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Virtual Screening of *Syzygium cumini* (L.) Skeels Flavonoid Compounds as SARS-CoV-2 Main Protease Therapy Candidates

Himyatul Hidayah [1,*], Ruswanto [2], Desri Lestari [1], Surya Amal [1], Neni Sri Gunarti [1]

[1] Faculty of Pharmacy, Universitas Buana Perjuangan Karawang, Karawang, West Java, Indonesia
[2] Faculty of Pharmacy, Universitas Bakti Tunas Husada, Tasikmalaya, West Java, Indonesia

* Corresponding author: himyatul.hidayah@ubpkarawang.ac.id

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Abstract

In December 2019, the first COVID-19 cases were in Wuhan, China. This case is a global concern and a threat to public health. Based on previous research using molecular docking methods, it was found that flavonoids exhibit strong inhibitory activity in SARS-CoV-2 main proteases. The study aims to determine the flavonoid compound *Syzygium cumini* (L.) Skeels can interact with the SARS-CoV-2 main protease receptor and can be used as a candidate for COVID-19 therapy with virtual screening. Myricetin 4"-O-acetyl-2-O-gallate has the lowest Gibbs free energy (ΔG) of -9.82 kcal/mol. The molecular dynamics of the best compound, Myrcetin 4-O-acetyl-2-O-gallate, RMSD, and RSMF values are quite stable. As a result of pharmacokinetic prediction and toxicity, the best compounds have a relatively good pharmacokinetic profile and are non-toxic. Thus, it can be concluded that the compound Myricetin 4"-O-acetyl-2-o-gallate in the *Syzygium cumini* (L.) Skeels is predicted to interact with the SARS-CoV-2 main protease receptor (7C6S) as a potential drug candidate for COVID-19 therapy.

1. Introduction

At the end of 2019, precisely in December, the first case of pneumonia occurred in Wuhan, Hubei Province, China. This case is linked to a seafood market that sells various live animals in the region, and the virus spreads through the air rapidly [1]. After his first case in Wuhan, COVID-19 cases continued to rise daily in China, reaching a peak between the end of January and the beginning of February 2020 [2].

The rapid mutation and adaptation capabilities of viruses encourage researchers to discover new therapeutic approaches. The primary strategy is to disrupt the virus's life cycle by blocking its replication or interfering with its function using membrane interactions [3, 4]. Another approach is the development of new drugs with computational research. Current computational research is the basis for saving time and improving accuracy in developing new pharmaceuticals [4, 5, 6]. One is the virtual screening approach used to identify the active compound with the target structure of an enzyme, with molecular docking as the backbone of this approach [7]. The main target of the researchers today is M^{pro} (main protease) because this SARS-CoV-2 is, according to previous research, similar to SARS-CoV-1 [8]. In addition, this enzyme plays an important role in the survival of the coronavirus, as this enzyme assists the transcription and replication processes [9]. Based on previous research [10], luteolin and quercetin [11] have strong activity as antivirals for influenza in vitro.

Flavonoids are secondary metabolite compounds in plants, whether vegetables, tea, wine, fruit, or parts of plants such as stems, roots, seedlings, barks, or even wood. One of the plants with a flavonoid content is the *Syzygium cumini* (L.) Skeels. According to literary research by Ramya *et al.* [12] [13, 14], brown leaves have at least 13 flavonoid compounds. Besides, according to the latest discovery, nine other flavonoid compounds exist in the *Syzygium cumini* (L.) Skeels [15]. According to a study by Cherrak *et al.* [16] using the molecular docking method, glycosylated flavonoids show strong inhibitory activity in SARS-CoV-2 main protease. Based on the discovery of Pratama *et al.* [17], the flavonoid compounds from seed

leaf roses (*Psidium guajava* L.) are epicatechin-3-O-gallate, ononin, and glycitin, potentially acting as inhibitors of the 3CL protease SARS-CoV-2.

This study aims to evaluate the potential inhibitory activity of the *Syzygium cumini* flavonoid composition against SARS-CoV-2 main protease receptors using the molecular docking method. *Syzygium cumini* was selected for its high flavonoid content, which has been shown in previous research to exhibit significant antiviral properties. Prior studies have demonstrated that flavonoids from *Syzygium cumini* possess strong inhibitory activity against various viral enzymes, suggesting it is a promising candidate for SARS-CoV-2 inhibition [18, 19, 20].

This research is expected to serve as an additional source of information, enhance existing knowledge, and aid in discovering alternative treatments for COVID-19 using natural materials. It also aims to provide a foundational basis for further investigation into the inhibitory activity of flavonoids from *Syzygium cumini* (L.) Skeels against SARS-CoV-2.

2. Experimental

2.1. Materials and Tools

The sample of the flavonoid compound of *Syzygium cumini* (L.) Skeels were used to refer to previous discoveries, and samples were taken as many as fifteen times. The sample was a three-dimensional structure obtained by downloading it from Pubchem's website (https://pubchem.ncbi.nlm.nih.gov/).

The materials used were three-dimensional structures of the SARS-CoV-2 of main protease (7C6S), ACE2 (6LZG), and spike protein (7LM8) obtained from the Protein Data Bank, as well as the two-dimensional flavonoid compound *Syzygium cumini* (L.) Skeels, which was downloaded from Pubchem's website (https://pubchem.ncbi.nlm.nih.gov/).

This study used hardware such as an Asus laptop with specifications for the Microsoft Windows 10 enterprise 64-bit operating system and a processor, the Intel (R) Core™ i3-5005U CPU @ 2.00 GHz, with 4.0 GB of RAM. The software used was Windows 10, Discovery Studio version 16.1, AutodockTools 1.5.7, Desmond of Schrödinger, MarvinSketch 5.2.5.0, and Molegro molecular viewer. Web-based programs such as pkCSM and Protein Data Bank (PDB) were also utilized.

2.2. Ligand Preparation

The flavonoid derivative structure was available and downloaded from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/). Protonation was performed at a pH of 7.4, and the conformation of the flavonoid derivative structure was modeled using MarvinSketch software version 5.2.5.0 by Chemaxon. The prepared structure was then saved in .mol2 file format [21]. This procedure was conducted for all flavonoid derivative ligands [22].

2.3. Receptor Preparation

The SARS-CoV-2 of main protease (7C6S), ACE2 (6LZG), and Spike Protein (7LM8) were downloaded from the Protein Data Bank website via the http://www.rcsb.org/ link in. pdb format. This receptor was then separated by its natural ligand and prepared by removing the water molecules and adding protons to the molecule. Then, the prepared receptor was stored in PDB format [23]. The three already prepared receptors were tested using the parameters below to select the best receptor.

The parameters used to select receptors were based on specific criteria to ensure the best possible fit for the study. The organisms selected were Homo sapiens and the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2). The resolution of the receptor structures was set to be less than 3 Å to ensure high stability and accuracy. The diffraction method employed was X-ray crystallography, which provided detailed and accurate structural data. The chosen receptors had to have similar poses to the original protein structure, ensuring that the native ligand data was considered. These stringent parameters were crucial in identifying the most suitable receptors for subsequent molecular docking and virtual screening processes.

2.4. Target Receptor Identification

Target receptor identification was done by reviewing the target receptor profile by entering the PDB code of the selected receptor on the website http://www.ebi.ac.uk/pdbsum/. Then, the data profile of the receptor appeared [24].

2.5. Docking Validation

This process was done to get a valid method. The subsequently downloaded target receptor was selected by its natural ligand as a validation process for the docking method. This data analysis parameter used root-mean-square-deviation (RMSD). The docking method was considered valid if the resulting RMSD value is ≤ 2 Å [24].

2.6. Virtual Screening and Docking ligand Against Target Receptors

After the ligand was prepared, it was converted from the.mol2 file format to the PDBQT file format using AutodockTools 1.5.7. The virtual screening process of all flavonoid derivatives was carried out using AutodockTools software, which is a tool for the docking process. Grid and docking parameter files were configured using autodock. Autodock and auto grid, integrated with Autodock4, were used to generate grid maps for each ligand atom. Each ligand was analyzed with the default docking setting, and the LGA was run for 100 runs on each ligand. In addition, the grid parameter file was also used to predict amino acid residues on the active site of the target receptor that interacted with the ligand [25]. The compound resulting from the docking with the next best candidate was visualized using Discovery Studio software version 16.1 by looking at a picture of its interactions in the form of 2 and 3-dimensions [22].

2.7. Molecular Dynamics Simulation

Main Pro's complex receptor structure, with the best candidate flavonoid compounds, simulated molecular dynamics using Desmond's software. The water model used was TIP3P. The process first parameterized ligands and proteins by adding style fields, then initial coordinate marking and system minimization. The second phase was gradual heating to balance the system from 0 K to 300 K. Molecular dynamics simulation was carried out with constant pressure and temperature conditions for 20 ns [22].

2.8. Analyzing drug-likeness (Drug scan)

Drug scan analysis was performed on artemisinin compounds to assess their potential as orally active drugs in humans, following Lipinski's Rule of Five (RO5). Using MarvinSketch software, the analysis focused on chemical and physical properties essential for oral bioavailability. The parameters evaluated included molecular weight (< 500 g/mol), lipophilicity (Log P < 5), hydrogen bond donors (< 5), hydrogen bond acceptors (< 10), and molar refractivity (between 40 and 130). These criteria help determine the likelihood of the compounds being effective oral medications [24].

2.9. Predicting pharmacokinetic and toxicity profiles

The absorption, distribution, metabolism, and toxicity tests were performed using the pkCSM program at https://biosig.lab.uq.edu.au/pkcsm/prediction. The composite structure of the best candidate was converted to the molfile (*.mol2) format before using the PkCSH program. The program automatically calculated the pharmacokinetic profile with human intestinal absorption (HIA), Caco-2, and plasma protein binding parameters [26].

3. Results and Discussion

3.1. Receptor Analysis and Identification

The receptor analysis was conducted on the main protease (7C6S), ACE2 (6LZG), and spike protein (7LM8) obtained from the protein data bank on the website https://www.rcsb.org/. In addition to the data presented in Table 1, which includes the profiles of the main protease (PDB code: 7C6S), ACE2 (PDB code: 6LZG), and spike protein (PDB code: 7LM8), further analysis was conducted to ensure the stability and suitability of the receptors for the study, a Ramachandran plot analysis of receptors was conducted using www.ebi.ac.uk/pdbsum. This Ramachandran plot profile is one of the parameters used to determine the stability of a protein. The protein structure is said to be well according to the Ramachandran plot when the non-glycine residue plot shows disallowed regions ≤ 0.8% [23]. The Ramachandran plot results for the 7C6S, 6LZG, and 7LM8 receptors can be seen in Figure 1.

ERRAT analysis was also done on protein structures using the SAVES (Structure Analysis and Verification Server) website at https://saves.mbi.ucla.edu. This analysis is valuable for detecting incorrect protein folding by statistically calculating the interactions between different types of atoms in the initial model [27]. The ERRAT analysis of main protease (7C6S), ACE2 (6LZG), and spike protein (7LM8) receptors are shown in Figure 2.

Table 1. Protein profile data

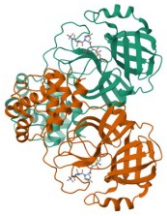
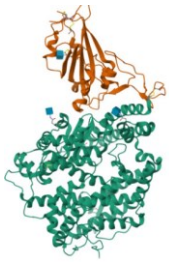
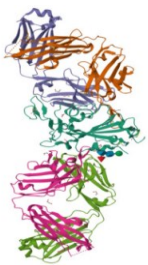
Code pdb	Protein		The natural ligand
	Name	Structure	
7C6S	Main protease		Boceprevir
6LZG	Angiotensin Converting Enzyme 2, Spike Protein		2-acetamido-2-deoxy-beta-D-glucopyranose
7LM8	Spike Protein, COVA1-16 Fab Heavy Chain, COVA1-16 Fab Light Chain, CV38-142 Fab heavy chain, CV38-142 Fab light chain		1,2-Ethanediol

Table 2. Grid box parameters

Code PDB	Center (Å)			Dimension (Å)			RMSD (Å)
	X	Y	Z	X	Y	Z	
7C6S	-19.838	-26.845	1.710	40	40	40	1.40
6LZG	-35.653	8.852	29.709	40	40	40	3.63
7LM8	8.131	-43.381	18.262	40	40	40	3.45

*All grids are set to have a spacing of 0.375.

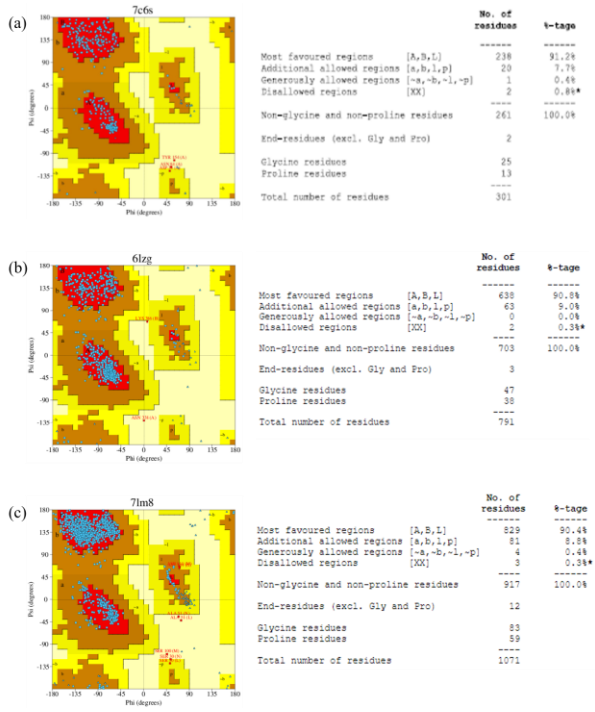


Figure 1. Ramachandran plots of (a) 7C6S, (b) 6LZG, and (c) 7LM8 (source: www.ebi.ac.uk/pdbsum)

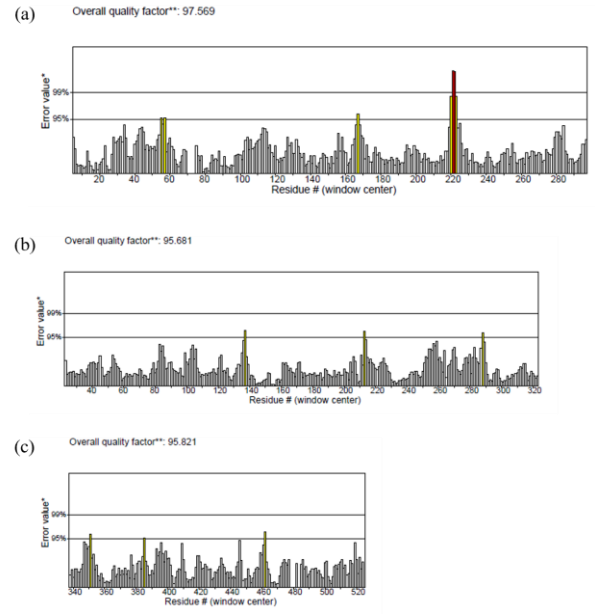


Figure 2. ERRAT results of (a) main protease, (b) ACE2, and (c) spike protein (Source: https://saves.mbi.ucla.edu)

The "y" axis of the ERRAT chart represents an error value, and the "x" axis represents the sequence of amino acid lipases. If the error value exceeds 99%, the area is poorly modeled. ERRAT calculations of the main protease receptors, ACE2, and spike protein showed an overall value of sequential factor quality of 97.569%, 95.681%, and 95.821%, respectively. Conscious of this result, it can be concluded that the protein structures are of high quality and resolution, making the error values of amino acid residues negligible [28]. Receptor identification was performed by examining the organism profile and the protein structure resolution obtained from the PDB website. The protease receptors with codes 7C6S, 6LZG, and 7LM8 are found in the organisms SARS-CoV-2 and Homo sapiens, with resolutions of 1.60, 2.50, and 1.94 Å, respectively. The results of the receptor analysis and identification show that the main protease (7C6S), ACE2 (6LZG), and spike protein (7LM8) receptors meet the required parameters and are suitable for proceeding to the stages of receptor preparation and docking validation.

3.2. Docking Method Validation

This docking method was validated through redocking, which involves reattaching the receptor to its natural ligand to determine the RMSD value, a key parameter. The similarity between the two natural ligand structures resulting from redocking is evaluated by measuring the distance between corresponding atoms [29]. Furthermore, the RMSD showed that the docking ligand aligns with the crystallographic ligand at the same active site. Method validation determines if the receptor can dock with the test ligand, and it is considered valid if the RMSD value is ≤ 2 Å [24]. A larger RMSD value indicates greater deviation in the ligand-receptor interaction, while a smaller RMSD value means the interaction is closer to the original conformation [24].

The grid box settings need to be done on the method validation to determine the coordinates of the ligands that will interact with the active side of the receptor. The setting is to set the size of the dimension and the center of the grid box with the points x, y, and z. The grid box settings and RMSD results can be seen in Table 2. Based on Table 2, RMSD values for main protease receptors with code 7C6S ≤ 2 Å, while for ACE2 (6LZG) and spike protein (7LM8) receptors indicate RMSD values of more than 2 Å. Based on these results, the validation of the main protease receptor docking method (7C6S) is valid. It can proceed to the virtual screening process as the target receptor. A visualization of the validation results can be seen in Figure 3.

Table 3. Virtual screening score

Compound	ΔG (kcal/mol)	Ki (μM)
Natural ligand (U5G)	-9.56	97.97
Remdesivir	-4.05	1.07
Myricetin 4”-O-acetyl-2-O-gallate	-9.82	63.25
Myricetin-3-(3-galloylrhamnoside)	-9.19	183.87
Myricetin 3-O-glucosides	-8.60	495.59
Myricitrin	-8.57	523.05
Isorhamnetin-3-β-D- galactopyranoside	-8.54	552.43
Quercetin-3-O-α-L-rhamnopyranoside	-8.46	628.61
Epiafzelechin_3_O_gallate	-7.84	1.80
Kaempferol 3-O-β-d-glucuronopyranoside	-7.84	1.79
Kaempferol-4’ rhamnoside	-7.49	3.26
Quercetin	-7.17	5.52
Kaempferol	-7.02	7.20
Epicatechin	-6.88	9.02
Catechin	-6.83	9.87
Isorhamnetin-3,4’ diglycoside	-6.77	10.89
Myricetin-3-O-(4”-O-malonyl)- α-L- rhamnopuranoside	-6.56	15.58

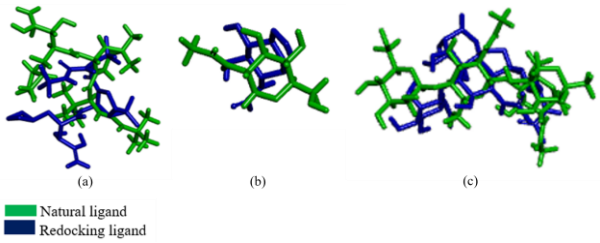


Figure 3. Visualization of the natural ligand position with the ligand after redocking (a) 7C6S, (b) 6LZG, (c) 7LM8

3.3. Virtual Screening

Virtual screening, or virtual filtering, is conducted to assess the binding affinity of 15 flavonoid compounds from *Syzygium cumini* (L.) Skeels to the main protease receptor and evaluate their potential as inhibitors. This stage is performed using AutodockTools software to dock each test compound against the receptor. The stable bond affinity of the docking result is seen based on the Gibbs free energy (ΔG) and the inhibition constant (Ki). The virtual screening process employed the Lamarckian Genetic Algorithm (LGA) docking algorithm, which performed 20 conformational runs. Remdesivir was selected as the comparison compound due to its broad therapeutic index, antiviral activity, and low toxicity [30]. Based on a study by Naik *et al.* [30], molecular docking results indicate that Remdesivir exhibits a strong binding affinity for the main protease receptor.

The virtual screening results of the flavonoid of *Syzygium cumini* (L.) Skeels against the main protease receptor (7C6S) are presented in Table 3. The top score is a compound with the best (lowest) ΔG value, which is the affinity parameter of ligand and receptor bonding. In addition, the lower the Ki value, the more stable the bonds formed. Based on virtual screening results, the compound myricetin 4”-O-acetyl-2-O-gallate has the best binding affinity to the main protease receptor (7C6S) seen from the ΔG value of -9.82 kcal/mol with a Ki of 63.25 μM. The results are better compared to natural ligands as well as remdesivir. Based on the results, the compound myricetin 4”-O-acetyl-2-O-gallate is likely to be used as a candidate for SARS-CoV-2 therapy that inhibits the main protease receptor of the virus. Compounds with ΔG and Ki values similar to those of the natural ligand are also expected to exhibit strong binding affinities. As shown in Table 3, all the flavonoid compounds demonstrate better ΔG values than Remdesivir, the reference drug.

3.4. Visualization of Docking Results

The docking results were visualized to analyze the interactions between the amino acid residues of the ligands. Ligands are considered to interact with the target receptor if amino acid interactions are present. The observed interactions include hydrogen bonds and hydrophobic bonds, both of which contribute to the stability of the ligand-receptor complex and affect the ΔG value [31]. The selected compound for visualization is myricetin 4”-O-acetyl-2-O-gallate. Visualization was carried out using Discovery Studio software. Hydrogen bonds, which involve hydrogen atoms (H) interacting with F, O, or N, are strong dipolar forces. Hydrophobic bonds also play a crucial role in stabilizing the binding conformation and can serve as a measure of the interaction strength between the amino acid ligands and receptors [32]. Observations of hydrogen and hydrophobic bonds are presented in Table 4.

Table 4. Hydrogen and hydrophobic bonds

Compound	Hydrogen Bonds	Hydrophobic Bonds		
		Pi-sigma	Alkyl/Pi-alkyl	Van der Waals
Natural ligand	HIS164, GLU166, LEU167	HIS41	CYS145, HIS163, MET165	ASN142, ARG188, GLN192, GLY143, GLY170, HIS172, LEU141, MET49, PRO168, PRO168, THR190
Remdesivir	ASP34, LYS88, SER81, TYR101, VAL35		LYS90	GLY79, HIS80, ILE78, LEU32, TYR37
Myricetin 4"-O-acetyl-2-O-gallate	ASN142, GLU166*, GLY143, LEU141, MET49, SER144		LEU167, MET165*, PRO168	ALA191, ARG188, ASP187, GLN189*, GLN192*, HIS41, HIS163, HIS164, PHE140, TYR54

* The amino acids that interact together on the test compounds and natural ligands

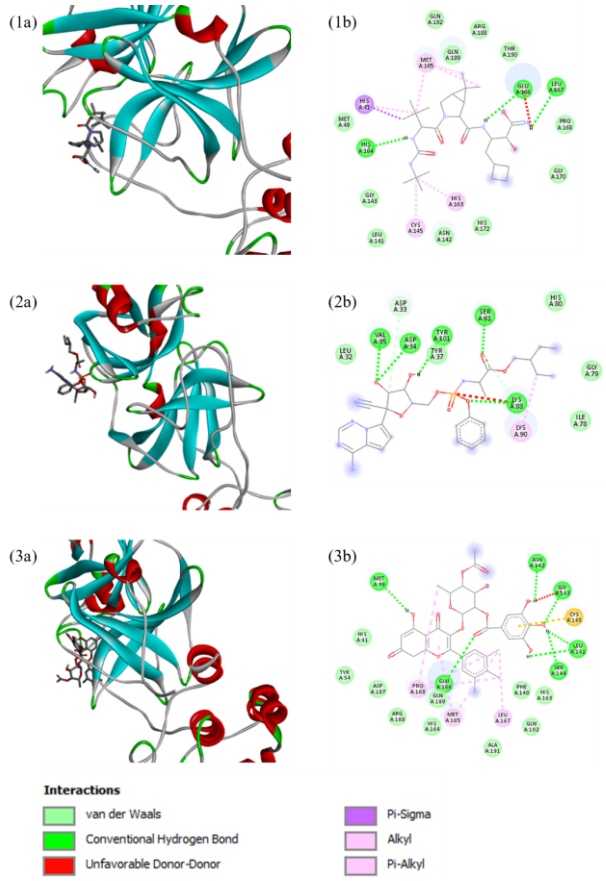


Figure 4. 3D and 2D visualizations of natural ligands (1a, 1b), Remdesivir (2a, 2b), and Myricetin 4"-O-acetyl-2-O-gallate (3a, 3b)

According to Table 4, the compound myricetin 4"-O-acetyl-2-O-gallate exhibits interactions similar to those observed with natural ligands. Specifically, it forms hydrogen bonds with GLU166 residues, alkyl hydrophobic bonds with MET165 residues, and van der Waals interactions with GLN189 and GLN192 residues.

The myricetin 4"-O-acetyl-2-O-gallate exhibits strong inhibitory activity due to its ability to form multiple hydrogen bonds and hydrophobic interactions with key residues like GLU166, GLY143, and MET165 in the receptor's binding pocket. These interactions are crucial for stabilizing the ligand-receptor complex, as demonstrated by the compound's low ΔG value of -9.82 kcal/mol. This implies a high binding affinity and potential effectiveness as a therapeutic agent. However, compounds like myricetin 3-(3"-galloylrhamnoside), which do not meet Lipinski's rules, may face challenges in bioavailability and permeability, necessitating further structural modifications or alternative delivery methods to enhance their drug-like properties.

3.5. Molecular Dynamics

Molecular dynamics simulation involves modeling the complex interactions between ligands and receptors under physiological conditions to observe drug behavior in the body over a specified period. This simulation employs a physical approach to particle dynamics and motion [22]. The ligands chosen for molecular dynamics simulation include natural ligands, best-test ligands, and comparators. The simulation was conducted using Desmond software from Schrödinger.

Molecular dynamics analysis is about the RMSD and RMSF values of ligands against receptors. RMSD is often used as a measure in three-dimensional geometry that can be used to measure changes or shifts in molecular conformation. The stability of the RMSD value is related to the protein's stability against the ligand in its binding position. Excess fluctuation of the RMSD indicates a free bond with the protein, resulting in protein denaturation [23]. Figure 5 represents the RMSD values for the molecular dynamics of the main protease (7C6S). It shows that the optimal ligands exhibit more stable RMSD values than natural and remdesivir ones.

Another parameter examined is the root mean square fluctuation (RMSF), which measures the shift of amino acid residues within proteins when interacting with ligand compounds. High RMSF values indicate considerable flexibility of the amino acid residues, suggesting that the interactions become unstable due to frequent positional changes during the simulation. Figure 6 shows RMSF results from natural ligands, best ligands, and comparators. As shown in Figure 6, the highest fluctuations for natural ligands are in the residues Gly2, Asp56, and Gly195, whereas for the stretched fluctuations of the ligands, the best are in the residues Gly23, Arg279, and Ser301. Based on this, the amino acid residues are unlikely to play an active role in ligand-receptor binding. Figure 7 shows a graph of the amino acid residues of the protein 7C6S interacting with the best ligands (myricetin 4"-O-acetyl-2-O-gallate).

Table 5. Drug screening results for flavonoid compounds from *Syzygium cumini* (L.) Skeels

Compound	Molecular weight (g/mol)	Log P	Hydrogen donor	Hydrogen acceptor	Molar refractivity	Meets 2 of 5 Lipinski's
	< 500	< 5	< 5	< 10	40-130	
Myricetin 4"-O-acetyl-2-O-gallate	651	2.8	5	14	158.60	Yes
Myricetin-3-(3-galloylrhamnoside)	641	-0.21	8	16	138.43	No
Myricetin 3-O-glucosides	504	-0.62	5	13	133.34	Yes
Myricitrin	426	-1.19	6	12	103.79	Yes
Isorhamnetin-3-β-D-galactopyranoside	476	-1.62	5	12	108.43	Yes

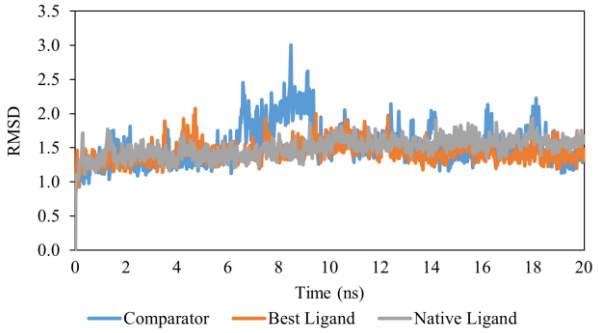


Figure 5. RMSD graph of molecular dynamics results (7C6S)

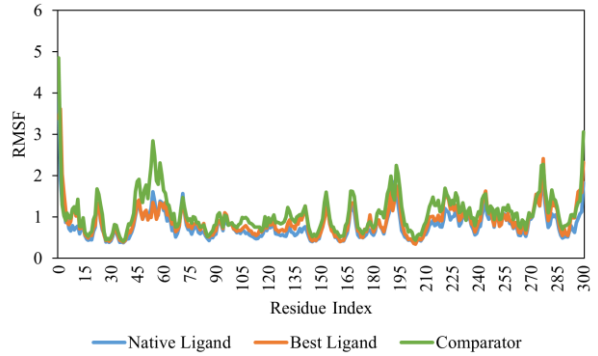


Figure 6. RMSF graph of molecular dynamics results (7C6S)

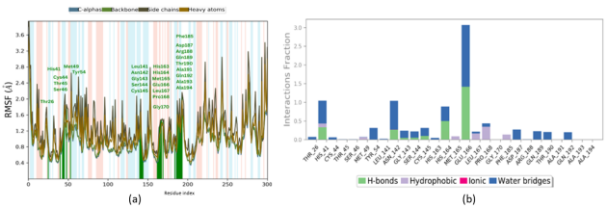


Figure 7. Molecular dynamics results of ligand contacts with the protein for the best ligand (7C6S)

3.6. Screening Ligand-based Drug-likeness (Drug Scan)

The drug scan was done by predicting a compound's physical and chemical properties to determine whether it can be used as an oral medicine. The parameters used follow Lipinski's Rule of Five, which consists of molecular weight (MW), partition coefficient (Log P), hydrogen donor, hydrogen acceptor, and molar refractivity [22, 33]. The results of the drug scan of the flavonoid compounds of the *Syzygium cumini* (L.) Skeels can be seen in Table 5.

The first of Lipinski's rules states that molecular weight is a key parameter influencing a drug compound's ability to pass through cell membranes, affecting drug distribution. If a molecule weighs more than 500 g/mol, the distribution process may be hindered as the drug struggles to diffuse across cell membranes [34]. The second Lipinski's rule of Log P relates to the drug's ability to dissolve in fats, oils, non-polar solvents, or lipophiles. A good range of lipofility is a log P ratio between -4.0 and 5. The solubility of fat and its ability to penetrate the cell membrane of the drug increase as the log P value increases [34]. The third and fourth Lipinski's rules are hydrogen bonds, consisting of the number of hydrogen donors and receptors related to the biological activity of the medicinal compound. Factors affecting biological activity are the chemical physics of the drug, such as water solubility, boiling point, melting point, clay-forming ability, and acidity [35].

Based on the drug scan results, several flavonoid compounds from *Syzygium cumini* exhibit promising potential as oral drug candidates, particularly myricetin 4"-O-acetyl-2-O-gallate. This compound meets several criteria outlined in Lipinski's Rule of Five, which are essential for drug-likeness, including optimal Log P values indicating good fat solubility and the ability to penetrate cell membranes. Additionally, it shows a favorable HIA percentage and lacks hepatotoxicity, making it a viable candidate for further development.

Previous studies have also demonstrated the antiviral properties of flavonoids from *Syzygium cumini*, reinforcing its potential for COVID-19 therapy. These characteristics suggest that myricetin 4"-O-acetyl-2-O-gallate could be effectively developed as an oral medication for inhibiting SARS-CoV-2 [36]. However, the compound myricetin 3-(3"-galloylrhamnoside), which does not conform to Lipinski's rules, may encounter challenges in drug development, particularly regarding bioavailability and permeability. Its molecular weight and hydrogen bonding characteristics might impede its ability to cross cell membranes efficiently, thereby limiting its effectiveness as an oral medication. Consequently, further modifications or alternative delivery methods may be required to improve its drug-like properties.

Table 6. Pharmacokinetic profile prediction results

Pharmacokinetic profile	Parameter	Myricetin 4"-O-acetyl-2-O-gallate	Myricetin-3-(3-galloylrhamnoside)	Myricetin 3-O-glucosides	Myricitrin	Isorhamnetin-3-β-D-galactopyranoside
Absorption	%HIA	63.311	27.931	33.394	43.334	49.713
	Caco-2 (mm/sec)	0.542	-1.398	-1.34	-0.982	0.333
Distribution	VDss (Log L/kg)	-0.183	0.44	1.542	1.552	1.246
	%BBB	-2.001	-2.907	-2.078	-1.811	-1.723
Metabolism	CYP 2D6 substrate	No	No	No	No	No
	3A4	No	No	No	No	No
	CYP 1A2	No	No	No	No	No
	2C19	No	No	No	No	No
	2C9 inhibitor	No	No	No	No	No
	2D6	No	No	No	No	No
	3A4	No	No	No	No	No
Excretion	Total clearance (Log mL/min/kg)	-0.632	0.214	0.413	0.303	0.492
	Kidney Substrate OCT2	No	No	No	No	No
Toxicity	Amest Test	No	No	No	No	No
	LD_{50} (mol/kg)	2.765	2.483	2.543	2.537	2.548
	Hepatotoxicity	No	No	No	No	No

3.7. Pharmacokinetic and Toxicity Predictions

Pharmacokinetic and toxicity predictions were made using the pkCSM program, which was conducted to determine the pharmacokinetic profile and whether a drug compound has potentially toxic properties. This prediction is made by observing a drug candidate's absorption, distribution, metabolism, excretion, and toxicity profiles.

Absorption prediction aims to determine that the drug candidate has absorption capabilities and good permeability to the body. The parameters used are HIA and Caco-2 (human colon adenocarcinoma) [37]. For the observed distribution prediction of VDss or distribution volume (Log L/kg), which is the theoretical volume of the total dose of the drug distributed evenly to obtain the same plasma concentration, a high volume of distribution affects the same concentration of the medicine as the plasma. The next parameter for the distribution profile is %BBB (blood-brain barrier), or blood-brain barrier. Knowing the ability of a drug to penetrate the brain becomes an important parameter in reducing the likelihood of side effects and the toxicity of a medication to the central nervous system [22].

Another pharmacokinetic profile observed is the metabolic profile of the drug candidate compound. Parameters seen on the metabolism profile are the metabolics of the medicinal compounds against several substrates. The enzyme that plays an important role in the metabolism of drugs in the liver is cytochrome P450. The cytochrome has several isoforms with substrate compounds, namely CYP1A2, CYP2C19, CYPs2C9, CYPS2D6, and CYPs3A. The excretion profile is also used for this pharmacokinetic prediction. The observed parameters are the prediction of the OCT2 substrate and total clearance. The kidneys have a transporter called organic cation transporter 2 (OCT2) that plays a role in kidney transport and clearance of drug compounds and endogenous compounds. This observation was done to identify authorization and potential contraindications because a compound with the properties of an OCT2 substrate results in unwanted effects if administered simultaneously with an OCT2 inhibitor [22]. Table 6 presents the forecasted pharmacokinetic profiles and toxicity assessments for five of the top 15 flavonoid leaf compounds based on virtual screening results.

The potential toxicity of the drug compounds is crucial in predicting their possible harmful effects. Key toxicity parameters include the Ames test, LD_{50}, and hepatotoxicity. The Ames test is commonly used to determine whether a compound is mutagenic. Another important parameter is oral rat acute toxicity (LD_{50}), which indicates the dose required to cause death in 50% of the test animals. Hepatotoxicity refers to the potential of a compound to cause liver dysfunction. Understanding the hepatotoxic properties of drug compounds is crucial for ensuring drug safety, as a medication is considered hepatotoxic if it induces any pathological or physiological changes in the liver [22].

4. Conclusion

Based on the study results, it can be concluded that flavonoid compounds from *Syzygium cumini* (L.) Skeels, particularly myricetin 4"-O-acetyl-2-O-gallate, are predicted to effectively bind to the main protease protein receptor (7C6S). This conclusion is supported by the ΔG

value of -9.82 kcal/mol for myricetin 4"-O-acetyl-2-O-gallate, which is lower than the ΔG values for natural ligands (-9.56 kcal/mol) and Remdesivir (-4.05 kcal/mol). The interaction between myricetin 4"-O-acetyl-2-O-gallate and the main protease protein is further evidenced by the presence of hydrogen and hydrophobic bonds. These findings suggest that myricetin 4"-O-acetyl-2-O-gallate from *Syzygium cumini* could be a promising drug candidate for COVID-19 therapy.

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