Berkala **PENELITIAN HAYATI**

ISSN: 08526834 | E-ISSN:2337-389X

Volume 30 | No. 2 | **August** | 2024

Original article

Composition of volatile bioactive compounds of sea cucumber (*Holothuria atra*) extract and their bioactivity as antivirals for SARS-CoV-2 COVID-19

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Abstract

COVID-19 is a pandemic that has an impact on all aspects of life. This pandemic is caused by the SARS-CoV-2 virus, which has a fast pathogenesis cycle, so alternatives are needed, such as incredibly natural and marine ingredients that can be used as drug candidates. *Holothuria atra* is an animal candidate that has great potential. This study aims to describe the potential of *H. atra* as an antiviral candidate for COVID-19 through *in silico* analysis. The research began with extracts and GC-MS tests to identify the compound content in *H. atra*. Molecular docking analysis, Lipinski druglikeness, and toxicity predictions were carried out to determine the potential of the compound as an antiviral candidate for COVID-19 through inhibition of RNA-dependent RNA-polymerase protein (RdRp) and human Angiotensin Converting Enzym-2 (hACE2). The research results showed that there were five volatile compounds in the methanol extract of *H. atra* with one potential compound as a COVID-19 antiviral, namely Cyclohexanone, 2,6-bis((4-methoxyphenyl)methylene)-. Lipinski's druglikeness analysis and toxicity prediction provide support as a COVID-19 antiviral. Further research, such as *in vitro* and *in vivo* testing, can be carried out to give an overview of living cells and its activity as an antiviral for COVID-19.

Keywords: COVID-19, SARS-CoV-2, *Holothuria atra*, antiviral

Received: December 13, 2023 Revised: May 4, 2024 Accepted: June 23, 2024

Introduction

Sea cucumbers, scientifically known as *Holothuria atra*, represent a marine organism with promising prospects for advancement within the field of medicine (Jayathilake et al., 2023; Migas & Klemenchenko, 1990; Yamada et al., 2000). This particular species of mammal is commonly observed inhabiting the aquatic environments spanning from the Red Sea and East Africa to Australia. Omnivorous organisms of this nature consume detritus and various forms of organic material as a means of sustenance (Ahmed et al., 2016). In response to predatory threats, these creatures possess a defensive mechanism wherein they release a toxic crimson fluid when their integument is subjected to rubbing or injury. This particular organism has the potential for biomedical applications due to its bioactive constituents, including lectins, sapogenins, steroids, and terpene glycosides, which exhibit therapeutic properties. Species belonging to the same family exhibit diverse bioactive constituents (Dhinakaran & Lipton, 2014; M.S., 2005; Puspitasari et al., 2023). For instance, *H. fuscocinerea* is known to possess Fuscocineroside C, a compound that demonstrates anticancer properties (Zhang et al., 2006). Similarly, *H. hilla* is recognized for its content of Hillaside C, which exhibits anti-leukemia and breast cancer activity (Wu et al., 2007). Telenata ananas, an alternative species of sea cucumber, exhibits promising potential as an HIV antiviral agent (Bordbar et al., 2011). The medical applications of *H. atra*, a type of sea cucumber, are being explored due to its potential in several areas, including its potential as an antiviral agent for COVID-19.

The COVID-19 pandemic has emerged in recent years as a global health crisis, characterized by the prevalence of the SARS-CoV-2 virus as the causative agent of this infectious disease. SARS-CoV-2 is an RNA virus that has remarkable transmissibility and primarily targets the respiratory system in humans (Zhou et al., 2020). SARS-CoV-2 is classified as an enclosed, single-stranded RNA virus, possessing a genomic size of around 30 kilobases. The genetic material of the virus contains instructions for the synthesis of many types of proteins, both structural and non-structural. Among these proteins is the spike (S) protein, which has a crucial function in facilitating the virus's entry into the cells of the host organism. The transmission of the virus is mostly facilitated through the dispersion of respiratory droplets, which are generated when an individual who is infected with the virus coughs, sneezes, or engages in verbal communication. Transmission can also occur through direct contact with surfaces that have been polluted (Hoffmann et al., 2020; K. Wang et al., 2020). The viral pathogenesis commences with the infection facilitated by the Spike protein, which then binds to the human ACE2 (Angiotensin Converting Enzyme 2) receptor (Walls et al., 2020). This interaction is followed by activation through the transmembrane protein serine 2 (TMPRSS2) (Hoffmann et al., 2020). The replication cycle is sustained by the involvement of two crucial

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http://dx.doi.org/10.23869/bphjbr.30.2.20243
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enzymes, notably $3CL^{pro}$ (3C-like protease) (Ahmad et al., 2021) and RdRp (RNA-dependent RNA polymerase) (Shannon et al., 2020). One potential strategy for impeding the infection and subsequent replication is to hinder the activity of three key components: $3CL^{pro}$, RdRp, and ACE2. The primary objective of this research is to determine the composition of bioactive chemicals present in sea urchin extract, with a particular focus on its essential oil. This will be achieved through the use of Gas Chromatography-Mass Spectrometry (GCMS) analysis. Furthermore, the study seeks to investigate the potential of these compounds as inhibitors for the SARS-CoV-2 viral enzymes $3CL^{pro}$ and RdRp, as well as the ACE2 receptor, using an *in silico* methodology.

Methods

Sampling technique

The sampling process in Gili Ketapang, located in the Sumberasih district of Probolinggo Regency in East Java, involved purposive sampling and a quadratic transect method. The primary organisms were preserved in refrigerated containers following collection, whereas echinoderms were sampled twice using the "square transect method" with a 1 x 1 m paralon frame. A perpendicular transect was delineated across the beach, covering 100 meters. Observation plots were positioned at regular intervals of 10 meters along the transect line. The transects were spaced 30 meters apart, while the stations were spaced 50 meters apart. Surveillance was conducted during the reduced water level, known as low tide (Nugroho, Ardiansyah, Kurniawan, & Rahayu, 2023).

Sample preparation and extraction

The sea cucumber samples obtained were then opened and cleaned inside. Next, clean and fresh samples were macerated using methanol solvent with a ratio of 1:4 (w/v) for 1x24 hours with three repetitions (Nugroho, Ardiansyah, Kurniawan, Rahayu, et al., 2023). The mixture was then separated using a Buchner funnel with a vacuum pump to separate the filtrate. The filtrate obtained was then concentrated using a rotary vacuum evaporator to get a concentrated extract.

GC-MS (Gas Chromatography-Mass Spectrophotometry) analysis

Analysis of volatile bioactive compound content was carried out using a GC-MS instrument. GC-MS instrument (Agilent 8890) using a column measuring 30 m x 250 µm x 0.25 µm (Agilent 19091S-433UI: 0236716H). 1 µL of the sample was injected, where the initial temperature was set at 100 ℃ and held for 2 minutes, then heated at a rate of 10 ℃/minute to a temperature of 200 ℃ which was held for 15 minutes. Next, it was heated at the same rate to 250℃, which was held for 18 minutes. The helium gas flow rate used was 1 mL/minute with a running time of 50 minutes. The separated compounds were then analyzed by MS with a molar mass between 50 to 550 g/mol with a source MS 230 ℃ (Max.=250 ℃) and MS Quad 150 ℃ (Max.=200 ℃). The libraries used as the basis for identification are NIST and Willey.

Preparation, docking, and visualization

The protein receptor structures RdRp (PDB ID: 7DFG) and ACE2 (PDB ID: 7VX5) were obtained from RCSB and prepared using Discovery Studio to remove unnecessary air molecules, protein chains, and other molecules. In addition, active site determination was carried out using Discovery Studio based on the drug ligand of the protein receptor (Sururi, Maharani, et al., 2023). Sterile proteins were then entered as macromolecules in the PyRx software. The compounds resulting from GC-MS analysis are then identified, and the 3D compound conformers are downloaded from the PubChem page. Next, it was minimized using OpenBabel in the PyRx program and input as a ligand (Sururi et al., 2022). Molecular docking analysis was carried out using Vina Wizard in the PyRx program where coordinates were used, namely ACE2 (X: 161.262000; Y: 204.724875; Z: 284.353208) and RdRp (X: 128.933609; Y: 131.912522; Z: 140.694652). Chloroquine and favipiravir were chosen as control comparison drugs to select potential compounds. Potential compounds with a more negative binding affinity than the control were visualized using Discovery Studio and PyMOL to determine the position and type of interaction formed.

Drug profil analysis with Lipinski's rules and toxicity prediction

The potential of the compound obtained from molecular docking was then analyzed for its potential as a medicinal compound using Lipinski's rule of five. Lipinski's drug similarity parameters include a molecular weight (MW) of less than 500 Da; lipophilicity (log P) less than 5; hydrogen bond acceptors less than 10; hydrogen bond donors less than 5; and molar refraction between 90-130 (Lipinski, 2004; Lipinski et al., 2001). The SWISSADME web server (swissadme.ch) is used for the analysis (Daina et al., 2017). To assess the possible toxicity of the chemical, a toxicity prediction analysis was carried out utilizing the ProTox-II web server (tox-new.charite.de/protox_II) (Banerjee et al., 2018). Hepatotoxicity, carcinogenicity, mutagenicity, and cytotoxicity are the four parameters used in the analysis.

Results

GC-MS Analysis

The results of the analysis in the form of a chromatogram (Figure 1) show that there are five compounds in the methanol extract of *H. atra*. The identified compound content (Table 1) shows that the dominant compound is Allylamine (36.55%). The five compounds identified were then analyzed using molecular docking to determine their potential as antivirals for COVID-19.

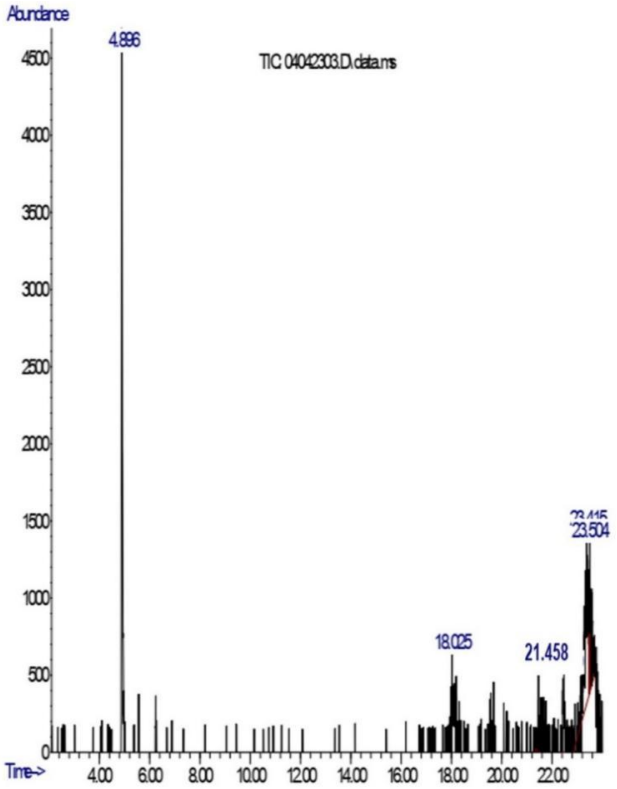

Figure 1. GCMS chromatogram of *H. atra* methanolic extract

Table 1. Identified Bioactive Compound by GC-MS of *H. atra* Methanolic Extract

Peak	RT (Minute)	Comp. (%)	Compound
1	4,896	36,55	Allylamine
2	18,025	14,51	Methyl nonanoate
3	21,458	1,28	Cyclohexanone, 2,6-bis((4-methoxyphenyl)methylene)-
4	23,415	28,51	2,3-Dimethyl-3-heptanol
5	23,504	13,25	N-Hexylacetamide

Molecular docking and visualization

The analysis results (Table 2) show that the five compounds have negative binding energies with a range of -3.0 to -6.2 kcal/mol for the RdRp receptor and -2.7 to -8.4 kcal/mol for ACE2, thus representing that the five compounds form a complex and have activity. Inhibition of each receptor. However, it is known that the binding affinity value of the control drug favipiravir is -5.1 kcal/mol and chloroquine is -5.8 kcal/mol, so only one compound from *H. atra* was found which has potential as an antiviral for COVID-19 by inhibiting RdRp and ACE2, namely Cyclohexanone, 2,6-bis((4-methoxyphenyl)methylene)- because it has a lower binding affinity value than the control drug.

Table 2 Molecular Docking Result

Compound	Binding Affinity (kcal/mol)	
	RdRp	ACE2
Favipiravir (Control drug)	-5.1	-
Chloroquine (Control drug)	-	-5.8
Allylamine	-3	-2,7
Methyl nonanoate	-3,9	-4,6
Cyclohexanone, 2,6-bis((4-methoxyphenyl)methylene)-	**-6,2**	**-8,4**
2,3-Dimethyl-3-heptanol	-4	-4,6
N-Hexylacetamide	-3,8	-4,2

Potential compounds are then visualized to determine the position and type of interaction formed with each receptor. The visualization results on the RdRp complex (Figure 2a) and ACE2 (Figure 2b) show that the potential compounds form complexes with several types of interactions, namely hydrogen bonds, hydrophobic bonds and electrostatic bonds. In the complex with RdRp, the potential compound has hydrogen bond interactions at residues Asn 543, Thr 565, and Tyr 689, as well as hydrophobic bonds at residues Ala 558, Ala 685, and Leu 576. Meanwhile, in the complex with ACE2, the potential compound has a hydrogen bond interaction at position Leu 391, as well as hydrophobic bonds at residues Leu 73, Phe 390, and Trp 69. 3D visualization of the complex with control and potential compounds is presented in Figure 3.

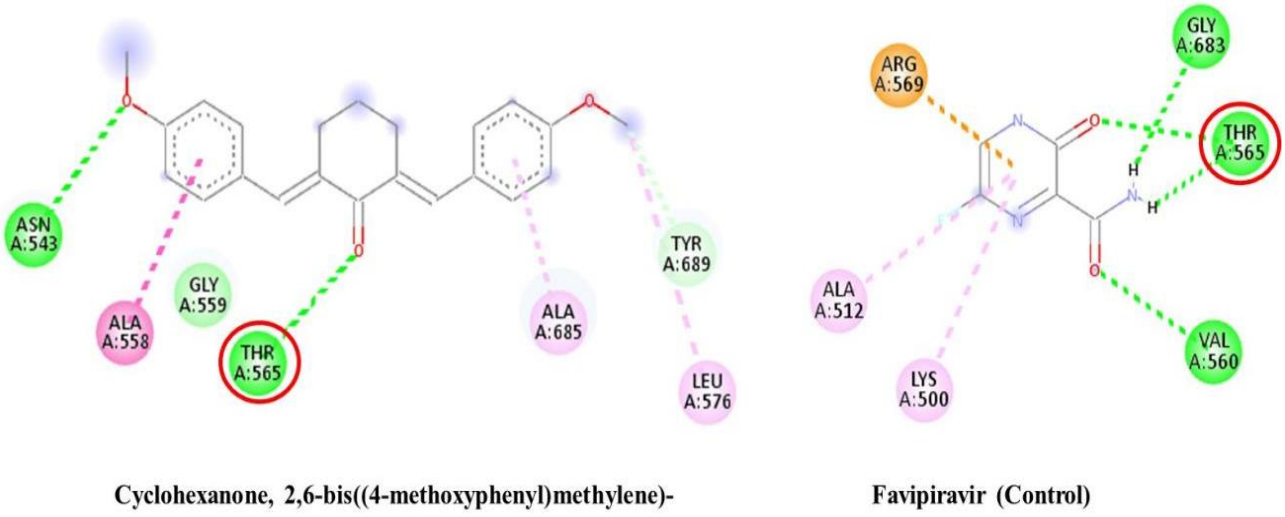

Figure 2a. Visualization of potent compound (a) and control drug (b) in RdRp. Note: red circle is simillar amino acid residues with control drug

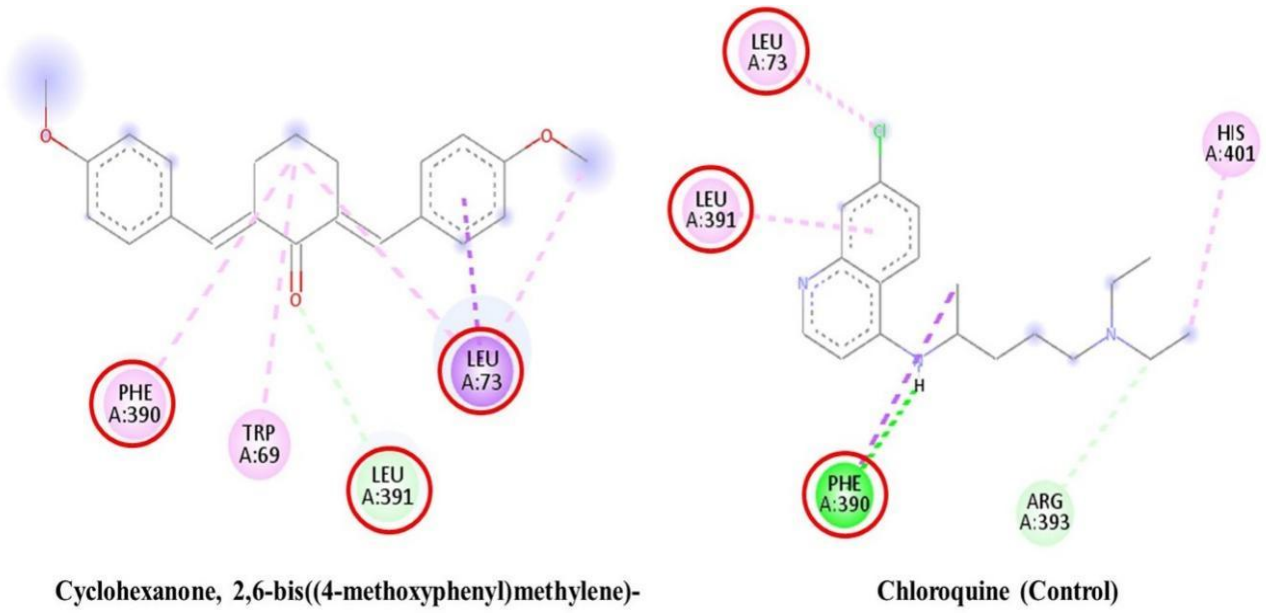

Figure 2b. Visualization of potent compound (A) and control drug (B) in ACE2 complex. Note: red circle is simillar amino acid residues with control drug

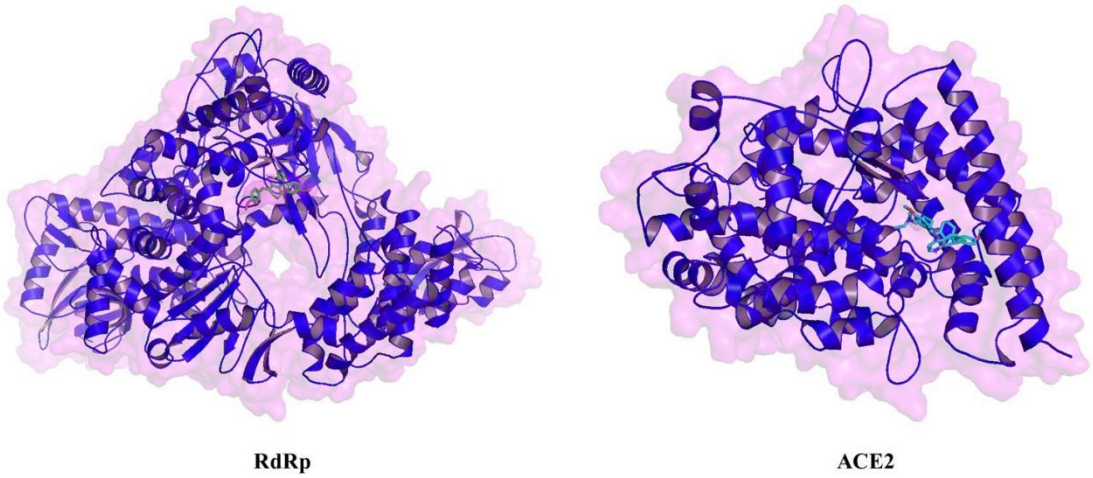

Figure 3. Surface Area Interaction of Each Complex

Druglikeness lipinski and toxicity prediction

Lipinski druglikeness analysis was performed to determine the profile of potential compounds as drug compounds. Lipinski's rule of five (Ro5) is a rule that is often used in selecting and investigating the potential of a compound as a drug compound. When a compound complies with Lipinski's five rules, the compound has the potential to be used as a drug compound, especially an oral drug compound. The first parameter is Lipinski's rule of five, a rule for predicting the potential of a compound as a drug compound, including the following parameters: molar mass < 500 Da, hydrogen bond donors < 5, hydrogen bond acceptors < 10, lipophilicity (log P) < 5, and molar refraction 40-130 (Lipinski, 2004). In addition, a toxicity prediction analysis was carried out to determine the toxic profile of potential compounds, including hepatotoxicity, carcinogenicity, mutagenicity parameters, and predicted LD_{50} values. The analysis results show that the potential compound has a good Lipinski druglikeness profile because it fulfils the five Lipinski rules, so the potential compound has potential as an oral drug. The potential compound has hepatotoxic potential but does not have the potential to be carcinogenic and mutagenic; apart from that, the LD_{50} value is 5000 mg/kg, which is included in the class 4 category (mildly toxic), so it has good potential as a medicinal compound.

Table 3. Druglikeness Lipinski and toxicity prediction result

Parameter	Result
Molecular weight (g/mol)	334.41
Hydrogen bond acceptor	3
Hydrogen bond donor	0
Molar refractivity	101.25
MLOGP	3.33
Druglikeness Lipinski	Yes
Bioavailability	0.55
Hepatotoxicity	Active
Carcinogenicity	Inactive
Mutagenicity	Inactive
LD_{50} (mg/kg)	5000

Discussion

H. atra is a marine biota that has the potential to be developed in the medical world because of its bioactive content (Awad et al., 2023; Ikhsan et al., 2023; Zayed et al., 2023). Generally, marine biota contains volatile compounds such as terpenoids and steroids, so one of the identification analyses that can be carried out is gas chromatography-mass spectrophotometry (GC-MS) analysis (Ralte et al., 2022). This analysis is based on separating compounds using GC and identifying the content that has been separated using an MS detector. GC separation begins when the concentrated extract is heated so that the compound content evaporated and is separated according to its boiling point (Heryanto et al., 2023). The output obtained from GC separation is a chromatogram with the dominant peak as the number of compounds that appear (Sparkman et al., 2011). The separated compounds are then identified using MS, where the compounds separated by GC will be passed to the detector and shot with electrons so that they will experience separation, and fragmentation patterns will appear (Sympli, 2021). The compound fragmentation pattern was then analyzed using the NIST and Willey libraries to identify the compounds present in the extract.

Molecular docking analysis is carried out to determine the potential of a compound to inhibit a particular target protein as a drug candidate at its active site (Sururi, Wati, et al., 2023). This analysis is a fundamental analysis before proceeding to the following analysis to reduce the risk of failure in developing a medicinal compound. The receptors used in this research are RdRp and ACE. RdRp has a role in the replication of the SARS-CoV-2 virus (Shannon et al., 2020), while ACE2 has a role as a gateway for the virus to enter host cells (Walls et al., 2020). Molecular docking analysis is carried out to determine the binding affinity value of a compound when it is a ligand for a receptor. Binding affinity is the free energy score of a complex formed (Anish et al., 2023). The lower the binding affinity, the more stable the complex formed will be. In this study, a compound is said to be potent if it has a lower binding affinity value than the control drugs favipiravir for the RdRp receptor and chloroquine for the ACE2 receptor.

Potential compounds are then visualized to determine the position and type of interaction formed with each receptor. Hydrogen bonds are strong interactions that are formed due to the attraction between hydrogen atoms and fluorine, oxygen, and nitrogen atoms (atoms with high electronegativity) (Głowacki et al., 2013; Rasyid et al., 2023). This bond is a stable and strong bond that is generally used as a basis for drug development in the world of drug discovery (Sharma et al., 2018). Hydrophobic bonds are interactions that are formed due to the meeting of the hydrophobic group of the receptor with the hydrophobic group of the ligand (Cheng et al., 2020; Joshi et al., 2023). Hydrophobic groups are groups that do not like water, such as carbon chains and benzene rings (Fan et al., 2004).

Meanwhile, electrostatic bonds are interactions due to the attraction of charge differences (between positive charges and negative charges) (Berenger et al., 2014; Njoroge et al., 2008). This interaction is an interaction that affects the stability and free energy of the complex formed. The more favorable interactions that are formed, the more stability will increase (Cheng et al., 2020). In addition, there are amino acid residues that are similar to the drug control (J. Wang et al., 2019). The similarity of these amino acid residues shows that the potential compound has an inhibitory position identical to the drug compound, thereby validating the potential compound's potential as an RdRp and ACE2 inhibitor agent for antiviral COVID-19. The similar amino acid residue in the RdRp complex is at position Thr 565, while in the ACE2 complex, it is found at positions Phe 390, Leu 391, and Leu 73. The more similarities in position, the more the validities of its potential as a receptor inhibitor will be similar to the drug control (Musfiroh et al., 2013).

Lipinski druglikeness analysis was performed to determine the profile of potential compounds as drug compounds. Lipinski's rule of five (Ro5) is a rule that is often used in selecting and investigating the potential of a compound as a drug compound (Lipinski, 2004; Lipinski et al., 2001). When a compound complies with Lipinski's five rules, the compound has the potential to be used as a drug compound, especially an oral drug compound. The first parameter is Lipinski's rule of five, a rule for predicting the potential of a compound as a drug compound, including the following parameters: molar mass < 500 Da, hydrogen bond donors < 5, hydrogen bond acceptors < 10, lipophilicity (log P) < 5, and molar refraction 40- 130 (Lipinski, 2004). Compounds have the potential as drug compounds when they meet at least three parameters of this rule. The potential compound fulfills the five Lipinski rules so that it has the potential as a drug compound. In addition, a toxicity prediction analysis was carried out to determine the toxic profile of potential compounds, including hepatotoxicity, carcinogenicity, mutagenicity parameters, and predicted LD_{50} values. A hepatotoxicity profile is a profile of whether a compound has a toxic potential to the human liver or liver (Chang & Schiano, 2007; Zahno et al., 2011). Carcinogenicity is the ability of a compound to induce tumor/cancer cells, causing the growth of tumor/cancer cells (Jamal et al., 2012; Paparella et al., 2017). Mutagenicity is the ability of a compound to induce genetic mutations, thereby causing normal cells to experience mutations (Rivera-Torres & Kmiec, 2017). The LD_{50} value or lethal dose 50 is the dose that produces a death response in 50% of the total population. The lower the dose, the compound is more toxic. The analysis results show that the potential compound has a good Lipinski druglikeness profile because it fulfills the five Lipinski rules, so the potential compound has potential as an oral drug. This is supported by a bioavailability value of 0.55, which is a good bioavailability category (Martin, 2005). A good bioavailability value represents that a compound can be absorbed quickly and well. The potential compound has hepatotoxic potential but does not have the potential to be carcinogenic and mutagenic; apart from that, the LD_{50}

value is 5000 mg/kg, which is included in the class 4 category (mildly toxic) so it has good potential as a medicinal compound. However, this compound has the potential to be hepatic or hepatotoxic. Hence, testing needs to be carried out to determine the lethal dose to damage the liver so that the minimum and maximum dose of the potential compound can be obtained.

Conclusion

Based on the research results, five compounds were found in the methanol extract of *H. atra* with potential compounds as COVID-19 antivirals, namely Cyclohexanone, 2,6-bis((4-methoxyphenyl)methylene)- because it has a lower binding affinity value than the drug control. In addition, Lipinski's druglikeness profile and predicted toxicity profile supports the potential for the compound to be a promising drug candidate. Further testing is needed to determine further its potential, especially *in vivo* testing to determine the toxic dose of potential compounds.

Acknowledgement

The authors extend their sincere appreciation to the students who provided valuable assistance during the fieldwork and the individuals residing in the vicinity of Gili Ketapang Island who generously facilitated the sampling process. Their contributions were indispensable in conducting this research, and their support is greatly acknowledged. This research is funded by the Ministry of Education, Culture, Research, and Technology (Grant No. 659/UN38/HK/PP/2022).

Disclosure of Interest

The authors report no conflict of interest.

References

Ahmad, B., Batool, M., Ain, Q. U., Kim, M. S., & Choi, S. (2021). Exploring the Binding Mechanism of PF-07321332 SARS-CoV-2 Protease Inhibitor through Molecular Dynamics and Binding Free Energy Simulations. In *International Journal of Molecular Sciences* (Vol. 22, Issue 17). https://doi.org/10.3390/ijms22179124

Ahmed, M. I., Aamer, M., & Lawrence, A. J. (2016). Identification of the Holothurian species of the Red Sea and Gulf of Aqaba using DNA barcoding technique. *Egyptian Journal of Aquatic Biology and Fisheries*, *20*(4), 1–7.

Anish, R. J., Mohanan, B., Aswathy, T. R., Nair, A., Radhakrishnan, K. V, & Rauf, A. A. (2023). An integrated approach to the structural characterization, long-term toxicological and anti-inflammatory evaluation of Pterospermum rubiginosum bark extract. *Journal of Ethnopharmacology*, *308*, 116262. https://doi.org/https://doi.org/10.1016/j.jep.2023.116262

Awad, S. N., Abd El Hafez, M. S., Abd Elwahab, M. G., Mady, R., Batiha, G. E.-S., Shaheen, H. M., & Ghareeb, D. A. (2023). Anti-inflammatory properties of *Holothuria atra* extract on lipopolysaccharide induced inflammation of White blood cells. *Damanhour Journal of Veterinary Sciences*, *9*(2), 9–16.

Banerjee, P., Eckert, A. O., Schrey, A. K., & Preissner, R. (2018). ProTox-II: a webserver for the prediction of toxicity of chemicals. *Nucleic Acids Research*, *46*(W1), W257–W263.

Berenger, F., Voet, A., Lee, X. Y., & Zhang, K. Y. J. (2014). A rotation-translation invariant molecular descriptor of partial charges and its use in ligand-based virtual screening. *J. Cheminf.*

Bordbar, S., Anwar, F., & Saari, N. (2011). High-value components and bioactives from sea cucumbers for functional foods—a review. *Marine Drugs*, *9*(10), 1761–1805.

Chang, C. Y., & Schiano, T. D. (2007). Drug hepatotoxicity. *Alimentary Pharmacology & Therapeutics*, *25*(10), 1135–1151.

Cheng, X., Shkel, I. A., O'Connor, K., & Record, M. T. (2020). Experimentally determined strengths of favorable and unfavorable interactions of amide atoms involved in protein self-assembly in water. *Proceedings of the National Academy of Sciences*, *117*(44), 27339 LP – 27345. https://doi.org/10.1073/pnas.2012481117

Daina, A., Michielin, O., & Zoete, V. (2017). SwissADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. *Scientific Reports*, *7*(1), 1–13.

Dhinakaran, D. I., & Lipton, A. P. (2014). Bioactive compounds from *Holothuria atra* of Indian ocean. *SpringerPlus*, *3*, 1–10.

Fan, K., Wei, P., Feng, Q., Chen, S., Huang, C., Ma, L., Lai, B., Pei, J., Liu, Y., Chen, J., & Lai, L. (2004). Biosynthesis, Purification, and Substrate Specificity of Severe Acute Respiratory Syndrome Coronavirus 3C-like Proteinase*. *Journal of Biological Chemistry*, *279*(3), 1637–1642. https://doi.org/https://doi.org/10.1074/jbc.M310875200

Głowacki, E. D., Irimia-Vladu, M., Bauer, S., & Sariciftci, N. S. (2013). Hydrogen-bonds in molecular solids – from biological systems to organic electronics. *Journal of Materials Chemistry B*, *1*(31), 3742–3753. https://doi.org/10.1039/C3TB20193G

Heryanto, R., Putra, C. A., Khalil, M., Rafi, M., Putri, S. P., Karomah, A. H., & Batubara, I. (2023). Antioxidant Activity and Metabolite Profiling of Xylocarpus granatum Extracts Using Gas Chromatography–Mass Spectrometry. *Metabolites*, *13*(2), 156.

Hoffmann, M., Kleine-Weber, H., Schroeder, S., Krüger, N., Herrler, T., Erichsen, S., Schiergens, T. S., Herrler, G., Wu, N.-H., Nitsche, A., Müller, M. A., Drosten, C., & Pöhlmann, S. (2020). SARS-CoV-2 Cell Entry Depends on ACE2 and TMPRSS2 and Is Blocked by a Clinically Proven Protease Inhibitor. *Cell*, *181*(2), 271-280.e8. https://doi.org/https://doi.org/10.1016/j.cell.2020.02.052

Ikhsan, I., Idroes, R., Azharuddin, A., Nasution, R., Yusnaini, R., & Iqhrammullah, M. (2023). Fatty Acid-Rich Extract from *Holothuria atra* for Hyperuricemia via Expressions Modulation of GLUT9a and GLUT9b in Rat Model. *Molecules*, *28*(10), 3981.

Jamal, Q., Lohani, M., Siddiqui, M., Haneef, D., Gupta, S., & Wadhwa, D. (2012). Molecular interaction analysis of cigarette smoke carcinogens NNK and NNAL with enzymes involved in DNA repair pathways: An in silico approach. *Bioinformation*, *8*, 795–800. https://doi.org/10.6026/97320630008795

Jayathilake, N. J., Wimalagunarathna, N. D., Wijesekara, I., & Gunathilake, V. K. (2023). *Holothuria atra*: An Underutilized Marine Resource for Nutritional and Collagen Benefits. *Asian Journal of Biotechnology and Bioresource Technology*, *9*(3), 41–51.

Joshi, K., Gupta, S. R. R., Verma, S., Sharma, R., Qureshi, S., Syed, M. A., & Nunia, V. (2023). Virtual screening of plant phytochemicals to discover potent Janus kinase-1 inhibitors against severe COVID-19 and sepsis. *International Journal of Computational Biology and Drug Design*, *15*(5), 391–411. https://doi.org/10.1504/IJCBDD.2023.133834

Lipinski, C. A. (2004). Lead- and drug-like compounds: the rule-of-five revolution. *Drug Discovery Today: Technologies*, *1*(4), 337–341. https://doi.org/https://doi.org/10.1016/j.ddtec.2004.11.007

Lipinski, C. A., Lombardo, F., Dominy, B. W., & Feeney, P. J. (2001). Experimental and computational approaches to estimate solubility and permeability in drug discovery and

development settings1PII of original article: S0169-409X(96)00423-1. The article was originally published in Advanced Drug Delivery Reviews 23 (1997) 3. *Advanced Drug Delivery Reviews*, *46*(1), 3–26. https://doi.org/https://doi.org/10.1016/S0169-409X(00)00129-0

M.S., K. (2005). *Echinoderms: Their Culture and Bioactive Compounds BT-Echinodermata* (V. Matranga (ed.); pp. 139–165). Springer Berlin Heidelberg. https://doi.org/10.1007/3-540-27683-1_7

Martin, Y. C. (2005). A Bioavailability Score. *Journal of Medicinal Chemistry*, *48*(9), 3164–3170. https://doi.org/10.1021/jm0492002

Migas, E. A., & Klemenchenko, E. G. (1990). Pharmacological effects of the sea-cucumber Stichopus japonicus extract. *Biol Morya-Mar Biol*, *5*.

Musfiroh, I., Muchtaridi, M., Muhtadi, A., Diantini, A., Hasanah, A. N., Udin, L. Z., Susilawati, Y., Mustarichie, R., Kartasasmita, R. E., & Ibrahim, S. (2013). Cytotoxicity studies of xanthorrhizol and its mechanism using molecular docking simulation and pharmacophore modelling. *Journal of Applied Pharmaceutical Science*. https://doi.org/10.7324/JAPS.2013.3602

Njoroge, F. G., Chen, K. X., Shih, N.-Y., & Piwinski, J. J. (2008). Challenges in Modern Drug Discovery: A Case Study of Boceprevir, an HCV Protease Inhibitor for the Treatment of Hepatitis C Virus Infection. *Accounts of Chemical Research*, *41*(1), 50–59. https://doi.org/10.1021/ar700109k

Nugroho, E. D., Ardiansyah, R., Kurniawan, N., Rahayu, A., & Sururi, A. M. (2023). *An in-silico study on the chemical compounds from Macrophiothrix longipedia as antiviral compounds against* COVID-19. *16*(4), 2380–2390.

Nugroho, E. D., Ardiansyah, R., Kurniawan, N., & Rahayu, D. A. (2023). Species identification of echinoderms from Gili Ketapang Island by combining morphology and molecular data. *Aquaculture, Aquarium, Conservation & Legislation*, *16*(1), 135–150.

Paparella, M., Colacci, A., & Jacobs, M. N. (2017). Uncertainties of testing methods: What do we (want to) know about carcinogenicity? *ALTEX-Alternatives to Animal Experimentation*, *34*(2), 235–252.

Puspitasari, Y. E., Tuenter, E., Foubert, K., Herawati, H., Hariati, A. M., Aulanni'am, A., Pieters, L., De Bruyne, T., & Hermans, N. (2023). Saponin and Fatty Acid Profiling of the Sea Cucumber *Holothuria atra*, α-Glucosidase Inhibitory Activity and the Identification of a Novel Triterpene Glycoside. *Nutrients*, *15*(4), 1033.

Ralte, L., Khiangte, L., Thangjam, N. M., Kumar, A., & Singh, Y. T. (2022). GC–MS and molecular docking analyses of phytochemicals from the underutilized plant, Parkia timoriana revealed candidate anti-cancerous and anti-inflammatory agents. *Scientific Reports*, *12*(1), 1–21. https://doi.org/10.1038/s41598-022-07320-2

Rasyid, H., Mardiyanti, R., Arief, I., & Saputri, W. D. (2023). An Insight of Cryptocarya Secondary Metabolites as Anticancer P388: Study of Molecular Docking and ADMET Properties. *Molekul; Vol 18 No 1 (2023)DO - 10.20884/1.Jm.2023.18.1.6364* . http://jos.unsoed.ac.id/index.php/jm/article/view/6364

Rivera-Torres, N., & Kmiec, E. B. (2017). A standard methodology to examine on-site mutagenicity as a function of point mutation repair catalyzed by CRISPR/cas9 and ssodn in human cells. *Journal of Visualized Experiments*. https://doi.org/10.3791/56195

Shannon, A., Selisko, B., Le, N. T. T., Huchting, J., Touret, F., Piorkowski, G., Fattorini, V., Ferron, F., Decroly, E., Meier, C., Coutard, B., Peersen, O., & Canard, B. (2020). Favipiravir strikes the SARS-CoV-2 at its Achilles heel, the RNA polymerase. *BioRxiv*, 2020.05.15.098731. https://doi.org/10.1101/2020.05.15.098731

Sharma, N., Sharma, M., Shakeel, E., Jamal, Q. M. S., Kamal, M. A., Sayeed, U., Khan, M. K. A., Siddiqui, M. H., Arif, J. M., & Akhtar, S. (2018). Molecular Interaction and Computational Analytical Studies of Pinocembrin for its Antiangiogenic Potential Targeting VEGFR-2: A Persuader of Metastasis. *Medicinal Chemistry*, *14*(6), 626–640. https://doi.org/10.2174/1573406414666180416125121

Sparkman, O. D., Penton, Z. E., & Kitson, F. G. (2011). *Chapter 3 - The GC/MS Interface* (O. D. Sparkman, Z. E. Penton, & F. G. B. T.-G. C. and M. S. (Second E. Kitson (eds.); pp. 85–88). Academic Press. https://doi.org/https://doi.org/10.1016/B978-0-12-373628-4.00003-4

Sururi, A. M., Maharani, D. K., & Wati, F. A. (2023). Potensi Senyawa Eugenol Dari Cengkeh (*Syzygium aromaticum*) Sebagai Inhibitor Protease Hiv-1 (PR). *Unesa Journal of Chemistry*, *Vol 12 No 1 (2023)*, 26–30. https://ejournal.unesa.ac.id/index.php/unesa-journal-of-chemistry/article/view/52025/42268

Sururi, A. M., Raihan, M., Aisa, E. R., Safitri, F. N., & Constaty, I. C. (2022). Anti-Inflammatory Activity of Stem Bark Dichloromethane Fraction Syzygium samarangense Extract as COX-2 Inhibitor: A Bioinformatics Approach. *Jurnal Kimia Riset*, *7*(2), 94–100. https://doi.org/10.20473/jkr.v7i2.39662

Sururi, A. M., Wati, F. A., & Maharani, D. K. (2023). A STUDI IN SILICO: POTENSI SENYAWA KATEKIN DAN TURUNANNYA DARI TEH HIJAU SEBAGAI INHIBITOR HGF SERTA PROFIL TOKSISITASNYA. *Unesa Journal of Chemistry*, *12*(2), 57–63.

Sympli, H. D. (2021). Estimation of drug-likeness properties of GC–MS separated bioactive compounds in rare medicinal Pleione maculata using molecular docking technique and SwissADME in silico tools. In *Network Modeling Analysis in Health Informatics and Bioinformatics* (Vol. 10, Issue 1). Springer Vienna. https://doi.org/10.1007/s13721-020-00276-1

Walls, A. C., Park, Y.-J., Tortorici, M. A., Wall, A., McGuire, A. T., & Veesler, D. (2020). Structure, Function, and Antigenicity of the SARS-CoV-2 Spike Glycoprotein. *Cell*, *181*(2), 281-292.e6. https://doi.org/10.1016/j.cell.2020.02.058

Wang, J., Ge, Y., & Xie, X.-Q. (2019). Development and Testing of Druglike Screening Libraries. *Journal of Chemical Information and Modeling*, *59*(1), 53–65. https://doi.org/10.1021/acs.jcim.8b00537

Wang, K., Chen, W., Zhang, Z., Deng, Y., Lian, J.-Q., Du, P., Wei, D., Zhang, Y., Sun, X.-X., Gong, L., Yang, X., He, L., Zhang, L., Yang, Z., Geng, J.-J., Chen, R., Zhang, H., Wang, B., Zhu, Y.-M., … Chen, Z.-N. (2020). CD147-spike protein is a novel route for SARS-CoV-2 infection to host cells. *Signal Transduction and Targeted Therapy*, *5*(1), 283. https://doi.org/10.1038/s41392-020-00426-x

Wu, J. U. N., Yi, Y.-H., Tang, H.-F., Wu, H.-M., & Zhou, Z.-R. (2007). Hillasides A and B, two new cytotoxic triterpene glycosides from the sea cucumber Holothuria hilla Lesson. *Journal of Asian Natural Products Research*, *9*(7), 609–615.

Yamada, K., Harada, Y., Miyamoto, T., Isobe, R., & Higuchi, R. (2000). Constituents of holothuroidea. 9. Isolation and structure of a new ganglioside molecular species from the sea cucumber Holothuria pervicax. *Chemical and Pharmaceutical Bulletin*, *48*(1), 157–159.

Zahno, A., Brecht, K., Morand, R., Maseneni, S., Török, M., Lindinger, P. W., & Krähenbühl, S. (2011). The role of CYP3A4 in amiodarone-associated toxicity on HepG2 cells. *Biochemical Pharmacology*, *81*(3), 432–441. https://doi.org/10.1016/j.bcp.2010.11.002

Zayed, M. A., Abd El Hafez, M. S., Abd Elwahab, M. G., Mady, R., Batiha, G. E.-S., Shaheen, H. M., & Ghareeb, D. A. (2023). Sea cucumber (*Holothuria atra*) ethyl-acetate extract exerts anti-cholinesterase properties. *Damanhour Journal of Veterinary Sciences*, *9*(1), 29–35.

Zhang, S.-Y., Yi, Y.-H., & Tang, H.-F. (2006). Bioactive triterpene glycosides from the sea cucumber Holothuria fuscocinerea. *Journal of Natural Products*, *69*(10), 1492–1495.

Zhou, P., Yang, X.-L., Wang, X.-G., Hu, B., Zhang, L., Zhang, W., Si, H.-R., Zhu, Y., Li, B., Huang, C.-L., Chen, H.-D., Chen, J., Luo, Y., Guo, H., Jiang, R.-D., Liu, M.-Q., Chen, Y., Shen, X.-R., Wang, X., … Shi, Z.-L. (2020). A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature*, *579*(7798), 270–273. https://doi.org/10.1038/s41586-020-2012-7.