Molecular Docking Studies of Marine Sulfated Polysaccharides: Exploring Green Seaweed's Role Against SARS-CoV-2 Spike Glycoprotein

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Abstract

SARS-CoV-2 (Severe Acute Respiratory Syndrome Coronavirus 2) is a virus responsible for the infectious disease of COVID-19 (Coronavirus Disease 2019), whose development is still being monitored. One way to deal with the virus's development is by searching for natural-based medicines that prevent and treat SARS-CoV-2 infection. The abundant biodiversity can be used as a source of treatment. Among many natural resources, seaweed is one of the natural resources rich in bioactive components. Sulfated polysaccharide is one of the potential bioactive compounds in seaweed because it has antiviral effects and the potential to treat SARS-CoV-2. This research aims at increasing the potential of Karimunjawa green seaweed sulfate polysaccharides for antiviral capabilities in SARS-CoV-2 through molecular docking. This research began with identifying the morphology of Karimunjawa seaweed. Identify the components of sulfated polysaccharide compounds based on literature studies according to the species that have been identified. Analysis of the antiviral ability of SARS-CoV-2 based on its binding ability to the SARS-CoV-2 target protein through a molecular docking computational program and testing drug compounds using the Lipinski rule. Based on the research results, it is known that the seaweed obtained from Karimunjawa based on morphology, belongs to the genus Kappaphycus. Molecular docking with a ligand and spike glycoprotein (6LZG) resulted in the hexadecanoic acid compound having a binding free energy of -5.3 kcal.mol^{-1}, which was the compound with the lowest yield compared to other test compounds. The prediction of the physicochemical properties of all test compounds fulfills Lipinski's five rules and has the potential to be used as medicinal compounds.

Keywords: *Seaweed, Sulfated Polysaccharide, Karimunjawa, Antiviral, SARS-CoV-2*

Introduction

The current post-pandemic management of COVID-19 generally focuses on making vaccines to suppress the development of SARS-CoV-2. The need for a COVID-19 vaccine is still limited, especially in several developing countries, where low-income countries show that only around 4% of the population has been successfully vaccinated. Apart from vaccines, developments in natural-based medicines are increasingly sought after, generally based on plants and algae components that are considered capable of fighting various diseases caused by viruses (Yim *et al.*, 2019). The search for viral therapeutic agents is tailored to the mechanism of viral infection to produce precise targets. SARS-CoV-2 infects host cells through a viral entry process mediated by transmembrane spike glycoproteins forming homotrimers protruding from the surface of the virus. The spike glycoprotein will bind to the ACE 2 (Angiotensin Converting Enzymes 2) receptor to be able to enter the host cell (Tortorici *et al.*, 2019; Zhang *et al.*, 2020). In this case, the spike glycoprotein is one of the specific target proteins in SARS-CoV-2 in the search for antiviral therapeutic agents in various research processes.

Natural medicine has many natural sources, one of which comes from marine waters, where 70% of the earth's area consists of waters that can accommodate natural wealth and their use still needs to be explored a lot. Marine plants, one of which is seaweed, are rich in bioactive components in the form of polyphenols, flavonoids, sulfated polysaccharides, proteins, peptides, vitamins, and minerals. Seaweed (marine macroalgae) is a source of natural polysaccharides which has not been widely explored for their use as antiviral agent. It contains

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https://ejournal.undip.ac.id/index.php/ijms
DOI: 10.14710/ik.ijms.29.4.567-575
Received : 27-09-2024
Accepted : 15-11-2024

many variants of sulfated polysaccharide-rich phytonutrients for pharmaceutical use (Muthukumar *et al.*, 2021). Bioactive components in seaweed have been reported to have significant pharmacological effects such as antiviral. Sulfated polysaccharides are natural complex polymers generally found in the cell walls of marine algae and not found in terrestrial plants. Several groups of essential sulfated polysaccharides are carrageenan and agar from red macroalgae, ulvan from green macroalgae, and fucoidan and laminarian from brown macroalgae (Gheda *et al.*, 2016). Green seaweed is a type of seaweed that contains polysaccharides in its cell walls of up to 45% of its dry weight. Green seaweed has a molecular weight of 34 kDa and is known as an effective antiviral agent against influenza virus (Kidgell *et al*., 2019). The urgency of this research is based on the development of COVID-19 drugs specifically designed to prevent cell binding and the entry process of SARS-CoV-2 into host cells. Therefore, the use of antiviral drugs represents a feasible strategy for preventing mild to moderate cases of COVID-19 (Backer *et al.*, 2020; Kumar *et al.*, 2020; Siukan *et al.*, 2020). This research aims at evaluating the antiviral activity of sulfated polysaccharides derived from green seaweed in inhibiting SARS-CoV-2 through molecular docking computational analysis, highlighting the importance of developing natural-based drugs for COVID-19 preventing treatment.

Materials and Methods

Sample collection

Seaweed samples from aquaculture in Kemujan Village, Karimunjawa District. Seaweed samples were cleaned from epiphytes attached to the surface of the thallus using water and documented for morphological analysis. Seaweed was first identified by studying morphological characteristics using a botanical approach. Morphological identification of seaweed is based on branching patterns, color, and thallus texture (Roleda *et al*., 2021; Yang *et al*., 2021).

Molecular docking

Molecular docking studies were carried out using receptor proteins as target proteins, specifically the RBD (Receptor Binding Domain) in the spike glycoprotein (PDB ID: 6LZG). The target protein structure was downloaded via the RCSB PDB (Protein Data Bank) database (http://www.rcsb.org/) without any mutations. The target protein used is prepared by removing water molecules, dimers, short bonds, and ligands using Biovia Discovery Studio software, resulting in a clean receptor (Salih *et al*., 2021; Alturki *et al*., 2022).

Ligands were selected from the gc-ms results of sulfated polysaccharides of the seaweed species *Kappaphycus striatus* in the form of furfural, 1H-imidazole, 2-furan methanol, 5-Hydroxy-methylfurfural, hydrazine, hexadecanoic acid, heptadecanoic acid, and 2-Heptanol (Bhuyar *et al*., 2020). The 3D structure of the ligand compound was obtained from PubChem (https://pubchem.ncbi.nlm.nih.gov/) (Salih *et al*., 2021).

Molecular docking was preceded by validation of the docking method with a redocking technique between the native ligand and the receptor protein using Autodock Tools 1.5 software. The parameter used is RMSD (Root Mean Square Deviation) to describe changes in ligand surface interactions before and after the docking process. This validation is said to be valid if it has an RMSD value <2 Angstrom (Å) and can be used for docking test compounds (Allen & Rizzo, 2014; Salih *et al*., 2021).

The active site of the protein was determined using the SiteMap module to identify potential receptor binding sites. Grid calculations were performed to fit all active sites and were further performed on molecular docking in the same grid file (Alturki *et al*., 2022).

Molecular docking between the ligand and receptor is carried out using AutoDock Tools software by placing the ligand on the binding site (grid box). Data on the molecular docking process are in the form of binding affinity energy, and RMSD < 2 as seen from the bond energy (kcal.mol^{-1}) (Dallakyan & Olson, 2015; Salih *et al*., 2021).

Lipinski's rule test

The drug-likeness analysis was conducted using Lipinski's rule of five, which includes evaluation molecular weight, Log P partition coefficient value, the number of hydrogen bond donors, the number of hydrogen bond acceptors, and molar refractivity. The Lipinski test was carried out via the website http://www.scfbio-iitd.res.in by entering compound files in *.pdb or *.sdf format (Lipinski, 2004; Jayaram *et al*., 2012).

Result and Discussion

Identification of Seaweed Morphology

Seaweed cultivated in the waters of Kemujan Village, Karimunjawa District is one type of seaweed that is commonly cultivated, especially in the waters of Karimunjawa. The morphology of seaweed can be seen in Figure 1. This seaweed has dark green thallus at the base and light green at the tip of the thallus, therefore it is grouped into green seaweed. According

to Nagarajan (2015), seaweed can be differentiated based on their pigmentation and green seaweed is included in the chlorophyta group. Chlorophyta is a diverse group of seaweeds with more than 7000 species distributed in various habitats. Another characteristic of this seaweed is that it has thick thallus with short branches several lateral branches and slightly rounded ends of the thallus. The surface of the thallus is uneven, smooth, and shiny. This seaweed thallus is 15-25 cm wide and 20-30 cm long. Research on the morphological identification of one of the Kappaphycus seaweed groups by Roleda (2021), explains that Kappaphycus seaweed is a type of seaweed that lives in subtidal areas attached to the substrate and has a fleshy, cartilaginous thallus texture, a slightly uneven and shiny surface. It has varying colors ranging from green, yellowish green, to yellowish brown. The thallus grows to form clumps measuring 10-30 cm with irregular branching patterns and is erratic in some parts. The thallus has small branches to the side with blunt ends that are round or conical in shape.

Molecular docking

Molecular docking is a common virtual screening approach for the process of searching for basic ingredients for drug compounds. Molecular docking is carried out to determine the binding affinity between a ligand or potential test compound and a macromolecule in the form of a receptor protein. The receptor protein used is a protein derived from the spike glycoprotein S1 of SARS-CoV-2 (ID: 6LZG). This receptor protein was used in this study to determine the potential compounds that are capable in inhibiting the attachment process of SARS-CoV-2 receptors to the ACE2 receptor protein on human cells. The selected ligand was observed for its binding ability to the SARS-CoV-2 spike glycoprotein receptor to prevent human cell receptors from binding to the SARS-CoV-2 receptor. This shows the ability of the compound in inhibiting the process of viral infection of human cells. Identification of the antiviral ability of sulfated polysaccharides sourced from marine waters can be observed through the binding of the spike protein which can suppress the attachment of the virus to ACE2 (SARS-CoV-2 S protein receptor) (Salih *et al*., 2021).

Molecular docking results were carried out taking into account the RMSD criteria. The RMSD value must be below 2 Å to indicate acceptable mooring results. The RMSD calculation aims at determining whether the method used is computationally capable of experimental procedures (Castro-Alvarez *et al.*, 2017). Based on the results of molecular docking of native ligands, positive controls, and the test ligands, all of them have RMSD values below 2 Å and showed valid computational process results. Molecular docking was carried out with test ligands in the form of seven sulfate polysaccharide component compounds derived from Kappaphycus green seaweed, native ligands, and remdesivir as a positive control. Remdesivir is a potential drug candidate for the treatment of COVID-19. On October22, 2020, remdesivir became the first drug approved

Table 1. SARS-CoV-2 Receptor Protein

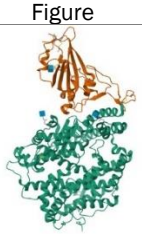
	PDB ID	Native Ligand	Figure
Spike Protein S1	6LZG	2-acetamido-2-deoxy-beta-D-glucopyranose	

Figure 1. Karimunjawa Green Seaweed Morphology

by the United States Food and Drug Administration (FDA) for the treatment of hospitalized COVID-19 patients (Lin *et al*., 2021). The results of molecular docking of all test ligands can be seen in Table 2. Analysis of molecular docking results were carried out by observing the binding free energy value (ΔG). A ΔG value with a small number or minus indicates the possibility of a bond forming between the ligand and the macromolecule (Dos Santos *et al.*, 2018). Based on the molecular docking results in Table 2, it can be seen that there is one compound with the lowest ΔG value, namely hexadecanoic acid with a ΔG value of -5.3 kcal.mol^{-1}. This was followed by the compound 2-furan methanol with a ΔG value of -4.8 kcal.mol^{-1}. The compounds furfural and 5-hydroxymethylfurfural have the same ΔG value, namely -4.5 kcal.mol^{-1}l, as well as the compounds heptadecanoic acid and 2-heptanol with a ΔG value of -4.3 kcal.mol^{-1}, and the compound with the highest ΔG value, namely 1H-imidazole, is - 3.3 kcal.mol^{-1}. When compared with the native ligand and positive control, there was no test ligand compound from the sulfated polysaccharide component that had a lower ΔG value. The native ligand has a ΔG value of -5.6 kcal.mol^{-1}, while remdesivir has a ΔG value of -6.2 kcal.mol^{-1}. The difference in the ΔG value of hexadecanoid acid and remdesivir is -0.9 kcal.mol^{-1}. This shows that the compounds tested still require structural modification and optimization to obtain potential results (Yasin *et al*, 2020). Several recent computational studies have shown the potential of sulfated polysaccharides originating from marine waters as SARS-CoV-2 antivirals, one of which comes from galactofucan sulfate and glucoronomannan sourced from Saccharina japonica showing strong binding affinity to the SARS-CoV spike glycoprotein by inhibiting the heparin-spike protein interaction (Jin *et al*., 2020). Similar research was found in Naufa *et al.* (2021) which produced the lowest binding affinity value of catechin compounds against SARS-CoV-2 spike glycoprotein (6LZG) of -5.2 kcal.mol^{-1}.

A 2D visualization observation of the receptor (protein) and ligand complex was conducted to identify molecules with potential to inhibit the spike protein of SARS-CoV-2. The visualization results are shown in Figure 2. A receptor is a protein molecule or a polymeric structure that specifically recognizes and binds to a ligand molecule, thereby causing some kind of cellular response. Ligands are typically small molecules such as small drug molecules, neurotransmitters, hormones, lymphokines, lectins, and antigens. Ligand act as complementary partner molecule in binding to the receptor for effective biomolecular response (Roy *et al*., 2015). Molecular docking results in an interaction between the receptor (protein) and ligand through various types of interactions, including hydrogen bonding, hydrophobic interactions, and electrostatic interactions. It also reveals the binding site location, which plays a key role in determining the effectiveness of ligand inhibition on protein function. The observation of the interaction outcomes is further supported by the ligand conformation, which shows the ligand's position in the protein binding site. This conformation reflects the ligand's adaptability to the protein environment, which in turn affects the strength and specificity of the interaction (Agu *et al.,* 2023). The visualization of molecular docking results aims at identifying interactions of ligands and amino acid residues in the SARS-CoV-2 spike glycoprotein. This research shows some participation of intramolecular interactions such as hydrogen bonding, alkyl pi, stacked pi-pi, and alkyl in Table 3. Hydrogen bonds are present in the interactions of the native ligand, reference ligand, and several test ligands. Test ligands that do not have hydrogen bonds are found in the compound's furfural and 1H-imidazole. The compound components hexadecanoic acid, heptadecanoic acid, and 2-heptanol have more bonds than the sum of pi-alkyl, pi-pi stacked, and alkyl bonds compared to the number of hydrogen bonds (conventional hydrogen bonds and carbon hydrogen bonds), while 2- methanol has the number of bonds

Table 2. Binding Energy of Native Ligand and Test Ligand

Sample	Bioactive Compound	Binding Affinity (kcal.mol^{-1})	RMSD	Number of hydrogen bonds	Number of amino acid residues
Native ligand	NAG601	-5.6	0	4	9
Positif control	Remdesivir	-6.2	0	3	15
Seaweed sulfated polysaccharides	Furfural	-4.5	0	0	13
	1H-imidazole	-3.3	0	0	12
	2-furanmethanol	-4.8	0	2	15
	5-Hydroxymethylfurfural	-4.5	0	4	12
	Hexadecanoic acid	-5.3	0	2	14
	Heptadecanoic acid	-4.3	0	2	11
	2-Heptanol	-4.3	0	2	10

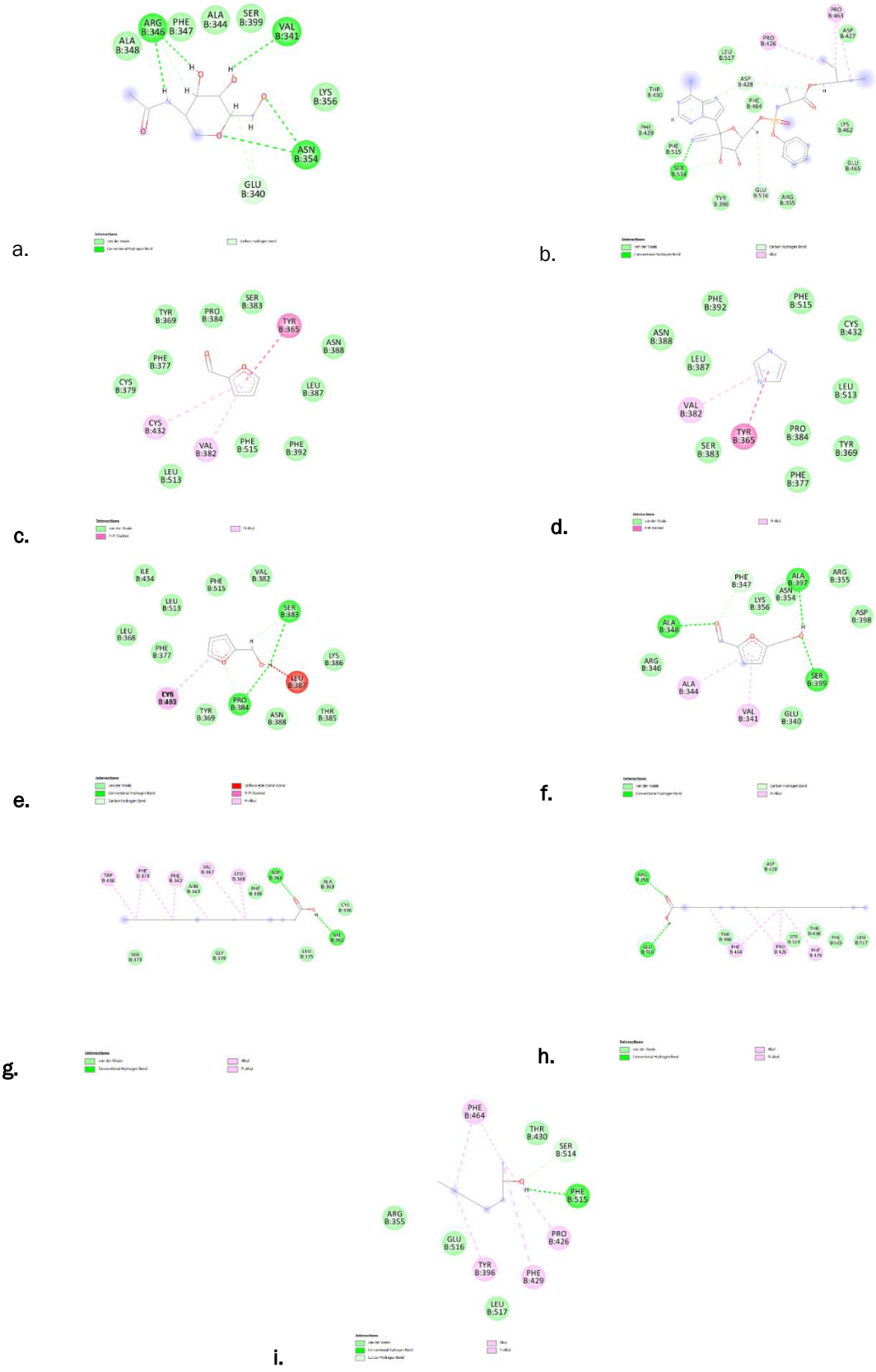


Figure 2. 2D Visualization of a) Native ligand, b) Remdesivir, c) Furfural, d) 1H-imidazole, e) 2-furan methanol, f) 5-Hydroxymethylfurfural, g) Hexadecanoic acid, h) Heptadecanoic acid, i) 2-Heptanol

from the sum of pi-alkyl and stacked pi-pi bonds equal to the number of conventional hydrogen bonds. Meanwhile, the 5-hydroxymethylfurfural compound has a greater number of hydrogen bonds (conventional hydrogen bonds and carbon hydrogen bonds) than the number of pi-alkyl bonds. Hydrogen bonds are interactions between molecules on the H atom and the N, O, or F atoms. Hydrogen bonds resulting from docking can function to support the stability of ligand and protein bonds. Meanwhile, hydrophobic bonds such as van der Walls, pi-pi stacked, alkyl, and pi-alkyl interactions function to increase conformational stability. So the higher the number of hydrogen bonds between the ligand-protein, the more stable the interactions between the ligand-protein that occur (Ferreira *et al*., 2017; Afriza *et al*., 2018). Computational analysis was able to identify sulfated polysaccharide ligands that were able to effectively bind to the SARS-CoV-2 spike protein and have the potential to inhibit the entry of the virus into host cells (Douma *et al*., 2021).

Lipinski's rule of five prediction

Lipinski's Rule of Five (RO5) is a parameter that is able to predict the suitability of a compound for its use as an oral drug. The parameters used to predict physicochemical properties are molecular weight (BM), logarithm of the octanol/water partition coefficient (Log P), hydrogen bond acceptors (HBA), and hydrogen bond donors (HBD) which are based on Lipinski's Rule of Five. Predictions of physicochemical properties are determined by looking at parameter values that violate no more than one rule. The results of screening for the physicochemical properties of sulfated polysaccharide derivative compounds can be seen in Table 4.

Table 3. Amino acid residues of ligand-protein interactions in spike glycoprotein

Bioactive Compound	Conventional Hydrogen Bond	Carbon Hydrogen Bond	Pi-Alkyl	Pi-Pi Stacked	Alkyl
Ligan Native	ARG B:346 VAL B:341 ASN B:354	GLU B:340			
Remedisivir	SER B:514	ASP B:428 GLU B:516			PRO B: 426 PRO B: 463
Furfural			CYS B:432 VAL B:382	TYR B:365	
1H-imidazole			VAL B:382	TYR B:365	
2-furanmethanol	PRO B:384 SER B:383		CYS B:432	TYR B:365	
5-Hydroxymethylfurfural	ALA B:348 ALA B:397 SER B:399	PHE B:347	ALA B:344 VAL B:341		
Hexadecanoic acid	VAL B:362 ASP B:364		TRP B:436 PHE B:374 PHE B:342		VAL B:367 LEU B:368
Heptadecanoic acid	ARG B:355 GLU B:516		PHE B:464 PHE B:429		PRO B:426
2-Heptanol	PHE B:515	SER B:514	PHE B:464 PHE B:429 TYR B: 396		PRO B:426

Table 4. Lipinski Test Results

Bioactive Compound	Molecular Mass	LogP	Hydrogen Bond Donor	Hydrogen Bond Acceptor	Molar Refractivity
Furfural	96.000000	1.092100	0	2	24.095499
1H-imidazole	68.000000	0.409700	1	1	18.587698
2-furanmethanol	98.000000	0.771900	1	2	24.630796
5-Hydroxymethylfurfural	126.000000	0.584400	1	3	30.018295
Hexadecanoic acid	256.000000	5.552299	1	2	77.947777
Heptadecanoic acid	270.000000	5.942400	1	2	82.564774
2-Heptanol	116.000000	1.947500	1	1	35.822788

The screening results indicated that all tested compounds, namely furfural, 1H-imidazole, 2-furanmethanol, 5-hydroxymethylfurfural, hexadecanoid acid, heptadecanoic acid, and 2-heptanol, adhered to the Lipinski's Rule of Five. A potential drug that complies with Lipinski's Rule of Five is likely to exhibit clinical activity when administered orally due to its favorable absorption and permeability. The measured molecular weights of all compounds were below 500,000 Daltons, suggesting that these compounds can pass through cellular membranes when consumed, enabling their pharmacological potential. The Log P values, representing solubility coefficients in water and lipids, ranged from -0.4 to 5. However, hexadecanoid acid and heptadecanoic acid exceeded the acceptable coefficient range. A higher Log P value indicates greater hydrophobicity, which can increase the toxicity of a molecule due to prolonged retention in the lipid bilayer and widespread distribution in the body. This may reduce binding selectivity to the intended biological target. Conversely, a more negative Log P value implies lower membrane permeability. Additionally, the other rules state that the number of hydrogen bond donors should not exceed five, and the number of hydrogen bond acceptors should not exceed ten. All tested compounds complied with the hydrogen bond donor and acceptor rules. Hydrogen bond donors are hydrogen atoms attached to highly electronegative atoms, and compounds with more than five donors tend to have difficulty penetrating cell membranes, leading to low bioavailability. Hydrogen bond acceptors are atoms capable of accepting hydrogen bonds from donors. Compounds with more than ten hydrogen bond acceptors typically exhibit poor solubility in lipophilic membranes, making them difficult to absorb orally. Lipinski's Rule of Five specifies a molar refractivity range of 40–130. Among the tested compounds, only hexadecanoid acid and heptadecanoic acid fell within this range. Molar refractivity values that are too low or too high can influence bioavailability and hinder drug absorption. Conversely, optimal molar refractivity enhances interactions with biological targets (Jayaram *et al.*, 2012; El *et al.,* 2016; Padmi *et al.,* 2022). The seven compounds derived from green seaweed each violated one aspect of Lipinski's rules. Nonetheless, all compounds demonstrated potential as orally administered therapeutic agents with antiviral activity.

Conclusion

Based on morphological identification, the green seaweed from Kemujan, Karimunjawa village, is grouped into the Kappaphycus genus. Molecular docking studies of sulfated polysaccharide compounds produced by the seaweed genus Kappaphycus against SARS-CoV-2 spike glycoprotein (6LZG) showed the lowest binding energy of -5.3 kcal.mol^{-1} of the hexadecanoid acid compound. In addition, the hexadecanoid acid compound has the highest number of hydrogen bonding and hydrophobic interactions compared to other test compounds as well as native ligands and the positive control remdesivir. The prediction of the physicochemical properties of all test compounds fulfills Lipinski's Rule of Five which can be interpreted as having potential for use as a medicinal compound.

Acknowledgment

This research was funded by DRTPM year 2024 in PDD program number SPK: 601-07/UN7.D2/PP/VI/2024 and contract number: 047/E5/PG.02.00.PL/2024 which is gratefully acknowledged.

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