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ANTIVIRAL POTENCY OF GLYCYRRHIZIN AND GLYCYRRHETIC ACID AGAINST SARS-COV-2 INFECTION OF THE NON-STRUCTURAL PROTEIN 3, MAIN PROTEASE, AND SPIKE GLYCOPROTEIN RECEPTORS

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Abstract

The efforts to develop antiviral for Covid-19 are still being conducted. One of them is the exploration of the antiviral potency of the compounds found in licorice namely glycyrrhizin and glycyrrhetic acid. They are used and developed further using molecular docking. The research aims to predict the antiviral potency of glycyrrhizin and glycyrrhetic acid against SARS-CoV 2 infection. The researcher employs SwissADME Tool in testing Lipinski's rule of five fulfillments to predict their physicochemistry. To get an optimum result, the researcher prepares the compounds and receptors. The receptors are validated with RMSD value <2Å, namely Non-Structural Protein 3 (6VXS), Main Protease (6W63), and Spike Glycoprotein (6VSB). The result shows that glycyrrhetic acid fulfills Lipinski's rule of five, in contrast with glycyrrhizin. Both compounds have good energy affinity and inhibition constant with the best value -13.6 kcal/mol and 0.000104 µM on the receptor 6W63. The interaction between Glycyrrhizin and amino acid residue is found in the interaction with the 6VXS, 6W63, and 6VSB receptors. Meanwhile, glycyrrhetic acid compound only interacts with the receptor 6VXS. The interaction between glycyrrhizin and glycyrrhetic acid and its target protein has antiviral activity potency against SARS-CoV 2 infection.

Keywords: Glycyrrhizin, Glycyrrhetic Acid, Non-Structural Protein 3, Main Protease, Spike Glycoprotein

Introduction

Severe acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) is a disease that causes acute respiratory syndrome until death. The use of vaccines is still limited in the society, several studies of development are required. Another approach is used to find new compounds that are effective in dealing with Covid-19. One of them is the computational method. The choice of this method is due to the fast and efficient data collection process with accountable results. This method is beneficial in the discovery of new drugs using computational device or also known as in silico. This molecular docking computation method is used to predict the interaction between ligands and proteins at the binding site (Monika *et al*,. 2010).

The selection of compounds for molecular docking is based on their antiviral activity, in this study using compounds with antiviral activity found in Glycyrrhiza glabra. Several studies have

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shown that the main components of the active triterpenoid saponin compounds located in the roots of the Glycyrrhiza glabra plant, namely Glycyrrhizin (GL) and Glycyrrhetic Acid (GA) with concentrations ranging from 6 -10%, have an important role, especially in their antiviral activity (Wang *et al*., 2015). In previous studies, it was found that Glycyrrhizin has an energy affinity for the ACE 2 receptor (PDB ID: IRP4L) and the Main Protease receptor (PDB ID: 6LU7) (Zhang *et al*., 2020).

In this study, molecular modeling was carried out using three different target receptors, including Non-Structural Protein 3 (PDB ID 6VXS), Main protease (PDB ID 6W63), and Spike glycoprotein (PDB ID: 6VSB). This research is very important to do as an antiviral development process for Covid-19 so that extracting the potential of licorice as an antiviral can be further utilized and developed after the molecular docking process is carried out. This study was different from the previous one by comparing the glycyrrhizin compound and glycyrrhetic acid as SARS-CoV 2 antiviral from licorice plants using different receptor variations, so that further research was carried out to find out how much potential it has in dealing with the SARS-CoV 2 virus.

Material and Methods

The device used is a laptop with Intel Core i3-6006U specifications with the Windows 10 operating system, 12GB RAM, 256GB SSD. The software used in this study is AutoDock Tools 1.5.6 ©, PyMol2.4 ©, LigPlot 4.5.3 ©, and Online SWISS ADME Tool ©

Ligand Preparation

The structure for the licorice ligand (Glycyrrhiza glabra) was obtained from https://pubchem.ncbi.nlm.nih.gov/ with the compound used Glycyrrhizin (PubChem CID: 34915), and glycyrrhetic acid (PubChem CID: 10114) with three comparative drugs Ritonavir (PubChem CID: 392622), Lopinavir (PubChem CID: 192725), Nelfinavir (PubChem CID 64143) then performed ligand preparation with AutoDock tools 1.4.2 with .pdbqt format.

Receptor Preparation

Receptors on SARS-CoV 2 were obtained from https://www.rcsb.org/ in PDB format with three tethering targets, namely Non-Structural Protein 3 (PDB ID: 6VXS), Main Protease (PDB ID: 6W63), and Spike Glycoprotein (PDB ID: 6VSB). Receptor preparation stages are carried out by removing water molecules, adding polar hydrogen atoms, and giving Kollman charges using MGL Tools software (Rizvi *et al*., 2013). The active site determination is obtained from the receptor binding with the native ligand based on the results of the grid box value determination (Gurung *et al*., 2016). The results are visualized in Table 1.

Receptor Validation

The receptor validation was replicated three times with the software and at the same time by looking at the position of the native ligand redocking results on each receptor target with the parameter RMSD value <2 Å. The smaller value indicates the position of the ligand results from redocking approaching the crystallographic ligand (Ramírez & Caballero, 2018).

Ligand-Protein Docking

Molecular docking was carried out in silico using AutoDock Vina software on two test compounds, Glycyrrhizin and glycyrrhetic acid, three comparison drugs ritonavir, lopinavir, and nelfinavir at three target receptors 6VXS, 6W63, and 6VSB. The molecular Docking data input process is done with the Command Prompt. PyMOL and LigPlot are used to visualize the three-dimensional and two-dimensional interactions between compounds and the target receptors on SARS-CoV 2.

Physicochemical Properties and Toxicity of Compounds

Determination of physicochemical properties using the Lipinski rule of five to see the prediction of oral bioavailability of drugs in the human body with Lipinski parameters in the form of molecular mass <500 g / mol, log P <5, Hydrogen Bond donor (HBD) <5 and Hydrogen Bond Acceptors (HBA) < 10. Predictions are carried out using the SWISSADME online tools http://www.swissadme.ch/index.php. The following process predicts the toxicity of compounds using the OSIRIS online tools (http://www.organicchemistry.org/prog/peo/) and https://tox-new.charite.de/protox_II. Toxicity analysis was performed to detect the risk of mutagenicity, Tumorigenic, irritation, hepatotoxicity, LD50, and toxicity class.

Table 1. Grid Box Size Dimensions

Macromolecule Target	Native Ligand	Grid Box Dimensions		
		No of grid points	Center XYZ	Grid Point Spacing
Non-Structural Protein 3 PDB ID: 6VXS	2-[n-cyclohexylamine]ethane Sulfonic Acid)	12x14x14	13.022 -6.691 -3.905	0.375
Main Protease PDB ID: 6W63	N-(4-tert-butylphenyl)-N-[(1R)-2-(cyclohexylamino)-2-oxo-1-(pyridin-3-yl)ethyl]-1H imidazole-4-carboxamide	20x16x22	-19.34 -18.376 -27.277	0.375
Spike Glycoprotein PDB ID: 6VSB	2-acetamido-2-deoxy-beta-D-glucopyranose	14x16x16	186.772 232.005 279.557	0.375

Result

Validation of Receptor

One of the receptor validation methods is the score function, and this method uses pose selection results from the redocking native ligand of each receptor on the active target site, which is represented by the value of Root Mean Square Deviation (RMSD) (Qi *et al*., 2020 ; Hevener, 2005). The results of receptor validation are shown in Figure 1. Based on the results of the docking validation, it was found that the three native ligands at each receptor had an RMSD value <2 Å.

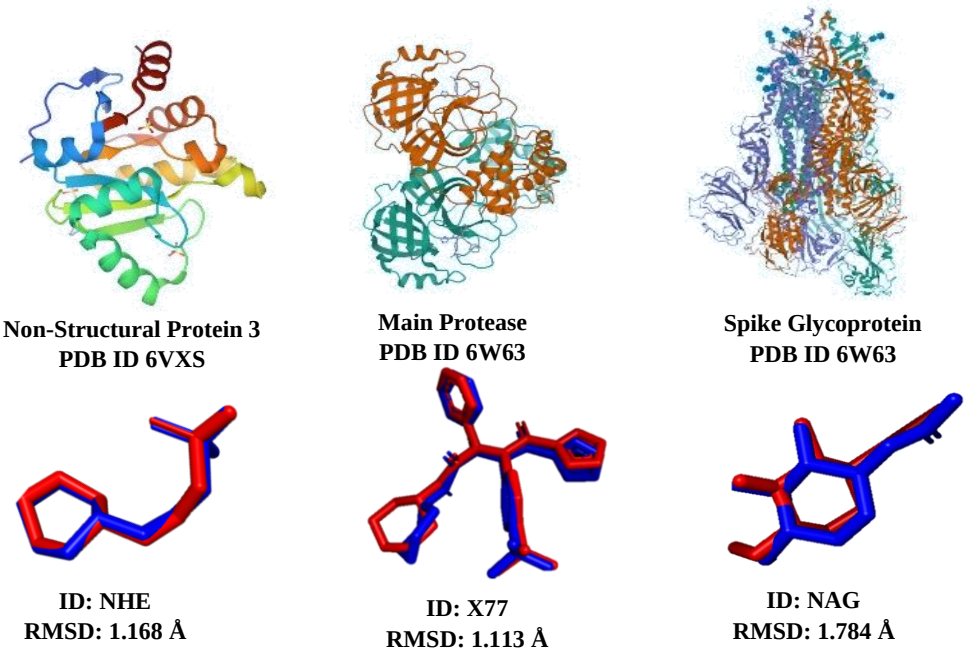

Figure 1. Receptor validation results. The RMSD value is obtained from the docking between the native ligand (Red) and the docked pose on the native ligand (Blue)

Ligand Protein Docking

The results of molecular docking in Table 2 show that Glycyrrhizin showed the lowest energy affinity value at all receptors compared to native ligands, glycyrrhetic acid compounds, and comparison drugs with the lowest energy affinity values shown at the main protease receptor 6W63. The result of Constant Inhibition is derived from the energy affinity value using the formula Ki = exp (ΔG / RT), the value of R is the ideal gas (1,985 × 10^{-3} kcal $mol^{-1}K^{-1}$), and the T value is the temperature (298.15K˚).

Table 2. Molecular docking results with AutoDock Vina

No	Compounds	Energy Affinity (kcal/mol)			Constant Inhibition (μM)		
		6VXS	6W63	6VSB	6VXS	6W63	6VSB
1.	Native ligand	-3.4	-8.3	-3.9	3199.01	0.79	1374.38
2.	Glycyrrhizin	-9.3	-13.6	-7.6	0.15	0.000104	2.648
3	Glycyrrhetic Acid	-8.4	-12.2	-7.2	0.69	0.0011	5.206
4	Ritonavir	-7.8	-	-	1.889	-	-
5	Lopinavir	-	-10.4	-	-	0.023	-
6	Nelfinavir	-	-	6.5	-	-	16.989

This form of binding to amino acid residues occurs at the pocket binding affinity of the active sites at all three receptors. The results of the interaction visualization of amino acid residues were visualized in two dimensions using LigPlot and three dimensions using PyMOL, interactions that can be visualized in the form of hydrogen bonds and hydrophobic interactions. The results of the interaction of amino acid residues can be seen in Figure 2.

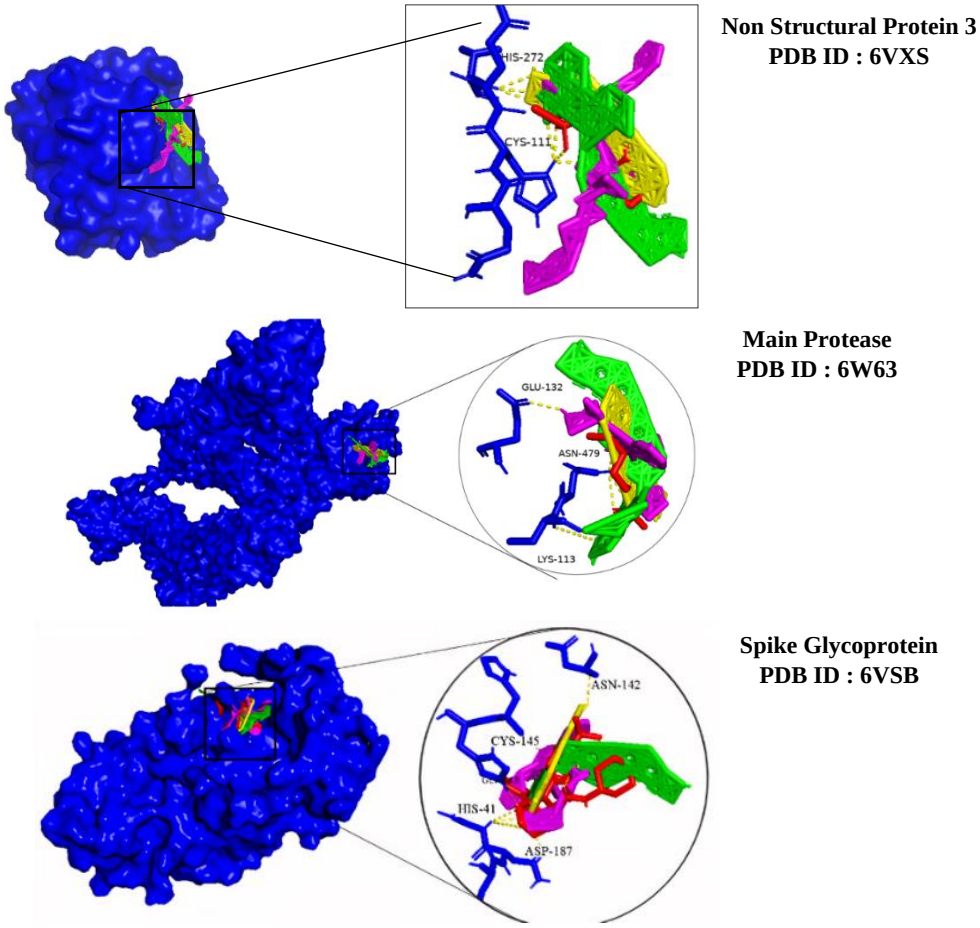

Figure 2. Hydrogen interactions of the compound glycyrrhizin (Green), glycyrrhetic acid (yellow), native ligand (red), and comparison drug (purple) with three receptors

The interaction of ligands and receptors is a representation of the molecular docking process. This form of binding to amino acid residues occurs at the pocket binding affinity of an active site on the NSP3 receptor with PDB ID: 6VXS, main protease with PDB ID: 6W63, and spike glycoprotein with PDB ID: 6VSB.

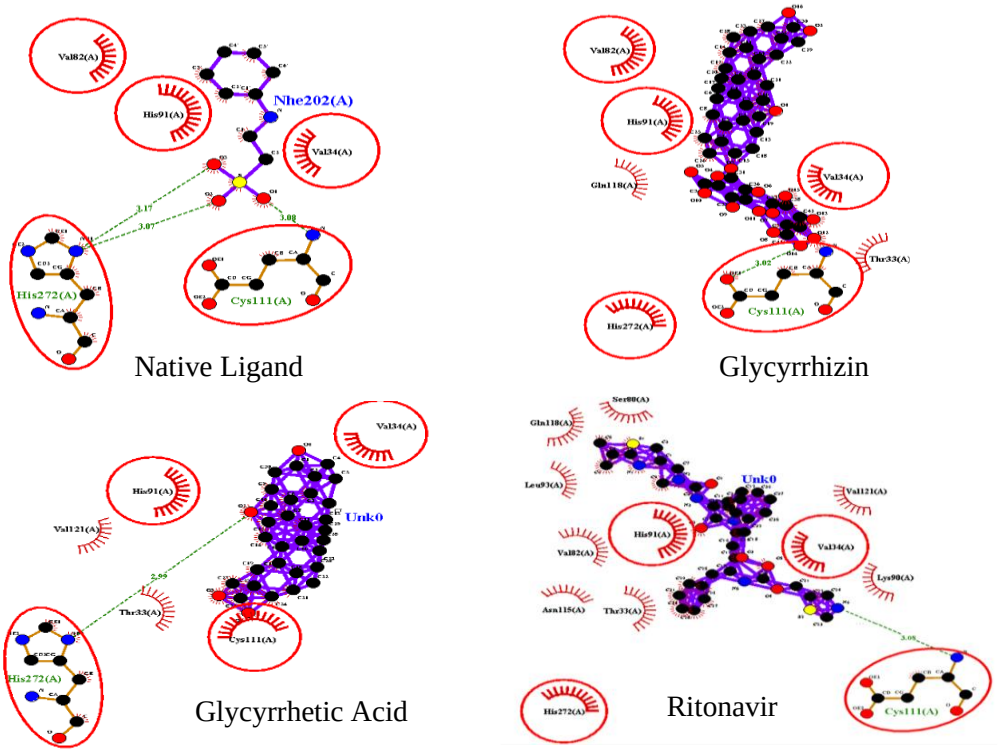

Figure 3. Interaction of ligand amino acid residues and non-structural protein 3 receptors with PDB ID: 6VXS in 2 dimensions

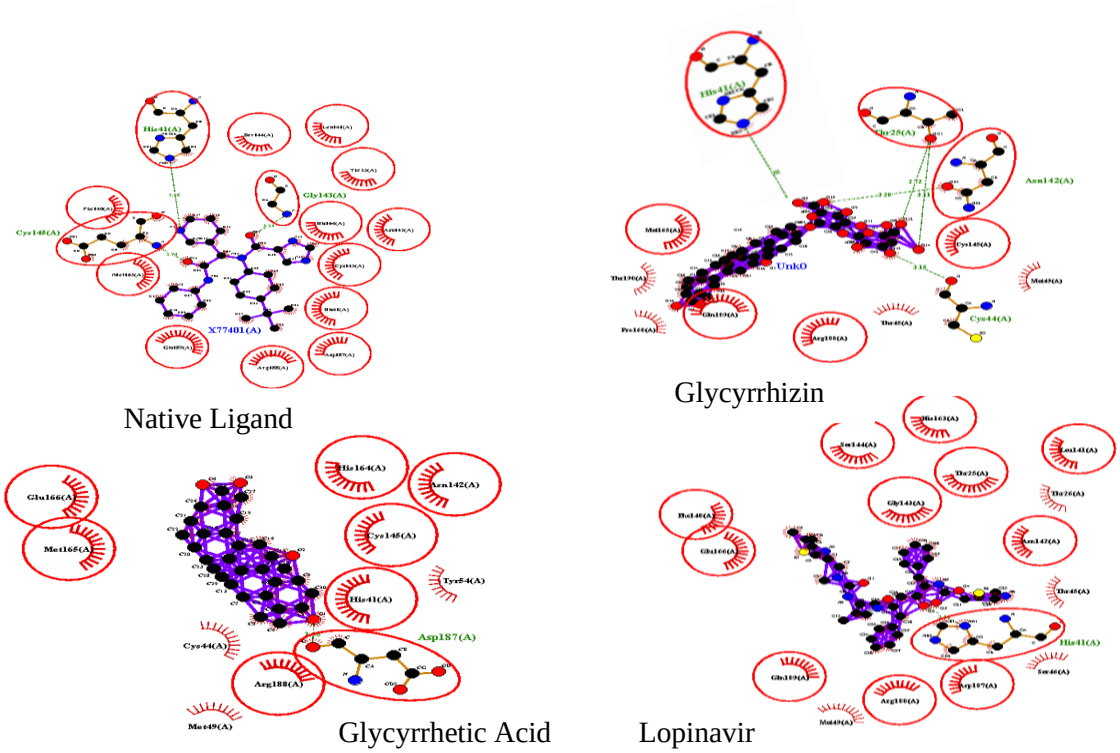

Figure 4. Interaction of ligand amino acid residues and non-structural protein 3 receptors with PDB ID: 6W63 in 2 dimensions

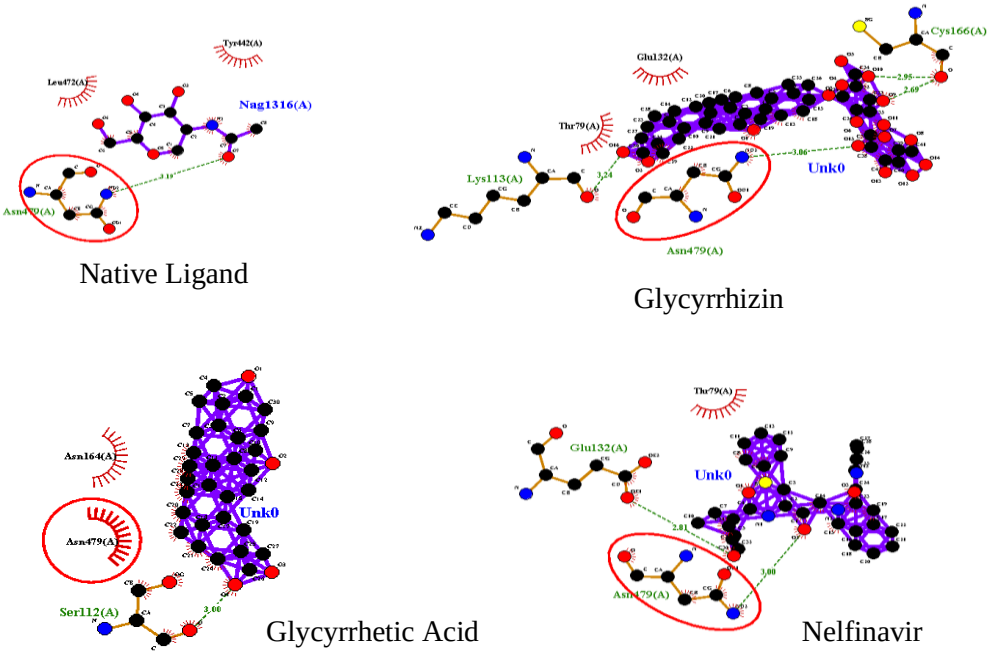

Figure 5. Interaction of ligand amino acid residues and non-structural protein 3 receptors with PDB ID: 6VXS in 2 dimensions

Physicochemical properties and toxicity of compounds

The results of the physicochemical prediction show that the glyceric acid compounds comply with the Lipinski rule of five while the glycyrrhizin compounds do not comply with the Lipinski's rule. The results of Glycyrrhizin and Glycyrrhetic acid have some different physicochemical properties. Glycyrrhizin had 3 violation and Glycyrrhetic acid had not violation.

Table 3. The results of physicochemical properties-based Lipinski rule of five

No	Compounds	Physicochemical Properties				Violation
		MW (g/mol) <500	Log P <5	HBD <10	HBA <5	
1	Glycyrrhizin	822.9	1.55	16	8	No
2	Glycyrrhetic Acid	470.68	0.02	2	4	Yes

Toxicity prediction results are shown in Table 4 with four toxicity parameters, namely mutagenic, tumorigenic, irritant, hepatotoxicity, LD_{50}, and toxicity class. There are two parameter using Osiris Tools to presented some parameter of Mutagenic, Tumorigenic, Irritation, and Hepatotoxicity The other parameter using ProTox Online to presented of LD_{50}.

Table 4. Results of toxicity parameters

No	Compounds	Toxicity Parameters				
		Mutagenic	Tumorigenic	Irritation	Hepatotoxicity	LD_{50} (mg/kg)
1	Glycyrrhizin	Low	Low	Low	Low	1750
2	Glycyrrhetic Acid	Low	Low	Low	Low	560

Discussion

SARS-CoV 2 has a variety of structures and genomics that are used as Receptor Binding Domain (RBD) which can be used in obtaining drug candidates that are effective in inhibiting infection with the SARS-CoV 2 Virus (Qi *et al*., 2020). In previous studies, it has been found that Glycyrrhizin has an energy affinity at IRP4L values of -9.6 kcal/mol and 6LU7 values of -9.3 kcal/mol (Zhang *et al*., 2020).

The initial process of data validation is done by looking at the Root Mean Square Deviation (RMSD) value which explains the similarity of the two ligand structures based on the results of the different atomic positions of the same type with a value < 2. The lower RMSD value indicates that the native ligand resulting from redocking is well validated so that it has a similar position with the native ligand resulting from crystallography on the active site (Leong *et al*., 2017). The results of the molecular docking process are known based on the value of energy affinity (ΔG) and inhibition constant (Ki). The energy affinity value is the potential energy to see equilibrium at constant temperature and pressure, the lower the free energy value, the more stable it is. The result of the Constant Inhibition value is directly proportional to the energy affinity (Ortiz *et al*., 2019).

The docking results were seen based on the affinity value for each dock pose, where the lowest energy was the best pose result on the active site. The value of the compound is predicted to have the ability to interact with the target receptor by having the same or lower energy affinity and inhibition constant than the control drug (Pantsar & Poso, 2018). Ligand and receptor interaction results represent the results of the molecular docking process to predict a binding between the ligand and the receptor (Meng, *et al* 2011).

Covid-19 has a structural and genomic complex consisting of single-stranded positive RNA encoding several structural and non-structural proteins (NSPs) including envelope proteins (E), spike protein (S) genes, membrane protein (M) genes, nucleocapsid protein (N) gene, replicase complex (ORF1ab) gene along with 3' and 5'- untranslated region (UTR). In this study, molecular modeling was carried out using three different target receptors, namely Non-Structural Protein NSP3 (PDB ID 6VXS), Main protease (PDB ID 6W63) and Spike glycoprotein (PDB ID: 6VSB) (Mousavizadeh & Ghasemi, 2020).

NSP 3 with PDB receptor code ID: 6VXS is a large multidomain. One of the units is ADP-ribose phosphatase which is predicted to interfere with the immune response in host cells. The antiviral therapy potential of ADP-ribosylation is a reversible modification of the transfer of ADP-ribose from NAD + to specific residues such as lysine, arginine, lysine, asparagine, phosphoserine, aspartate, glutamate, and cysteine (Michalska *et al*., 2020). The interaction with NSP 3, in two hydrogen bonds of amino acid residues of Cys111 and His272 (Figure 4). Both are part of the active site amino acids that interact between ligands and receptors on non-structural protein 3, namely Cys111, His272, and Asp286. This residue exerts an inhibitory effect of SARS CoV 2-PLpro with GRL-0617 on the cytopathogenic destruction induced by the SARS-CoV-2 virus as well as host cell evasion activity (Li *et al*., 2021).

Main Protease with the code 6W63, a broad spectrum dipeptidyl Mpro inhibitor, efficiently blocks the proliferation of SARS-CoV, making it an effective way of treating COVID-19 in humans (Stobart *et al.,* 2013). At the 6W63 receptor, there is a crystallographic ligand on the active site, namely the non-covalent inhibitor X77, so that the target of receptor tethering is easier to obtain (Fiorucci *et al.,* 2020). Interaction on the main protease obtained three hydrogen bonds of amino acid residues, namely Gly143, His41, Cys145. The SARS-CoV and SARS CoV-2 main protease receptors show very similar positions at the two catalytic sites. The Cys-His residue (Cys145 and His41) consists of active catalytic binding sites in SARS-CoV-2 potent in inhibiting the virus replication process (Shin *et al*., 2020; Chitranshi *et al*., 2020).

Spike Glycoprotein is the entrance to the Covid-19 virus. The selection of the receptor with the code PDB ID: 6VSB is because this receptor is a single up domain receptor found in the receptor binding domain, so it is estimated that it can inhibit the spread of the Covid-19 virus. The process of attaching proteins to the host cell utilizes densely glycosylated spikes to enter the host. This process occurs when the S1 subunit binds to the receptor host cell, the receptor binding will become unstable so that the RBD S1 domain undergoes a conformational movement (Wrapp *et al*., 2019). The interaction of the glycoprotein spike receptor, in a hydrogen bond of the amino acid Asn479 residue. This residue is considered part of the amino acid active site that interacts between the ligand and the receptor. RBD S-protein has 70% similarity with SARS-CoV with key

residues (Tyr442, Leu472, Asn479, Asp480, and Thr487). These residues are predicted to play a role in Spike glycoprotein Covid-19 with human ACE2 (Choudhary *et al.*, 2020).

Prediction of physicochemical properties is carried out using Lipinski's five laws in evaluating the bioavailability of a compound so that selectivity can be carried out optimally. Prediction of physicochemical properties are seen based on the Lipinski rule of five to evaluate the bioavailability of oral drugs, whether a compound has good permeability and can penetrate the body's biological membranes. The parameters of the Lipinski rule of five are molecular weight <500 g/mol Log P <5, HBD <10, and HBA <5 with the minimum condition that one deviation violates the Lipinski rule of five (Lipinski *et al*., 2012). Based on BCS (Biopharmaceutical Classification System) Licorice assessment on Glicyrrhizin compounds and glycyrrhetic acids, including in class IV (Chemistry, 2010). BCS is a classification in the form of an experimental model on the measurement and solubility, and permeability of substances under certain conditions in oral drug use. A drug must be having high solubility and permeability requirements. BCS class IV shows the most solubility and permeability. Therefore, the development of this compound requires a more compatible and efficient delivery system with several more feasible and effective approaches to improve and redesign drug formulations concerning carrier, encapsulation, and target systems *(*Kumar *et al*., 2018; Markovic *et al*., 2020).

ADMET is needed to determine the bioavailability of compounds and the risk of compound toxicity, the compound toxicity prediction process can use several software, namely Osiris for testing Mutagenic, Tumorigenic, Irritation, and Hepatotoxicity. toxicity prediction. The bioavailability of indicated low risk of toxicity using the Osiris tool (Kumar *et al*., 2017). In testing using ProTox online, the data obtained showed toxicity indicating that the toxic dose was often given as the LD_{50} value in mg/kg body weight. The LD_{50} is the mean lethal dose which means the dose at which 50% of the test subjects die after exposure to a compound. The representation of LD_{50} values between 500-5000 mg/kg BW is included in low toxicity (Banerjee *et al*., 2018).

Conclusion

Glycyrrhizin and glycyrrhetic acid compounds have better activity than native ligand and comparative drugs at the three receptors, there are Non-Structure Protease 3 (6VXS), Main Protease (6W63), and Spike Glycoprotein (6VSB), so it is thought that these compounds have potential in inhibiting infection. SARS-CoV 2.

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