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Analysis of Molecular Docking and Dynamics Simulation of Mahogany (*Swietenia macrophylla* King) Compounds Against the PLpro Enzyme SARS-COV-2

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Abstract

Background*: Using natural ingredients as antivirals can be considered a treatment for SARS-CoV-2. One of the potential plants, mahogany (Swietenia macrophylla King), is widely used in various countries as an antiviral treatment. Paparin-like protease (PLpro) is an essential cysteine protease that regulates viral replication and interferes with the regulation of immune sensing.* ***Objective:*** *This study aims to predict which compounds in the mahogany plant have good affinity, patterns, and stability interaction against the target protein of SARS-CoV-2.* ***Methods:*** *The drug-likeness parameter using SwissADME was used to screen compounds that will be docked against PLpro using the Autodock program. The parameters observed in molecular docking analysis are the value of bond energy and interaction model to amino acid residues. The compounds in mahogany plants that have the best interactions were then analyzed using molecular dynamics simulation methods to determine the stability of their bonds based on the values of Root Mean Square Deviation (RMSD) and Root Mean Square Fluctuation (RMSF).* ***Results:*** *Twenty-two compounds met the drug-likeness requirements. Molecular docking analysis showed that the compounds predicted to have the best binding affinity and have an interaction pattern similar to natural ligands towards the molecular target of PLpro are 7-deacetoxy-7-oxogedunin and 3β-hydroxy-stigmast-5-en-7-one. The molecular dynamics simulation results revealed that based on the RMSD and RMSF values, the compound 3β-hydroxy-stigmast-5-en-7-one showed higher stability than 7-deacetoxy-7-oxogedunin.* ***Conclusion:*** *3β-hydroxy-stigmast-5-en-7-one and 7-deacetoxy-7-oxogedunin were predicted to have good interaction with PLPro; however, 3β-hydroxy-stigmast-5-en-7-one showed the higher interaction stability.*

Keywords*: antivirus, mahogany, molecular docking, molecular dynamics simulation, SARS-CoV-2*

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INTRODUCTION

Coronavirus disease 2019 (Covid-19) is caused by Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2). This disease emerged in Hubei Province, China (Yu *et al*., 2020). SARS-CoV-2 causes respiratory problems similar to those caused by the Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV) in 2003 and the Middle East Respiratory Syndrome Coronavirus (MERS-CoV) in 2012. All three come from the Coronaviridae, a family of viruses that have an RNA genome with a single positive sense strand (Tu *et al*., 2020). Globally, the weekly number of COVID-19 cases was from October 31 to November 6, 2022, with more than 2.1 million new cases reported. Weekly death toll, with around 9400 deaths reported. As of November 6, 2022, 629 million confirmed cases and 6.5 million deaths have been reported globally (WHO, 2022). According to data from the COVID-19 Handling Task Force, as of November 16, 2022, there were 6 million positive cases and 159 thousand deaths (Satgas Penanganan Covid-19, 2022).

Several treatment approaches have been made to inhibit SARS-CoV-2. Remdesivir can inhibit SARS-CoV-2 infection, as can nafamostat, which is a MERS-CoV inhibitor, which can prevent membrane fusion and inhibit SARS-CoV-2 infection (Wang *et al*., 2020). The re-use of drugs, including antiviral agents (ivermectin, nitazoxanide, lopinavir, remdesivir, tocilizumab), supporting agents (azithromycin, corticosteroids, vitamin C, vitamin D), and vaccines, is being tried to meet the urgent demand against the COVID-19 pandemic (Chen *et al*., 2022).

Apart from using synthetic antivirals, the use of natural ingredients as antivirals can also be considered as a treatment for SARS-CoV-2. A number of active compounds from natural products have shown potential antiviral activity (Septiana, 2020). One of them worthy of research is the mahogany plant (*Swietenia macrophylla* King). Mahogany belongs to the Meliaceae family. Mahogany is widely used in various countries as a treatment for antivirals. Toona sinensis leaves, which are also included in the Meliaceae family, have antiviral effects against SARS-CoV (Petrera, 2015). There are various compounds in mahogany, such as polyphenols, fatty acid esters, essential oils, steroids, lignans and limonoids (Moghadamtousi *et al*., 2013). In general, polyphenols are believed to have various uses, such as antioxidants, anti-inflammatories, antivirals, and antibacterials (Mulu *et al*., 2021). Limonoids, as triterpenoid derivatives, have activity as antiviral, antifungal, antibacterial, anticancer, and antimalarial (Vardhan & Sahoo, 2020). As an antiviral, 3-hydroxy caruilignan C (3-HCL-C) isolated from *S. macrophylla* stems causes a decrease in protein and RNA levels, thereby interfering with hepatitis C virus replication (Musarra-Pizzo *et al*., 2021). From various databases and references, 22 compounds contained in the mahogany plant have been collected. However, it is not yet known how the antiviral mechanism action of the mahogany plant. Studies are needed to determine which of these compounds play a role in antiviral activity and the target proteins of these compounds.

Drug development efforts can be carried out by molecular modelling or in silico tests, which play a role in designing, discovering, and optimizing bioactive compounds in the drug development process. The in silico test can be carried out by means of molecular docking, which functions to predict the activity of a compound in target cells. The docking will align the ligand into the target cell and produce a bond energy value indicating the amount of energy required to form the bond between the ligand and the receptor. The lower the bond energy, the stronger the bond. The stronger the link between the ligand and the receptor, the more active it is. (Kesuma *et al*., 2018). It is now known that there are many potential targets for anti-SARS-CoV-2 work, one of which is PLpro (paparin-like protease), which functions to split polyprotein replication into non-structural proteins (Chen *et al*., 2022). After carrying out molecular docking, proceed with Molecular Dynamics (MD) Simulations. The MD simulation aims to determine the stability of the ligand-receptor interaction; this is done because molecular docking has not been able to provide information regarding the stability of the ligand-receptor interaction in space and time (Dewi *et al*., 2022).

This study will carry out an analysis of the molecular docking of compounds in mahogany plants that have been screened for drug-likeness as test ligands for the protein PLpro of the SARS-CoV-2 virus. Molecular docking was carried out using Autodock 4 software, the MGL tool, and Biovia Discovery Studio as visualization tools. Ligand-protein interactions and the best pattern of amino acid residues will be followed by MD simulation testing using Yasara software so that the stability value of the ligand-protein bond is obtained.

MATERIALS AND METHODS

Materials

The material used in this study was the SMILES code of the test ligand obtained from PubChem, which was made in a three-dimensional structure using the

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VEGA ZZ application. The three-dimensional structure of the macromolecule, namely PLpro (PDB ID: 7QCG), was downloaded from the Protein Data Bank (RCSB PDB).

Tools

The tools used in this study were a set of TOSHIBA Dynabook B35/Y with Intel(R) Core(TM) i5-5200U Processor specifications, 8.0 Giga Byte RAM, 500 Giga Byte SSD hard disk, Intel(R) HD Graphics Graphics Card 5500, AutoDock 4.0, AutoDockTool, Biovia Discovery Studio, VEGA ZZ, Notepad++, SwissADME (http://www.swissadme.ch/), and YASARA Dynamics.

Method

Test ligand screening

The drug-likeness parameter of the mahogany plant compound was conducted using the SwissADME webserver (http://www.swissadme.ch/) to screen the compounds before the molecular docking analysis process. Canonical SMILES of mahogany plant compounds were obtained from PubChem. The Canonical SMILES code was copied and pasted into the "Enter a list of SMILES here" box, and the "Run!" button was clicked. The drug-likeness profile of the compound was carried out to determine whether a compound meets the requirements as an oral drug candidate or not (Daina *et al*., 2017).

Preparation of the three-dimensional structure of the test ligand

The three-dimensional structure of the test ligands was created using the VEGA ZZ application. The first step was to copy the Canonical SMILES test ligand from PubChem and then paste it into Vega ZZ by selecting the Edit > Build > SMILES menu. The SMILES code obtained from PubChem was then pasted into the dialogue box that appears, then clicked Build. After that, select the Calculate > Charge & Potential menu, and a dialogue box will appear. In the Force Field section, select "AUTODOCK", and in the Charges section, select "Gasteiger", then click "Fix". Then, minimize it by choosing the Calculate > Ammp > Minimization menu. In the Minimization Steps box, enter 10,000 and then run. The three-dimensional structure obtained is then saved in the.pdb format for molecular anchoring; if it is still in the form of a two-dimensional structure, it must first be converted into a three-dimensional structure by clicking Edit > Coordinates > Convert to 3D.

Test ligand preparation

The preparation of the test ligand structures was carried out using AutoDockTools to add hydrogen atoms and charges to the ligands. First, open the three-dimensional structure of the test ligand by selecting the File menu > Read molecule, then selecting Edit > Hydrogens > Add menu. After that, select "All Hydrogens", "noBondOrder", and "Yes" in the Add Hydrogens dialogue box that will appear. Then select the Edit menu again > Charges > Compute Gasteiger. The next step is to create a ligand file in the pdbqt format by selecting the Ligand > Input > Choose menu, selecting the prepared ligand file, and then selecting "Select Molecule for AutoDock4". Then select the Ligand menu > Torsion tree > Detect root to identify the ligand root. After that, select the Ligand > Torsion tree > Choose Root menu, then select the Ligand > Torsion tree > Choose Torsions > Done menu to identify the number of the torque. Then select Ligand > Torsion tree > Set Number of Torsions > Dismiss. Then select the Ligand menu > Output > Save as file with the pdbqt format.

Macromolecules download

The 3D structure of the receptor used, the PLpro protein (PDB ID: 7QCG) was downloaded from the RCSB PDB website (https://www.rcsb.org/). The 3D structure of the protein is downloaded in ".pdb" file format. The file is then saved in the work folder.

Macromolecular preparation

Macromolecule preparation was carried out using Biovia Discovery Studio. First of all, open the macromolecule file by selecting File > Open from the menu. Then pressed CTRL+H simultaneously so that the protein molecules appeared. Clicked Water > Delete to remove the water molecules. The next step is to separate the macromolecular complex into individual protein (receptor) and ligand files by clicking on Ligand Groups > selecting native ligand > Copy > Paste in the new Molecule Window, and then saving as (ligand name).pdb, the resulting ligand file is obtained. separated from the protein. After that, return to the initial molecule window and select Ligand Group > Delete, to remove natural ligands. Then select Hetatm > Delete to remove other residues (if any).

Then, the protein files that have been separated from their natural ligands are added hydrogen and cargo using AutoDockTools by selecting the menu File > Read Molecule > Edit > Hydrogens > Add. After that, select "Polar Only", "noBondOrder", and "Yes" in the Add Hydrogens dialogue box that will appear. Then select the Edit menu again > Charges > Add Kollman Charges. The next step is to create a protein file with the .pdbqt format, which will be used to determine the grid parameters, by selecting the Grid > Macromolecule > Choose menu, selecting the protein file that has been

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prepared, then selecting "Ok" and save the file with the format name .pdbqt.

Furthermore, the preparation of the native ligand structure first opens the three-dimensional structure file of the native ligand, which has been separated from the protein by selecting the File > Read molecule menu, then selecting Edit > Hydrogens > Add menu. After that, select "All Hydrogens", "noBondOrder", and "Yes" in the Add Hydrogens dialogue box that will appear. Then select the Edit menu again > Charges > Compute Gasteiger. The next step is to create a ligand file in the .pdbqt format by selecting the Ligand > Input > Choose menu, selecting the prepared ligand file, and then selecting "Select Molecule for AutoDock4". Then select the Ligand menu > Torsion tree > Detect root to identify the ligand root. After that, select the Ligand > Torsion tree > Choose Root menu, then select the Ligand > Torsion tree > Choose Torsions > Done menu to identify the number of the torque. Then select Ligand > Torsion tree > Set Number of Torsions > Dismiss. Then select the Ligand menu > Output > Save as file with the .pdbqt format. The .pdbqt files of macromolecules and ligands, the autodock4.exe and autogrid4.exe application files, and the AD4.1_bound.dat files (obtained from https://autodocksuite.scripps.edu/force-fields/) are placed in the same folder.

Molecular tethering method validation

In this validation process, we will compare the conformation of the natural ligand to the receptor in the experimental crystallographic structure with the conformation of the natural ligand that is redocked to the receptor using AutoDockTools by setting the Grid box x, y, z, centre x, y, z, spacing by default. The results of this comparison are expressed by the root mean square deviation (RMSD) value. The docking method is said to be valid if the RMSD value is $\leqslant$ 2Å. If the RMSD value obtained is greater than 2 Å, then the procedure used is invalid, so the Grid box x, y, z, and centre x, y, z spacing values are adjusted manually until RMSD $\leqslant$ 2Å is obtained.

Molecular docking process

The molecular docking process was carried out using AutoDock4.0 (AD4.0) and AutoDockTools (ADT). Protein and ligand structures that have been optimized separately are stored in the same folder. Before carrying out the docking process, a grid parameter file is first prepared with the following steps: select Grid > Macromolecule > Open: protein file format .pdbqt. Then select the menu Grid > Set Map Types > Open: ligand file (native ligand during the Validation process) format .pdbqt. Then select the Grid > Grid box menu, then select the Center > Center on ligand menu (for Default validation) and set the size x, y, z, centre x, y, z, and spacing in the Grid Options dialogue box that will appear (Following the Grid value Validation box for test ligands). Then select the File menu > Close saving current, then select the Grid > Output > Save GPF menu and save it in the .gpf format. You need to pay attention to naming the file because wrong naming will not make docking work. The next step is to run Autogrid by clicking the Run menu > Run AutoGrid, then on "Program Pathname" select the file "autogrid4.exe" while on "Parameter Filename" select the file with the ".gpf" format earlier, then click "Launch" and wait for the process walk to finish. After the autogrid process is complete, the next step is to prepare a docking parameter file with the following steps, selecting the Docking > Macromolecule > Set Rigid Filename menu and selecting a protein file with the .pdbqt format. Then select the Docking menu > Ligand > Choose > Select the ligand > Select Ligand > Accept. Next, determine the docking parameters by selecting the Docking menu > Search Parameters > Genetic Algorithm, setting the Number of GA Runs to 100, and setting the Population Size to 150 in the dialogue box that will appear, then clicking Accept. After that, select the Docking menu > Docking Parameters > Accept. Then select the Docking menu > Other Options > AutoDock4.2 Parameters > a Set Autodock4.2 Options box will appear > in the Include Parameter_file in dpf click "Yes" > in the Enter Parameter_File section, it is written "AD4.1_bound.dat". Then select the Docking > Output > Lamarckian GA menu and save it in the .dpf format. The next step is to run Autodock by clicking the Run menu > Run AutoDock, then on "Program Pathname" select the file "autodock4.exe" while on "Parameter Filename" select the file with the ".dpf" format earlier, then click Launch and wait for the process to run until finished. The docking results obtained were then analyzed and visualized using the Biovia Discovery Studio.

Molecular dynamics simulation process

The two best test ligands that have the smallest bond-free energy values and amino acid residues that are similar to the native ligands (If a compound has the best or smallest bond-free energy values but does not interact with amino acid residues that are similar to the native ligand, then the compound has the best interaction pattern and cannot be said to have the same activity as

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natural ligands) are followed by MD simulation tests. The MD simulation process is carried out using YASARA Dynamics. The structures of the test-protein ligand complex and the native-protein ligand complex are placed in the same folder. Before running the program, set the script to "md_run.mcr" with 0.9% NaCl, pH 7.4, at a temperature of 298K, and the simulation duration is 20 ns and uses ForceField AMBER14 (Parihar *et al*., 2022; Shree et al., 2022). After that, proceed with running the YASARA program. Then select the menu Options > Macro & Movie > Set Target (select the target you want to analyze in .pdb format). Next, re-select the menu Options > Macro & Movie > Play Macro > md_run.mcr > OK (pre-set script) and wait for the process to run until it finishes automatically. After the process is complete, an analysis of the ligand-protein is carried out by selecting the Options menu > Macro & Movie > Set Target (selecting the target to be analyzed in .pdb format). Then select Option > Macro & Movie > Play Macro > md_analyze.mcr > OK, wait for the process to run until it's finished. The data obtained were analyzed for RMSD and RMSF values.

RESULTS AND DISCUSSION

Test ligand screening

Drug-likeness screening qualitatively assesses the possibility of a molecule becoming an oral drug in terms of bioavailability using the SwissADME website. At SwissADME, there are five Drug-likeness filters, namely Lipinski, Ghose, Veber, Egan, and Muegge. In this study, the Lipinski filter was chosen because Lipinski analyzed 2,245 drugs from the World Drugs Index database, and this filter is known as Lipinski's rule-of-five (Lipinski *et al*., 1997).

Based on Table 1, it can be seen that there were 22 compounds tested for drug-likeness that were predicted to have the opportunity to become oral drugs, although of the 22 compounds, there were some that violated or did not meet the requirements. However, this is tolerable because each compound only violates one rule. According to Lipinski's rules, in general, a drug can be administered in oral if it does not violate more than one criterion (Lipinski *et al*., 1997). Drug-likeness is based on oral drugs because oral administration of drugs is one of the most commonly used methods in clinical practice. Oral medications can be taken easily by patients, do not require special medical assistance, and usually provide greater convenience compared to other routes of administration (Santos *et al*., 2016).

Preparation of three-dimensional structures and preparation of test ligands and macromolecules

The canonical SMILES code of 22 compounds in mahogany obtained from Pubchem was then made into a three-dimensional structure using the VegaZZ application. The three-dimensional structure of the target protein was obtained from the Protein Data Bank with a PDB code and 7QCG 1.75 Å (PLpro) resolution. This macromolecule meets the criteria, namely having a three-dimensional structure obtained from crystallographic X-ray results with a resolution of < 3 Å (Mukherjee *et al*., 2010; Sándor *et al*., 2010). The macromolecule used is already complex with its natural ligands, so it is easy to determine the active side of the macromolecule.

Molecular docking method validation

Method validation was carried out to determine whether the molecular docking method used was reliable or valid by comparing the crystallographic conformation of the natural ligand and the natural ligand that was reddocked against the target protein using AutodockTools. The validation process needs to determine the grid box or central coordinates, where the interaction of the ligand and protein is known as the active site of the protein. The centre of the grid box is generally determined based on the centre of mass of the naturally occurring ligand, while the dimensions of the grid box are based on the size of the ligand and the binding site on the protein that contains the essential amino acids for the protein's activity. The centre and gridbox dimensions of the macromolecules used in this study can be seen in Table 2. Parameters observed in the validation process were RMSD values and interactions that occurred between the crystallographic ligands and the redocked ligands with residues of target protein amino acids. The smaller the RMSD value, the closer the ligand position is to the natural ligand conformation. An RMSD value < 2 Å indicates that the error of the calculation results is smaller, so that the calculation can be said to be more accurate, whereas an RMSD value > 2 Å indicates that the deviation from the calculation results is more remarkable so that the docking results obtained cannot be used as a reference (Mukherjee *et al*., 2010; Sándor *et al*., 2010).

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Table 1. Drug-likeness prediction results (Moghadamtousi *et al*., 2013) & (http://ijah.apps.cs.ipb.ac.id/)

Compound	Lipinski Rules Parameter				Results
	Molecular weight ≤ 500	MLOGP ≤ 4,15	N or O ≤ 10	NH or OH ≤ 5	
Secomahoganin	×	✓	✓	✓	*Yes*
12α-Acetoxyswietephragmin D	×	✓	×	✓	*No*
12α-Acetoxyswietephragmin C	×	✓	×	✓	*No*
Swietenialide D	×	✓	×	✓	*No*
7-deacetoxy-7-oxogedunin	✓	✓	✓	✓	*Yes*
6-O-Acetylswietephragmin E	×	✓	×	✓	*No*
Swietenitin A	×	✓	×	✓	*No*
Swietenitin B	×	✓	×	✓	*No*
Swietenitin C	×	✓	×	✓	*No*
Swietenitin D	×	✓	×	✓	*No*
Swietenitin E	×	✓	×	✓	*No*
Swietenitin F	×	✓	×	✓	*No*
Swietenitin G	×	✓	×	✓	*No*
Swietenitin H	×	✓	×	✓	*No*
Swietenitin I	×	✓	×	✓	*No*
Swietenitin J	×	✓	×	✓	*No*
Swietenitin K	×	✓	×	✓	*No*
Swietenitin L	×	✓	×	✓	*No*
Swietenitin M	×	✓	×	✓	*No*
Swietenolide	✓	✓	✓	✓	*Yes*
Swietenine	×	✓	✓	✓	*Yes*
Swietenolide diacetate	×	✓	✓	✓	*Yes*
3β,6-Dihydroxydihydrocarapin	✓	✓	✓	✓	*Yes*
Augustineolide	×	✓	×	✓	*No*
Andirobin	✓	✓	✓	✓	*Yes*
Proceranolide	✓	✓	✓	✓	*Yes*
3,6-O,O-diacetylswietenolide	×	✓	✓	✓	*Yes*
Swietenolide monohydrate	×	✓	✓	✓	*Yes*
Swietemacrophine	×	✓	×	✓	*No*
1-O-Acetylkhayanolide B	×	✓	×	✓	*No*
Epoxyfebrinin B	×	✓	×	✓	*No*
Roxburghiadiol A	✓	×	✓	✓	*Yes*
β-Sitostenone	✓	×	✓	✓	*Yes*
3β-hydroxy-stigmast-5-en-7-one	✓	×	✓	✓	*Yes*
β-Sitosterol	✓	×	✓	✓	*Yes*
Stigmasterol	✓	×	✓	✓	*Yes*
γ-Himachalene	✓	×	✓	✓	*Yes*
Cadina-1,4-diene	✓	×	✓	✓	*Yes*
Swietemacrophyllanin	✓	✓	✓	×	*Yes*
Catechin	✓	✓	✓	✓	*Yes*
Epicatechin	✓	✓	✓	✓	*Yes*
Scopoletin	✓	✓	✓	✓	*Yes*
3-Hydroxy Caruilignan C	✓	✓	✓	✓	*Yes*

Information: Yes = Meets the requirements (violated 1 rule), No = Does not meet the requirements (violated > 1 rule), ✓ = complies with Lipinski's rule, × = does not comply with Lipinski's rule

Table 2. Gridbox parameters

Macromolecular Code	Gridbox					
	Center			Dimensions (Å)		
	X	Y	Z	X	Y	Z
7QCG	69,93	28,744	-29,08	40	40	40

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Table 3. Redocking result from RMSD value

Target Protein Name	Natural ligands	PDB code	RMSD (Å)	Condition
PLpro	AKOS003853619	7QCG	1.391	< 2 Å

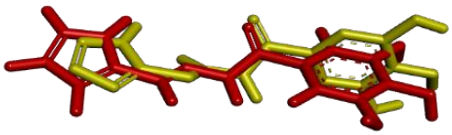

Figure 1. Crystallographic (red) and Redocked (yellow) ligand overlay results

Table 4. Redocking result from RMSD value

Compound	**Bond Energy (kcal/mol)**	**Amino Acid Residues Hydrogen Bonds**	**Involved in Interactions Besides Hydrogen Bonds**
AKOS003853619 (native ligand)	-4,32	Met206, Ser170, Arg166	Gln174, Met208, Tyr207, Arg183, Leu199, Glu203, Val202
7-Deacetoxy-7-oxogedunin	-7,55	**Arg166,** Lys232	**Val202, Gln174, Ser170, Met208,** Phe216, Leu185, **Tyr207, Leu199, Arg183, Met206, Glu203**
3β-hydroxy-stigmast-5-en-7-one	-7,36	-	Tyr171, **Gln174, Glu203,** Leu185, Lys232, **Leu199, Tyr207, Met208, Met206, Val202, Arg166, Ser170,** Glu167

Bold: similarity of amino acids interacting with natural ligands.

Based on the RMSD values and the overlay of the redocking results of the four natural ligands on their target proteins in Table 3 and Figure 1, it can be seen that the positions of the redocked ligands are close to those of the crystallographic ligands and occupy the same active sites because they interact with amino acids, which also interact with the crystallographic ligands. The redocking process is important because it helps validate the docking method and assess its accuracy. In molecular docking, the process of redocking occurs when the ligand (molecule under test) is placed back into the active site of the target protein after calculating its initial energy and placement. If the redocking ligand approaches the crystallographic ligand position, occupies the same active position, and interacts with amino acids that also interact with crystallographic ligands, this indicates consistency between the docking results and the crystallographic structure (de Oliveira *et al*., 2022; Venkatesh, 2022). These results suggest that the docking method used is valid and that the protein and ligand docking processes can be carried out using AutodockTools.

Analysis of molecular docking results

Based on this study, 22 compounds in the mahogany plant were tethered to the target protein using the molecular docking method, namely AutodockTools. Molecular docking aims to predict the binding mode and affinity of a small molecule for the active site of a particular target protein, and the result is a value that describes the bond-free energy, i.e., the amount of energy required by the ligand to form a bond with the receptor (Guedes *et al*., 2013). Evaluation of the test ligand in molecular docking involves assessing a number of factors, including the free bond energy and the similarity of the amino acid residue to the natural ligand. These two factors play a role in the selection of ligands that have the potential to bind specifically and effectively to the target protein. Bond energy is one of the most critical factors in determining the stability of the ligand-receptor complex (de Oliveira *et al*., 2022). Lower free bond energy values tend to be better at molecular docking. The lower the free bond energy value between the target molecule and the ligand, the more stable the bond complex is (Kurczab, 2017). The similarity of the amino acid residues of the test ligands with the natural ligands in the target protein can increase the chances of producing, suitable binding complexes. This is because the binding of natural ligands to the target protein is determined by specific interactions between the ligand and amino acid residues in the active site of the protein (Wu & Huang, 2023).

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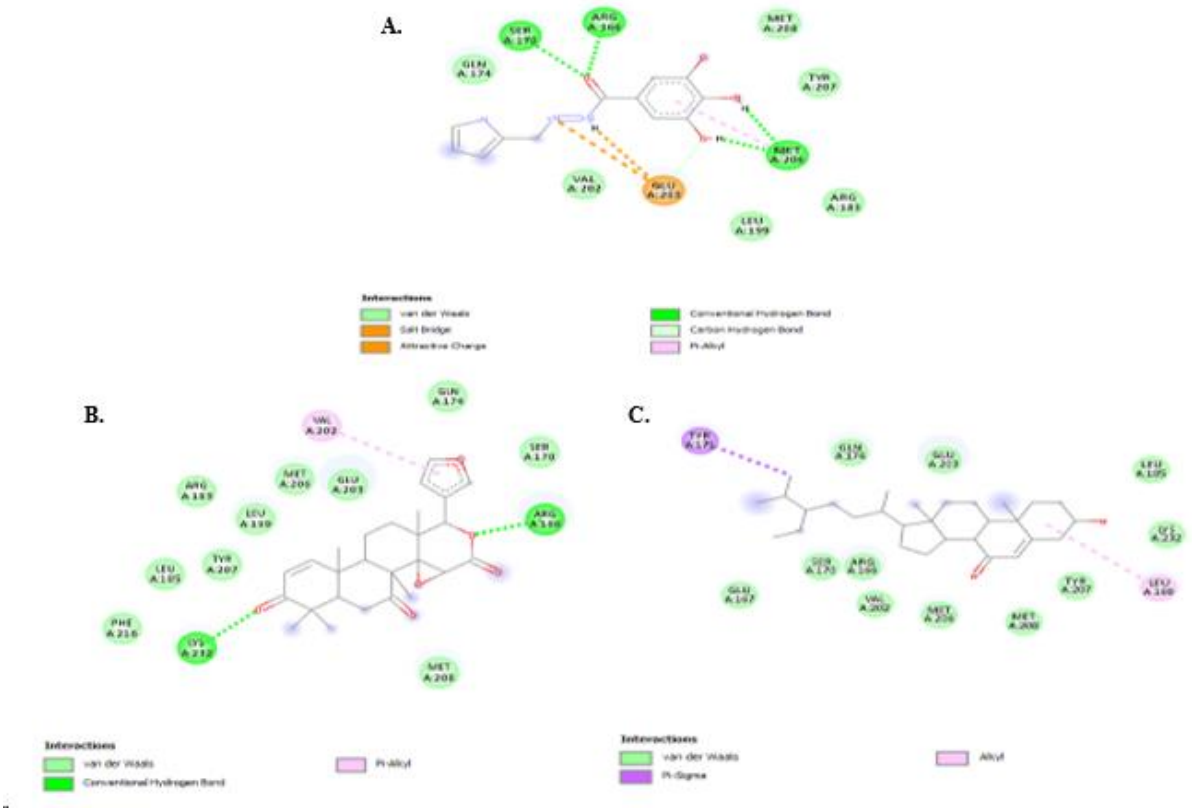

Figure 2. Interaction diagram of the test ligand compared to the natural ligand; (**A**) AKOS003853619 (natural ligand), (**B**) 7-Deacetoxy-7-oxogedunin, and (**C**) 3β-hydroxy-stigmast-5-en-7-one

Based on Table 4, two compounds have lower bond energies and interact with amino acid residues that are similar to natural ligands. AKOS003853619, which is a natural inhibitor and ligand of 7QCG, interacts with amino acid residues present on the active side through hydrogen bonds, van der Waals bonds, π-alkyl bonds, and salt bridges (Figure 2). According to Ewert *et al*. (2022), natural ligands in 7QCG form hydrogen bonds with Arg166, Ser170, and Met206 and form additional side chains with Gln174. n. The interaction of the Glu203 side chain achieves further stabilization. In the context of protein structure, the number associated with an amino acid, such as "Glu203", refers to the position of that amino acid in the protein sequence. This number is used to identify and refer to specific amino acids in protein structures, especially when discussing their functional properties or locations (Pulido *et al*., 2014).

Compound 7-deacetoxy-7-oxogedunin has the lowest bond-free energy value in PLpro protein, followed by 3β-hydroxy-stigmast-5-en-7-one. The values of the two test ligands were smaller when compared to AKOS003853619 (Table 4) because there were more amino acid residues that interacted with the two test ligands than with the natural ligands. Compound 7-deacetoxy-7-oxogedunin has a smaller free bond energy value compared to 3β-hydroxy-stigmast-5-en-7-one because 7-deacetoxy-7-oxogedunin has the same amino acid residue as the natural ligand, while 3β -hydroxy-stigmast-5-en-7-one lacks one residue similar to that of the natural ligand (Table 4).

Based on the results of molecular docking, the two best test ligands on the target protein have the potential as anti-SARS-CoV-2 candidates because these test ligands interact with the identical amino acid residues as the natural ligands of the target protein. The results of molecular docking indicated that several compounds from the mahogany plant had interactions with the tested molecular targets. Some of them have better free bond energy values than natural ligands. A compound can be known to have activity against a target protein by observing two parameters, namely bond energy and interaction pattern. If a compound has the best bond energy value but does not interact with amino acid residues that are similar to natural ligands, then the compound does not have the best interaction pattern and cannot be said to have the same activity as natural ligands (Salem *et al*., 2023). Next, the two best test ligands will be subjected to MD simulation tests to find

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out whether the tested ligands have good bond stability with macromolecules compared to natural ligands.

Analysis of molecular dynamics simulation results

Based on this study, two compounds in the mahogany plant were tethered to the target protein using the MD simulation method, namely YASARA Dynamics. MD simulation aims to study the movement and interaction of atoms in a molecular system. These simulations provide a deeper understanding of the physical and chemical properties of molecules, including their structure, stability, dynamics, and reactivity. The stability of the ligand-protein bond can be determined by calculating the RMSD and RMSF values (Patel *et al*., 2021). Prior to the start of the analysis, the simulation conditions were set with 0.9% NaCl, pH 7.4, at a temperature of 298K, and the duration of the simulation was 20 ns. Setting 0.9% NaCl, pH 7.4, at 298K is often used in laboratory practice to try to replicate environmental conditions similar to those of the human body. A simulation time of 20 ns can provide initial insight into the stability of the RMSD and RMSF (Parihar *et al*., 2022; Shree et al., 2022).

RMSD analysis involves a comparison of the conformational change of the simulated system with the initial or experimental structure. Monitoring RMSD over time makes it possible to assess system stability and identify periods of equilibrium and fluctuation. Higher RMSD values indicate more significant structural deviation from the reference structure, indicating increased flexibility or conformational changes. RMSF measures the flexibility or local mobility of individual atoms or residues in a biomolecular system during an MD simulation. It provides information about regions that undergo significant conformational changes or exhibit high flexibility. Atoms with a low RMSF show stability and a lack of fluctuation, while atoms with a high RMSF show greater flexibility or movement (Wu *et al*., 2022).

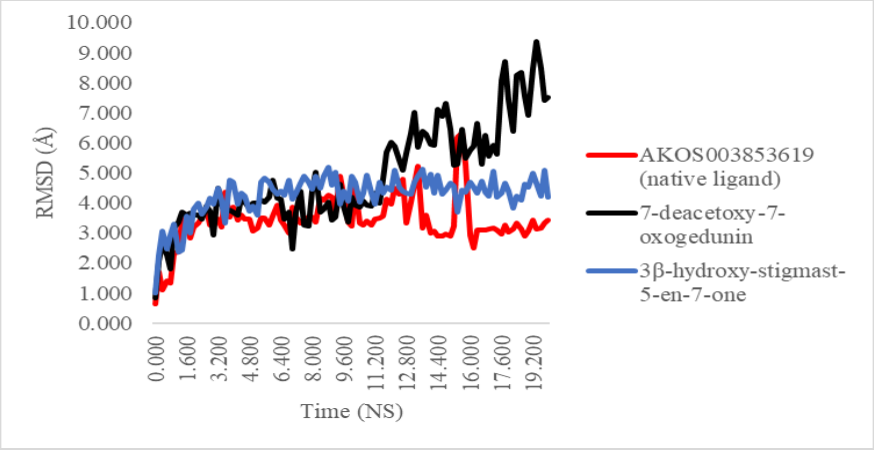

Figure 3. Comparison of RMSD values between native ligand (red), 7-deacetoxy-7-oxogedunin (black), and 3β-hydroxy-stigmast-5-en-7-one (blue)

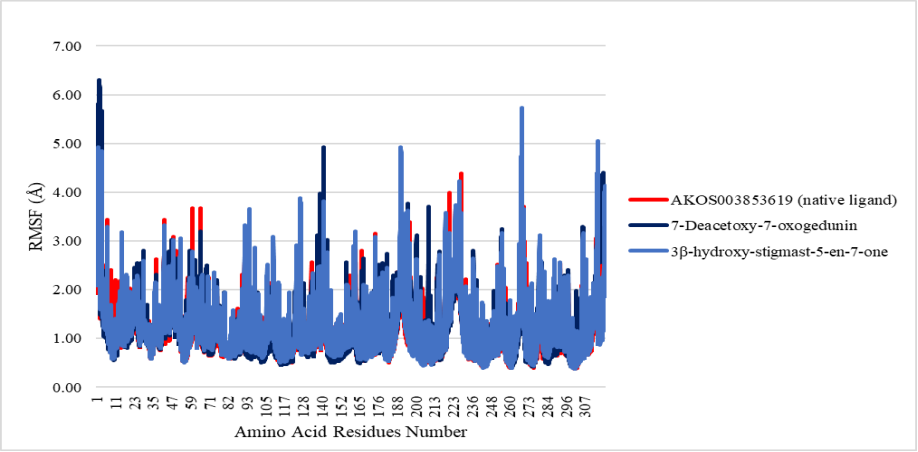

Figure 4. Comparison of RMSF values between AKOS003853619 (red), 7-Deacetoxy-7-oxogedunin (black), and 3β-hydroxy-stigmast-5-en-7-one (blue). (amino acid residue number = Met206, Ser170, Arg166, Gln174, Met208, Tyr207, Arg183, Leu199, Glu203, and Val202)

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Table 5. Redocking result RMSD

	Average (Å)		
	Native Ligand	**7-deacetoxy-7-oxogedunin**	**3β-hydroxy-stigmast-5-en-7-one**
RMSD (Å)	3.423 ± 0.833	4.902 ± 1.697	4.250 ± 0.684
RMSF (Å)	1.182 ± 0.668	1.230 ± 0.727	1.247 ± 0.678

CONCLUSION

Based on Figure 3, the natural protein-ligand complex is more stable than 7-deacetoxy-7-oxogedunin and 3β-hydroxy-stigmast-5-en-7-one. This result can also be proven by the RMSD average value of the natural ligand, which is smaller than 7-deacetoxy-7-oxogedunin and 3β-hydroxy-stigmast-5-en-7-one (Table 5). Based on the results of the graphs and averages, it can be seen that the stability of the 3β-hydroxy-stigmast-5-en-7-one ligand with the PLpro protein is close to the stability of the bond of the natural protein-ligand complex. Meanwhile, the graph of 7-deacetoxy-7-oxogedunin began to increase at 11 ns, which means that the stability of the bond decreased. If the RMSD value is below 5 Å (Angstrom), it can be said to be a relatively stable value in molecular dynamics simulations. In contrast, if it is above 5 Å, it indicates significant conformational changes and large structural changes and means low stability (Rudnev *et al*., 2022). The flexibility of the structure of each compound can influence conformational changes over time. Compounds with stable and well-defined structures throughout the simulation are likely to have better stability. The presence of intermolecular forces involved, such as hydrogen bonds, van der Waals interactions, and electrostatic interactions, can also influence the stability and bond energy values. High RMSD values can be affected by various conditions, such as temperature and pH, and also by interactions between the ligand-protein; this can change the structural conformation of the ligand-protein bond so that the stability becomes poor (Kordzadeh & Saadatabadi, 2022; Oliwa & Shen, 2015). The lower the RMSD value, the more stable the bond between the ligand and the protein, and it shows that the two structures (natural ligand and 3β-hydroxy-stigmast-5-en-7-one) are more similar to each other (Guterres & Im, 2020).

Based on Figure 4, the test ligands 7-deacetoxy-7-oxogedunin and 3β-hydroxy-stigmast-5-en-7-one have RMSF graphs that are almost similar to the test ligands. The natural protein-ligand complexes were more stable than the tested ligands, as indicated by the lower RMSF values of natural ligands than 7-deacetoxy-7-oxogedunin and 3β-hydroxy-stigmast-5-en-7-one (Table 5). The RMSF value will outline the conformational shifts of each amino acid residue, which gives an idea of its flexibility. The lower the RMSF value, the more stable the positions of the ligands and amino acids are (Elfita *et al*., 2022). Based on the RMSF results, the amino acid residues of natural ligands such as Met206, Ser170, Arg166, Gln174, Met208, Tyr207, Arg183, Leu199, Glu203, and Val202 did not change and remained stable. Likewise, the amino acid residues in the test ligands 7-deacetoxy-7-oxogedunin and 3β-hydroxy-stigmast-5-en-7-one were similar to the natural ligands, did not change and remained stable. Docking and MD results may differ because docking only evaluates binding energy or binding affinity. MD, on the other hand, stresses the ligand-protein complex's long-term stability (Chen, 2014).

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Based on the research that has been done, it can be concluded that 3β-hydroxy-stigmast-5-en-7-one and 7-deacetoxy-7-oxogedunin were predicted to have good interaction with PLPro. Compound 3β-hydroxy-stigmast-5-en-7-one showed higher interaction stability, so it is expected to maintain a better inhibitory effect of PLpro than 7-deacetoxy-7-oxogedunin.

AUTHOR CONTRIBUTIONS

Conceptualization, L. S. W. F. A., N. H., A. P., R. H.; Methodology, L. S. W. F. A., N. H., A. P., R. H.; Software, L. S. W. F. A., N. H., A. P., R. H.; Validation, L. S. W. F. A., N. H., A. P., R. H.; Formal Analysis, L. S. W. F. A., N. H., A. P., R. H.; Investigation, L. S. W. F. A., N. H., A. P., R. H.; Resources, L. S. W. F. A., N. H., A. P., R. H.; Data Curation, L. S. W. F. A., N. H., A. P., R. H.; Writing - Original Draft, L. S. W. F. A., N. H., A. P., R. H.; Writing - Review & Editing, L. S. W. F. A., N. H., A. P., R. H.; Visualization, L. S. W. F. A., N. H., A. P., R. H.; Supervision, L. S. W. F. A., N. H., A. P., R. H.; Project Administration, L. S. W. F. A., N. H., A. P., R. H.; Funding Acquisition, L. S. W. F. A., N. H., A. P., R. H.

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CONFLICT OF INTEREST

The authors declared no conflict of interest.

REFERENCES

Chen, Y.-C. (2014). Beware of docking!. *Trends in Pharmacological Sciences*; *36*; 1–18. doi: 10.1016/j.tips.2014.12.001.

Chen, T.-H., Tsai, M.-J., Chang, C.-S., Xu, L., Fu, Y.-S. & Weng, C.-F. (2022). The Exploration of Phytocompounds Theoretically Combats SARS-CoV-2 Pandemic Against Virus Entry, Viral Replication and Immune Evasion. *Journal of Infection and Public Health*; *16*; 1–39. doi: 10.1016/j.jiph.2022.11.022.

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*; 1–13. doi: 10.1038/srep42717.

de Oliveira, T. A., Medaglia, L. R., Maia, E. H. B., Assis, L. C., de Carvalho, P. B., da Silva, A. M. & Taranto, A. G. (2022). Evaluation of Docking Machine Learning and Molecular Dynamics Methodologies for DNA-Ligand Systems. *Pharmaceuticals*; *15*; 1-15. doi: 10.3390/ph15020132.

Dewi, R. S., Anggraeni, A., Bahti, H. H., Yusuf, M., Hardianto, A. & Mutholib, A. (2022). Simulasi Dinamika Molekuler Ligan Disekunderbutil ditiofosfat (DSBDTP) untuk Ekstraksi Logam Tanah Jarang. *SainsMath: Jurnal MIPA Sains Terapan*; *1*; 1–8.

Elfita, L., Apriadi, A., Supandi, S. & Dianmurdedi, S. (2022). Studi Penambatan Molekuler dan Simulasi Dinamika Molekuler Senyawa Turunan Furanokumarin terhadap Reseptor Estrogen Alfa (ER-α) Sebagai Anti Kanker Payudara. *Jurnal Sains Farmasi & Klinis*; *9*; 255–264. doi: 10.25077/jsfk.9.3.255-264.2022.

Ewert, W., Günther, S., Miglioli, F., Falke, S., Reinke, P. Y. A., Niebling, S., Günther, C., Han, H., Srinivasan, V., Brognaro, H., Lieske, J., Lorenzen, K., Garcia-Alai, M. M., Betzel, C., Carcelli, M., Hinrichs, W., Rogolino, D. & Meents, A. (2022). Hydrazones and Thiosemicarbazones Targeting Protein-Protein-Interactions of SARS-CoV-2 Papain-like Protease. *Frontiers in Chemistry*; *10*; 1–13. doi: 10.3389/fchem.2022.832431.

Guedes, I. A., de Magalhães, C. S., & Dardenne, L. E. (2013). Receptor–ligand Molecular Docking. *Biophysical Reviews*; *6*; 75–87. doi: 10.1007/s12551-013-0130-2.

Guterres, H. & Im, W. (2020). Improving Protein-Ligand Docking Results with High-Throughput Molecular Dynamics Simulations. *Journal of Chemical Information and Modeling*; *60*; 2189–2198. doi: 10.1021/acs.jcim.0c00057.

Kesuma, D., Siswandono, S., Purwanto, B. T. & Hardjono, S. (2018). Uji In Silico Aktivitas Sitotoksik dan Toksisitas Senyawa Turunan N-(Benzoil)-N'- Feniltiourea Sebagai Calon Obat Antikanker. *JPSCR: Journal of Pharmaceutical Science and Clinical Research*; *3*; 1-11. doi: 10.20961/jpscr.v3i1.16266.

Kordzadeh, A. & Saadatabadi, A. R. (2022). Effects of the Temperature and the pH on the Main Protease of Sars-Cov-2: A Molecular Dynamics Simulation Study. *Biointerface Research in Applied Chemistry*; *12*; 7239–7248. doi: 10.33263/BRIAC126.72397248.

Kurczab, R. (2017). The Evaluation of QM/MM-Driven Molecular Docking Combined with MM/GBSA Calculations as a Halogen-Bond Scoring Strategy. *Acta Crystallographica Section B: Structural Science, Crystal Engineering and Materials*; *73*; 188–194. doi: 10.1107/S205252061700138X.

Lipinski, C. A., Lombardo, F., Dominy, B. W. & Feeney, P. J. (1997). Experimental and Computational Approaches to Estimate Solubility and Permeability in Drug Discovery and Development Settings. *Advanced Drug Delivery Reviews*; *23*; 3–25. doi: 10.1016/S0169-409X(96)00423-1.

Moghadamtousi, S. Z., Goh, B. H., Chan, C. K., Shabab, T. & Kadir, H. A. (2013). Biological Activities and Phytochemicals of *Swietenia macrophylla* King. *Molecules*; *18*; 10465–10483. doi: 10.3390/molecules180910465.

Mukherjee, S., Balius, T. E., & Rizzo, R. C. (2010). Docking Validation Resources: Protein Family and Ligand Flexibility Experiments. *Journal of Chemical Information and Modeling*, *50*(11), 1986–2000. https://doi.org/10.1021/ci1001982

Mulu, A., Gajaa, M., Woldekidan, H. B. & Wmariam, J. F. (2021). The Impact of Curcumin Derived Polyphenols on the Structure and Flexibility

COVID-19 Main Protease Binding Pocket: A Molecular Dynamics Simulation Study. *PeerJ*; *9*; 1–16. doi: 10.7717/peerj.11590.

Musarra-Pizzo, M., Pennisi, R., Ben-Amor, I., Mandalari, G. & Sciortino, M. T. (2021). Antiviral Activity Exerted by Natural Products against Human Viruses. *Viruses*; *13*; 1-30. doi: 10.3390/v13050828.

Oliwa, T. & Shen, Y. (2015). cNMA: A Framework of Encounter Complex-Based Normal Mode Analysis to Model Conformational Changes in Protein Interactions. *Bioinformatics*; *31*; i151–i160. doi: 10.1093/bioinformatics/btv252.

Parihar, A., Sonia, Z. F., Akter, F., Ali, M. A., Hakim, F. T. & Hossain, M. S. (2022). Phytochemicals-Based Targeting RdRp and Main Protease of SARS-CoV-2 using Docking and Steered Molecular Dynamic Simulation: A promising Therapeutic Approach for Tackling COVID-19. *Computers in Biology and Medicine*; *145*; 1-13. doi: 10.1016/j.compbiomed.2022.105468.

Patel, A. H., Patel, R. B., Memon, M. J., Patel, S. S., Desai, S. A. & Meshram, D. B. (2021). Docking, Binding Free Energy Estimation, and MD Simulation of Newly Designed CQ and HCQ Analogues Against the Spike-ACE2 Complex of SARS-CoV-2. *International Journal of Quantitative Structure-Property Relationships*; *6*; 77–89. doi: 10.4018/IJQSPR.2021100105.

Petrera, E. (2015). Antiviral and Immunomodulatory Properties of Meliaceae Family. *Journal of Biologically Active Products from Nature*; *5*; 241–254. doi: 10.1080/22311866.2015.1081569.

Pulido, R., Baker, S. J., Barata, J. T., Carracedo, A., Cid, V. J., Chin-Sang, I. D., Davé, V., den Hertog, J., Devreotes, P., Eickholt, B. J., Eng, C., Furnari, F. B., Georgescu, M.-M., Gericke, A., Hopkins, B., Jiang, X., Lee, S.-R., Lösche, M., Malaney, P., … & Leslie, N. R. (2014). A Unified Nomenclature and Amino Acid Numbering for Human PTEN. *Science Signaling*; *7*; 1-10. doi: 10.1126/scisignal.2005560.

Rudnev, V. R., Nikolsky, K. S., Petrovsky, D. V., Kulikova, L. I., Kargatov, A. M., Malsagova, K. A., Stepanov, A. A., Kopylov, A. T., Kaysheva, A. L. & Efimov, A. V. (2022). 3β-Corner Stability by Comparative Molecular Dynamics Simulations. *International Journal of Molecular Sciences*; *23*; 1-14. doi: 10.3390/ijms231911674.

Salem, I. M., Mostafa, S. M., Salama, I., El-Sabbagh, O. I., Hegazy, W. A. H. & Ibrahim, T. S. (2023). Design, Synthesis and Antitumor Evaluation of Novel Pyrazolo[3,4- d]Pyrimidines Incorporating Different Amino Acid Conjugates as Potential DHFR Inhibitors. *Journal of Enzyme Inhibition and Medicinal Chemistry*; *38*; 203–215. doi: 10.1080/14756366.2022.2142786.

Sándor, M., Kiss, R. & Keserű, G. M. (2010). Virtual Fragment Docking by Glide: a Validation Study on 190 Protein−Fragment Complexes. *Journal of Chemical Information and Modeling*; *50*; 1165–1172. doi: 10.1021/ci1000407.

Santos, G. B., Ganesan, A. & Emery, F. S. (2016). Oral Administration of Peptide-Based Drugs: Beyond Lipinski's Rule. *ChemMedChem*; *11*; 2245–2251. doi: 10.1002/cmdc.201600288.

Satgas Penanganan Covid-19. (2022). Data Sebaran Situasi Virus COVID-19. https://covid19.go.id/

Septiana, E. (2020). Prospek Senyawa Bahan Alam Sebagai Antivirus Dalam Menghambat SARS-CoV-2. *Bio Trends*; *11*; 30–38.

Shree, P., Mishra, P., Selvaraj, C., Singh, S. K., Chaube, R., Garg, N. & Tripathi, Y. B. (2022). Targeting COVID-19 (SARS-CoV-2) Main Protease through Active Phytochemicals of Ayurvedic Medicinal Plants–Withania Somnifera (Ashwagandha), *Tinospora cordifolia* (Giloy) and *Ocimum sanctum* (Tulsi)–a Molecular Docking Study. *Journal of Biomolecular Structure and Dynamics*; *40*; 190–203. doi: 10.1080/07391102.2020.1810778.

Tu, Y., Chien, C., Yarmishyn, A. A., Lin, Y., Luo, Y.-H., Lin, Y.-T., Lai, W.-Y., Yang, D.-M., Chou, S.-J., Yang, Y.-P., Wang, M.-L., & Chiou, S.-H. (2020). A Review of SARS-CoV-2 and the Ongoing Clinical Trials. *International Journal of Molecular Sciences*; *21*; 1-19. doi: 10.3390/ijms21072657.

Vardhan, S. & Sahoo, S. K. (2020). In Silico ADMET and Molecular Docking Study on Searching Potential Inhibitors from Limonoids and Triterpenoids for COVID-19. *Computers in Biology and Medicine*; *124*; 1-12. doi: 10.1016/j.compbiomed.2020.103936.

Venkatesh. (2022). Molecular Docking of Ganomestenol with Sars-Cov-2 Mpro. *Asian Journal of Pharmaceutical and Clinical Research*; *15*; 46–47. doi: 10.22159/ajpcr.2022.v15i2.43679.

Wang, M., Cao, R., Zhang, L., Yang, X., Liu, J., Xu, M., Shi, Z., Hu, Z., Zhong, W. & Xiao, G. (2020). Remdesivir and Chloroquine Effectively Inhibit

P-ISSN: 2406-9388
E-ISSN: 2580-8303

the Recently Emerged Novel Coronavirus (2019-nCoV) In Vitro. *Cell Research*; *30*; 269–271. doi: 10.1038/s41422-020-0282-0.

World Health Organization (WHO). (2022). World Health Organization. Coronavirus disease 2019 (COVID-19) Weekly Epidemiological Update, Edition 117, published 9 November 2022. https://www.who.Int/publications/m/item/weekly-Epidemiological-Update-on-Covid-19---9-November-2022.

Wu, J., Zhou, Y., Zhang, J., Zhang, H.-X., & Jia, R. (2022). Molecular Dynamics Simulation Investigation of the Binding and Interaction of the EphA6–Odin Protein Complex. *The Journal of Physical Chemistry B*; *126*; 4914–4924. https://doi.org/10.1021/acs.jpcb.2c01492

Wu, Q. & Huang, S.-Y. (2023). HCovDock: an Efficient Docking Method for Modeling Covalent Protein–Ligand Interactions. *Briefings in Bioinformatics*; *24*; 1-10. doi: 10.1093/bib/bbac559.

Yu, R., Chen, L., Lan, R., Shen, R., & Li, P. (2020). Computational Screening of Antagonists Against the SARS-CoV-2 (COVID-19) Coronavirus by Molecular Docking. *International Journal of Antimicrobial Agents*; *56*; 106012. doi: 10.1016/j.ijantimicag.2020.106012.

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