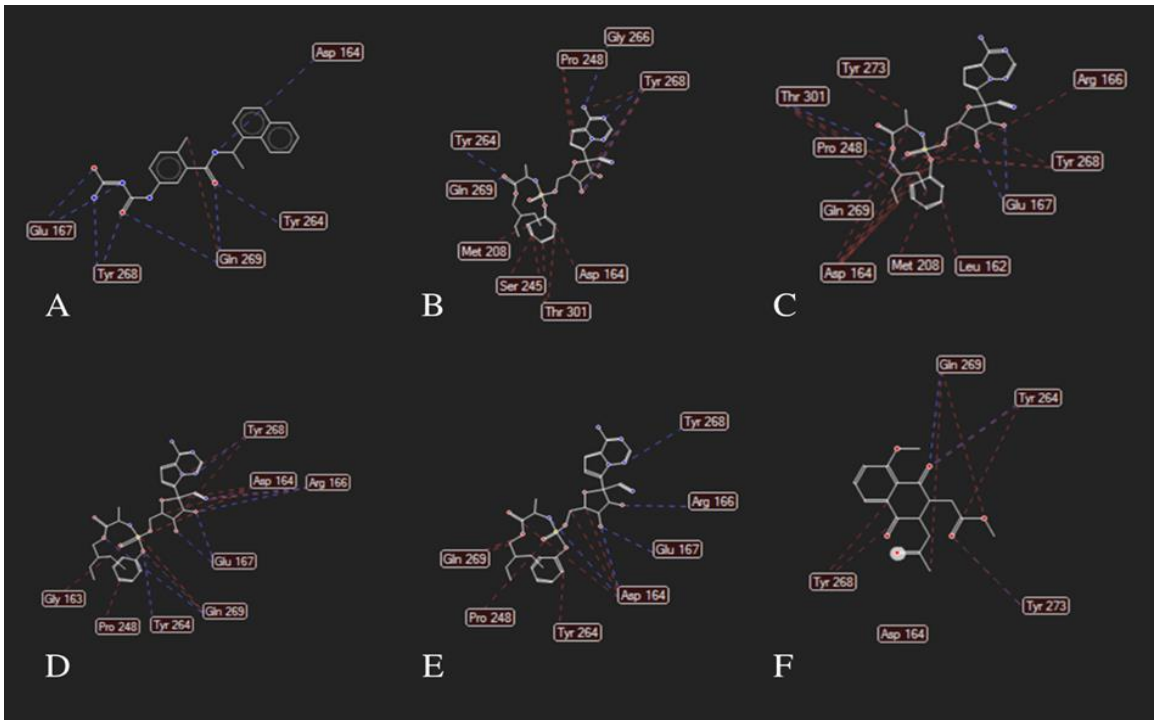
Figure 1 Receptor preparation and validation

3.2. Docking molecular and interaction

Rerank Score, Moldock Score, and H-bond with 3 replications each were the docking parameters as a result of molecular docking. The Rerank Score was a critical parameter for determining the bond energy between the ligand and the receptor as well as the interaction.

Table 1 Rerank score, hydrogen bonds and steric bonds

Ligands	Rerank Score	Interaction with Amino Acid	
		Hydrogen Bonds	Steric Bonds
Eleutherine	-102.853	Gly 266 (3.30); Tyr 268 (3.34 & 3.19); Tyr 264 (2.94); Gln 269 (2.68)	Gly 266; Tyr 264; Gln 269; Pro 248 (3.06 & 2.81); Tyr 268 (3.16, 3.14 & 3.13); Asp 164 (2.83); Thr 301 (3.12, 3.08 & 3.17); Ser 245 (3.07); Met 208 (2.71)
Isoeleutherine	-108.493	Glu 167 (3.09 & 3.09); Gln 269 (2.56); Thr 301 (2.84)	Arg 166 (3.05); Tyr 268 (3.15, 2.86 & 3.14); Glu 167; Leu 162 (3.09); Met 208 (3.13); Thr 301 (2.90, 2.59 & 3.16); Gln 269 (3.13); Tyr 273 (3.00 & 2.44); Pro 248 (2.87); Asp 164 (3.19, 3.03, 2.52, 2.83, 3.10, 3.16 & 3.19)
Eleuthinone	-108.005	Tyr 268 (3.25); Arg 166 (2.75 & 3.10); Glu 167 (3.05 & 3.06); Gln 269 (2.70 & 2.99); Tyr 264 (3.22)	Tyr 268 (3.09 & 2.95); Arg 166 (3.19); Asp 164 (2.37, 3.06, 3.11 & 2.87); Glu 167; Tyr 264; Pro 248 (3.14); Gly 163 (2.92); Gln 269 (2.88, 2.85 & 2.97)
Elecanacine	-104.640	Tyr 268 (3.44); Arg 166 (3.09); Glu 167 (3.29); Asp 164 (2.99 & 3.11)	Tyr 268; Arg 166; Glu 167; Asp 164 (3.18, 2.59 & 2.58); Tyr 264 (2.78); Pro 248 (2.99); Gln 269 (3.12, 2.94 & 2.72)
Ribavirin	-94.195	Tyr 264 (2.94); Gln 269 (2.85); Tyr 273 (3.10)	Tyr 264 (3.06 & 3.19); Tyr 273 (3.05); Gln 269 (2.58 & 3.18); Asp 164; Tyr 268 (3.02 & 3.17)
Native Ligand	-114.433	Asp 164 (3.11); Gln 269 (2.66 & 2.60); Tyr 264 (3.10); Tyr 268 (2.68 & 2.81); Glu 167 (2.75 & 3.07)	Gln 269 (3.19); Glu 167; Tyr 268; Tyr 264; Tyr 264

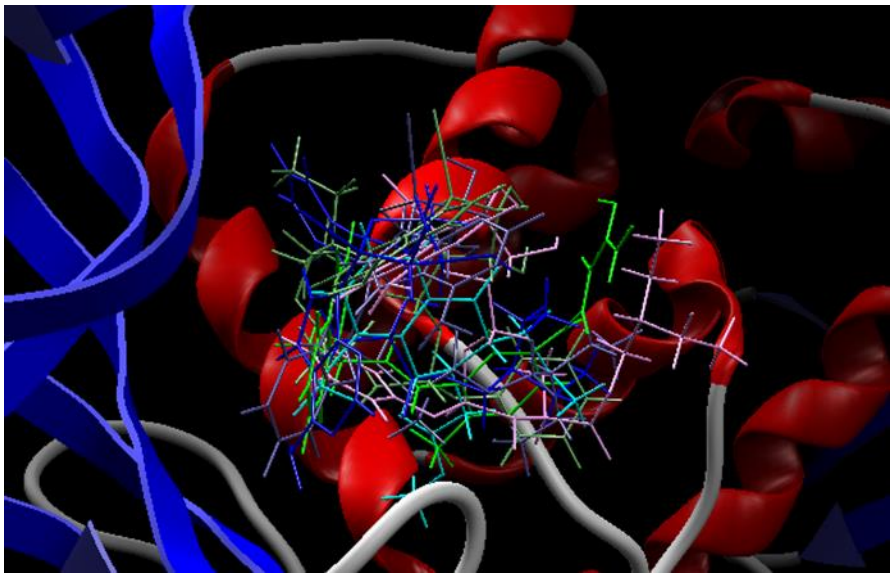


Hydrogen bonds (blue dotted line) and steric bonds (red dotted line) of native ligand compounds (A), eleutherine (B), isoeleutherine (C), eleuthinone (D), elecanacine (E), and Ribavirin (F) in the 7JIT receptors.

Figure 2 2D Forms of hydrogen bonds and steric bonds of eleutherine, isoeleutherine, eleuthinone, elecanacine, and Ribavirin

The Rerank Score assessment was used to estimate ligand binding, with a lower value indicating higher affinity. The lower the Rerank Score, the stronger and more active the ligand-receptor bond [19]. Table 1 displays the Rerank Score results for all compounds. The compound with the lowest Rerank Score, -114,433 Kcal/mol, is native ligand. The four compounds in this research, on the other hand, had lower Rerank Scores than the comparator drug Ribavirin.

The docking process required determining the interaction between the ligand and the amino acid in addition to determining the bond energy. The presence of amino acid residues in the receptor was one of its characteristics, and they played a role in determining the interaction between the ligand-receptor bond. There were two interactions involved in this research: hydrogen bonds and steric bonds. Table 1 and Figure 2 show the amino acids and functional groups involved in hydrogen bonding and steric bonding at the 7JIT receptor. Figure 3 depicts docking results for the native ligand, the test compounds such as eleutherine, isoeleutherine, eleuthinone, elecanacine, and the comparator Ribavirin against receptors with residues amino acids.



Compounds eleutherine (blue flame), isoeleutherine (dark green color), eleuthinone (pink color), elecanacine (gray blue color), Ribavirin (tosca green color) and native ligand (green flame) against 7JIT receptors with acid residues amino shown stick (thin) style.

Figure 3 Compound docking results

3.3. Prediction of physicochemical properties and toxicity

Table 2 Prediction of physicochemical properties compounds in *Eleutherine palmifolia* (L.) Merr

Ligands	MW (g/mol)	Log P	HBA	HBD	Torsion	TPSA (Å²)	Number of violation of Lipinski Rules of 5
Eleutherine	288.34	2.38	4	0	2	52.60	0
Isoeleutherine	288.34	2.39	4	0	2	52.60	0
Eleuthinone	318.32	1.61	6	0	6	86.74	0
Elecanacine	272.30	2.04	4	0	1	52.60	0
Ribavirin	244.20	-2.14	7	4	3	143.72	0
Standard Value	≤ 500	≤ 5	≤ 10	≤ 5	≤10	≤140	>1

The physicochemical prediction of the test compound and the reference drug was used to assess the test compounds similarity to the drug based on the Lipinski's rule of five (Ro5). The Lipinski's Ro5 parameter was made up of molecular weight (MW), number of hydrogen bond donors (HBD), number of hydrogen bond acceptors (HBA), oil to water partition coefficient (Log P), and the number of bonds that can be rotated (Torsion) [15]. Other research incorporated topological polar surface area (TPSA) parameters [16]. According to the results of the compounds studied, all test

compounds met the Lipinski's Ro5 (Table 2). However, with a value of 143.72 Å^2, the comparator drug Ribavirin did not meet the good TPSA parameter.

The toxicity of the test compounds must also be predicted in this research. ProTox-II and the pkCSM online server were used to analyze the predicted results of the LD_{50} value, toxicity class, and toxicity based on AMES toxicity, hepatotoxicity, and skin sensitization of the test compounds. Table 3 describes it in detail. Eleutherine, isoeleutherine, eleuthinone, and elecanacine were classified as having class IV toxicity and the comparator drug Ribavirin belongs to class V toxicity in this research. Furthermore, all test compounds were predicted to be safe in terms of AMES toxicity, hepatotoxicity, and skin sensitization, with the exception of the elecanacine compound, which was predicted to be mutagenic and the eleuthinone compound, which was predicted to be hepatotoxic.

Table 3 Prediction of toxicity compounds in *Eleutherine palmifolia* (L.) Merr

Ligands	LD_{50} (mg/kg)	Toxicity Class	AMES Toxicity	Hepatotoxicity	Skin Sensitization
Eleutherine	500	IV	No	No	No
Isoeleutherine	500	IV	No	No	No
Eleuthinone	1000	IV	No	Yes	No
Elecanacine	1000	IV	Yes	No	No
Ribavirin	2700	V	No	No	No

4. Discussion

Severe Acute Respiratory Syndrome Coronavirus 2 has a long history of causing respiratory illness in humans. Clinical management, infection prevention, control measures, drug and vaccine development are the primary focus for limiting disease spread. World Health Organization (WHO) currently approves many drugs and vaccines to treat and prevent disease. The disadvantage is that drugs and vaccines have dangerous side effects. As a result, it is critical to identify and characterize new drug candidates in order to combat this pandemic.

The Non-structural protein 3 has opened up new avenues for identifying new candidates who can inhibit viral replication and thus become effective therapies against SARS-CoV-2. We used four compounds as new drug candidates that were predicted to inhibit the NSP3 protein: eleutherine, isoeleutherine, eleuthinone, and elecanacine. Molecules with high binding affinity, hydrogen and steric interactions, good physicochemical properties, and low toxicity can be used as lead molecules in drug development. As a result, a molecular docking research of these four compounds was performed to determine binding affinity and predict hydrogen and steric interactions. As previously stated, the Rerank Score was used in this research to interpret the interaction of the SARS-CoV-2 protease with the ligand. The Rerank Score is a value that describes the bond energy required to form a bond between the ligand and the receptor in order to predict the activity of a compound [20].

Based on the Rerank Score, the eleutherine compound had an affinity of -102,853 Kcal/mol, isoeleutherine -108,493 Kcal/mol, eleuthinone -108,005 Kcal/mol, elecanacine -104,640 Kcal/mol, native ligand -114,433 Kcal/mol, and the comparator drug Ribavirin -94,195 Kcal/mol for the NSP3. This means that, when compared to the four test compounds and Ribavirin, the native ligand had the highest affinity. However, the four tested compounds had a higher affinity than Ribavirin, and even those four compounds far exceeded Ribavirins binding energy value. Thus, the four compounds investigated in this research were expected to provide antiviral activity that acted as an inhibitor of the NSP3 for SARS-CoV-2, outperforming Ribavirin. This can be interpreted to mean that the compounds eleutherine, isoeleutherine, eleuthinone, and elecanacine were predicted to have potential as antiviral drug candidates capable of inhibiting SARS-CoV-2 replication. Other research had revealed that the NSP3 is one of the most complex proteins, with numerous functional domains. Furthermore, it may play a significant role in viral RNA replication/transcription.

There are two types of interactions in this research: hydrogen bonds and steric bonds. Hydrogen bonds are denoted by a blue dashed line that also shows the atomic distance (Å), whereas aesthetic bonds are denoted by a red dashed line that also shows the bond distance (Å). Can be seen in Figure 2. When the bond distance decreases, it indicates that the bond is becoming stronger and more stable. If the bond distance is greater, however, the bond will be easily broken. The energy of hydrogen bonds between proteins and ligands is referred to as hydrogen bond energy, whereas steric bonds are the energies of steric interactions between proteins and ligands [21]. According to other research, an interaction

with the 7 amino acid residues Gly163, Asp164, Glu167, Pro248, Tyr264, Tyr268 and Gln269 indicates that the compound under research binds amino acids important for inducing inhibition of the NSP3 receptors. Tyr268, in addition to forming a hydrogen bond with the amino acid, plays an important role in inhibiting the NSP3 receptor [22]. So, if the compound in the research interacts with the amino acid residue, it has a stable ligand-protein bond in inhibiting the NSP3 receptor. The results showed that in hydrogen bond, the native ligand binds to the key amino acid residue in the hydrogen bond, Tyr268. In addition, 3 compounds consisting of eleutherine, eleuthinone and elecanacine which also have hydrogen bonds with the amino acid Tyr268. Meanwhile, isoeleutherine and ribavirin do not bind to the amino acid Tyr268.

According to the research data, the native ligand binds to 5 amino acid residues in steric bonding, namely Gln269, Glu167, Tyr268, Tyr264, and Asp164. Eleutherine binds to four amino acid residues that have been identified as compatible in the literature: Tyr264, Gln269, Tyr268 and Asp164. According to the literature, isoeleutherine binds to four amino acid residues: Tyr268, Glu167, Gln269, and Asp164. Eleuthinone binds to five amino acid residues that have been identified as compatible in the literature: Tyr268, Asp164, Glu167, Tyr264, and Gln269. Elecanacine binds to five amino acid residues that have been identified as compatible in the literature: Tyr268, Glu167, Asp164, Tyr264, and Gln269. Ribavirin binds to four amino acid residues that have been identified as compatible in the literature: Tyr264, Gln269, Asp164, and Tyr268. According to the literature, the four compounds in this research have more stable ligand-protein bonds, as evidenced by the presence of hydrogen bonds and steric bonds in amino acids.

The prediction of physicochemical properties and toxicity is the next step of the in silico studies. The SwissADME online tool program was used to predict the physicochemical properties of all test compounds and reference drugs. SwissADME is a free online tool for calculating the pharmacokinetic properties, drug likeness, and therapeutic chemistry of a drug molecule [23]. The compounds physicochemical predictions are used to assess the similarity of the tested compounds to drugs based on the Lipinski's rule of five (Ro5).The Lipinski's Ro5 is an in silico tool for evaluating physicochemical properties, which is critical in determining drug safety prior to marketing as an identification process during pre-clinical trials. Other studies have found that, in addition to the Lipinski's Ro5 parameter, the descriptor of a test compounds physicochemical properties is topological polar surface area (TPSA) [16].

According to the Lipinski's Ro5, the test compound cannot violate more than one predetermined rule, namely molecular weight (MW) less than 500, number of hydrogen bond donors (HBD) less than 5, number of hydrogen bond acceptors (HBA) not exceeding 10, Log P number not exceeding 5, and the number of bonds that can be rotated (Torsion) not exceeding 10. Meanwhile, the topological polar surface area (TPSA) parameter should not be greater than 140 $Å^2$. According to the literature, if the TPSA value exceeds 140 $Å^2$, the drug molecule will be unable to penetrate the cell membrane [16]. This is supported by other studies, which show that TPSA ≤ 140 $Å^2$ has a ≤ 20% oral bioavailability [24]. The results of this research revealed that the four test compounds met the parameters or did not violate any of the Lipinski's Ro5 rules while meeting the good TPSA parameters. This means that the compounds tested in this research could be used as drug candidates to inhibit SARS-CoV-2 replication, particularly in the NSP3 protease. Surprisingly, the comparator drug in this research did not meet the good TPSA parameters.

Safety is usually the most important consideration during the drug development process. As a result, before testing in experimental animals, it is necessary to predict toxicity with the goal of predicting the amount of tolerability. In this research, ProTox-II and pkCSM online tools were used to predict toxicity. ProTox-II is critical for determining oral toxicity based on the 50% lethal dose (LD_{50}) and toxicity class. Furthermore, ProTox-II can predict the toxicity class (I-VI) based on the toxic dose [25]. Meanwhile, AMES toxicity, hepatotoxicity, and skin sensitisation were used to determine toxicity using pkCSM. To assess the mutagenicity of compounds, prediction of AMES toxicity was performed in this research. Hepatotoxicity testing was performed to determine a compounds hepatotoxicity, a compound was considered hepatotoxic if it interfered with the normal physiological function of the liver [26]. Furthermore, skin sensitization was performed to identify compounds that could cause an allergic reaction on the skin.

Ribavirin had the highest LD_{50} value of 2700 mg/kg (toxicity class V), followed by eleuthinone, and elecanacine, all of which had LD_{50} values of 1000 mg/kg (toxicity class IV). According to GHS (Globally Harmonized System) standards, toxicity class IV compounds are harmful if swallowed orally, while toxicity class V compounds are may be harmful if swallowed orally. Ribavirin is in toxicity class V, while the four test compounds are in toxicity class IV. However, the drug Ribavirin can still be used because its benefits outweigh its toxicity. According to the pkCSM online tool, all tested compounds were predicted to be safe with the exception of elecanacine, which was predicted to be mutagenic and eleuthinone, which was predicted to be hepatotoxic.

5. Conclusion

Molecular docking studies, physicochemical predictions, and toxicity of *Eleutherine palmifolia* (L.) Merr compounds indicate the potential for effective inhibition of the SARS-CoV-2 NSP3 receptor. Except for the elecanacine compound, which is predicted to be mutagenic and the eleuthinone compound, which is predicted to be hepatotoxic, all tested compounds have toxicity in class IV and toxicity based on AMES toxicity, hepatotoxicity, and skin sensitization is predicted to be in the safe category. As a result, this research is highly relevant for drug development against SARS-CoV-2. This research, however, still requires validation of in vitro and in vivo test studies to determine its true potential as an anti-SARS-CoV-2 agent.

Compliance with ethical standards

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Disclosure of conflict of interest

We warrant that the article is the Author's original work and ensure no conflicts of interest to declare. All co-authors have seen and agree with the contents of the manuscript and there is no financial interest to report. We certify that the submission is not under review at any other publication.

References

[1] Shereen MA, Khan S, Kazmi A, Bashir N, Siddique R. COVID-19 infection: Emergence, transmission, and characteristics of human coronaviruses. J Adv Res. 2020 Jul, 24:91–8.

[2] Susilo A, Rumende CM, Pitoyo CW, Santoso WD, Yulianti M, Herikurniawan H, et al. Coronavirus Disease 2019: Tinjauan Literatur Terkini. Jurnal Penyakit Dalam Indonesia. 2020 Apr 1, 7(1):45.

[3] Handayani D, Hadi DR, Isbania F., Burhan E, Agustin H. Corona Virus Disease 2019. Jurnal Respirologi Indonesia. 2020 Apr, 40(2):119-29.

[4] Narkhede RR, Cheke RS, Ambhore JP, Shinde SD. The Molecular Docking Study of Potential Drug Candidates Showing Anti-COVID-19 Activity by Exploring of Therapeutic Targets of SARS-CoV-2. Eurasian Journal of Medicine and Oncology. 2020, 4(3):185–195.

[5] Naqvi AAT, Fatima K, Mohammad T, Fatima U, Singh IK, Singh A, et al. Insights into SARS-CoV-2 genome, structure, evolution, pathogenesis and therapies: Structural genomics approach. Biochim Biophys Acta BBA - Mol Basis Dis. 2020 Oct, 1866(10):165878.

[6] Carvalho MF, Lazarowitz SG. Interaction of the Movement Protein NSP and the Arabidopsis Acetyltransferase AtNSI Is Necessary for Cabbage Leaf Curl Geminivirus Infection and Pathogenicity. J Virol. 2004 Oct 15, 78(20):11161–71.

[7] Suzuki H., Rotavirus Replication: Gaps of Knowledge on Virus Entry and Morphogenesis. Tohoku J. Exp. Med. 2019, 248(4):285-296.

[8] Norazharuddin H, Lai NS. Roles and Prospects of Dengue Virus Non-structural Proteins as Antiviral Targets: An Easy Digest. The Malaysian journal of medical sciences. 2018, 25(5):6-15.

[9] Jahan I, Onay A. Potentials of plant-based substance to inhabit and probable cure for the covid-19. Turkish Journal of Biology. 2020, 44(1):228–241.

[10] Insanu M, Kusmardiyani S, Hartati R. Recent Studies on Phytochemicals and Pharmacological Effects of Eleutherine americana Merr. Procedia Chemistry. 2014, 13:221-228.

[11] Narko T, Permana B, Prasetiawati R, Soni D, Khairiyah F. Molecular Docking Studyof Bulb Of Bawang Dayak (*Eleutherine palmifolia* (L) Merr) Compound as Anti Servical Cancer. Jurnal Ilmiah Farmako Bahari. 2017, 8(2):1-14.

[12] Babula P, Mikelova R, Patesil D, Adam V, Kizek R, Havel L, Sladky Z. Simultaneous Determination Of 1,4 Naphtoquinone, Lawsone, Juglone and Plumbgin by Liquid Chromathography With UV Detection. Biomedical Paper. 2005, 149:1-25.

[13] Siswandono. Kimia Medisinal 1. Edisi Kedua. Surabaya: Airlangga University Press, 2016.

[14] Sumaryada T, Pramudita CA. Molecular Docking Evaluation of Some Indonesian's Popular Herbals for a Possible COVID-19 Treatment. Biointerface Research in Applied Chemistry. 2021, 11(3):9827-9835.

[15] Dhameliya TM, Nagar PR, Gajjar ND. Systematic virtual screening in search of SARS CoV-2 inhibitors against spike glycoprotein: pharmacophore screening, molecular docking, ADMET analysis and MD simulations. Mol Divers. 2022 Oct, 26(5):2775–92.

[16] Yergaliyeva E, Bazhykova K, Abeuova S, Vazhev V, Langer P. In silico drug-likeness, biological activity and toxicity prediction of new 3,5-bis(hydroxymethyl)tetrahydro-4H-pyran-4-one derivatives. Chem Bull Kazakh Natl Univ. 2022 Dec 3, (4):14–20.

[17] Manavi MA. In-silico study for Identification of Potential SARS-CoV-2 main protease inhibitors: Virtual Drug Screening and Molecular Docking with AutoDock Vina and molegro virtual docker. J Cell Mol Res. 2022, 13(2):108-112.

[18] Mateev E, Valkova I, Angelov B, Georgieva M, Zlatkov A. Validation Through Re-Docking, Cross-Docking and Ligand Enrichment In Various Well-Resoluted MAO-B Receptors. International Journal of Pharmaceutical Sciences and Research. 2022, 13(3):1000-1008.

[19] Prasetiyo A, Kumala S, Mumpuni E, Tjandrawinata, RR. Validation of Structural-Based Virtual Screening Protocols with The PDB Code 3G0B and Prediction of The Activity of Tinospora crispa Compounds as Inhibitors of dipeptidyl-peptidase-IV. Research Results in Pharmacology. 2022, 8(1):95-102.

[20] Lee C, Mar A. Computer Screening of Natural Compounds as Potential Inhibitors of SARS-CoV-2 Major Proteases: Target of COVID-19. Journal of Contemporary Medical Practice. 2022, 4(5).

[21] Bhaliya J, Shah V. Identification of potent COVID-19 Main Protease (Mpro) inhibitors from Curcumin analogues by Molecular Docking Analysis. International Journal of Advance Research, Ideas and Innovations in Technology. 2020, 6(2):664-672.

[22] Patel R, Prajapati J, Rao P, Rawal RM, Saraf M, Goswami D. Repurposing the antibacterial drugs for inhibition of SARS-CoV2-PLpro using molecular docking, MD simulation and binding energy calculation. Mol Divers. 2022 Aug, 26(4):2189–209.

[23] Sahu N, Mishra S, Kesheri M, Kanchan S, Sinha RP. Identification of Cyanobacteria-Based Natural Inhibitors Against SARS-CoV-2 Druggable Target ACE2 Using Molecular Docking Study, ADME and Toxicity Analysis. Indian J Clin Biochem. 2022 Jul 5:1-13.

[24] Yalçın S, Yalçınkaya S, Ercan F. In silico detection of inhibitor potential of Passiflora compounds against SARS-Cov-2(Covid-19) main protease by using molecular docking and dynamic analyses. J Mol Struct. 2021 Sep, 1240:130556.

[25] Arulanandam CD, Hwang JS, Rathinam AJ, Dahms HU. Evaluating different web applications to assess the toxicity of plasticizers. Sci Rep. 2022 Nov 16, 12(1):19684.

[26] Nema S, Verma K, Mani A, Maurya NS, Tiwari A, Bharti PK. Identification of Potential Antimalarial Drug Candidates Targeting Falcipain-2 Protein of Malaria Parasite—A Computational Strategy. BioTech. 2022 Nov 30, 11(4):54.