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Emodin from Rhubarb (*Rheum officinale* Baill.) as An Antiviral against SARS-CoV-2 via Inhibitor Pathway: An *In Silico* Study

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ABSTRACT

Coronavirus 2019 (COVID-19) is a respiratory disease that was first detected in Wuhan, China in December 2019 and spread throughout the world. This disease is caused by Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2). This study aimed to determine the potential of the rhubarb emodin compound (*Rheum officinale* Baill.) as an anti-SARS-CoV-2 via computational study. Sample collections were taken from PubChem and PDB databases. Sterilization is conducted using AutoDock and Notepad++. Molecular docking was performed using PyRx. Data visualized performed using PyMol and LigPlot+. Rhubarb emodin (*R. officinale* Baill.) has a binding affinity value of -7.6 kcal/mol and an RMSD value of 0. The result showed that emodin has the potential as an inhibitor of the 3CLpro activity of SARS-CoV-2. However, the results obtained are computational predictions, so further validation is needed in a wet laboratory study.

1. INTRODUCTION

Coronaviruses are a large group of enveloped viruses, positive sense, and have single-stranded RNA that can cause damage and infection in the respiratory tract of mammals, including humans [1]. Coronavirus 2019

(COVID-19) is a respiratory disease initially detected in Wuhan, China in December 2019 and then spread rapidly throughout the world. This disease is caused by Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) that remains in the same genus as an infectious virus in animals and humans. Genetic analysis shows that SARS-CoV-2 is closely related to bat-SL-CoVZC45 and bat-SL-CoVZXC21 as well as SARS-CoV and MERS-CoV [1- 3].

SARS-CoV-2 has spike proteins on the surface of the virus that binds to host receptors throughout infection [4]. This protein has a strong affinity for human angiotensin-converting enzyme-2 (ACE2) as its receptor and is extremely efficient in infecting [5]. After infection, SARS-CoV-2 has a polypeptide yield of approximately 800 kDa during transcription. These polypeptides have proteolytic properties that can form many proteins. This process is mediated by papain-like protease (PL^{pro}) and 3-chymoptrypsin-like protease ($3CL^{pro}$). $3CL^{pro}$ is able to hydrolyze ppl and pplab viral polyproteins to produce functional proteins during coronavirus replication. These proteases play a critical role in replication and so have sequences that are essential for drug development. Therefore, $3CL^{pro}$ is widely studied as a potential target inhibitor for treating SARS, MERS, and COVID-19 [6- 9].

Recent reports indicate several drugs that function as $3CL^{pro}$ inhibitors against COVID-19 infection, namely: paxlovid and molnupiravir [10], [11]. Cases of COVID-19 that continue to mutate cause some of the drugs and vaccines that have been given to be less effective. Herbal plants have high potential as anti-COVID19 agents [12]. Therefore, a therapeutic agent is required to reduce the mortality and morbidity effects of COVID-19 [13].

Indonesia has a large diversity of plant species within the world. It is recorded that 5,000 medicinal plants have essential uses in traditional medicine since ancient times [14- 16]. This traditional medicine has been applied for a protracted time in Indonesia to treat viral infections such as flu, herpes, and hepatitis [17], [18]. According to several studies, there are significant concentrations of antiviral compounds in several plants widely studied [19], [20]. One of the potentials that can be used as an antiviral for SARS-CoV-2 is rhubarb (*R. officinale* Baill.*)*. Rhubarb is known as a plant that is used as medicinal plants, herbal ingredients, and fragrances. In addition, emodin in rhubarb (*R. officinale* Baill.) has been investigated as a candidate for SARS-CoV drug [21], [22]. Therefore, this study aimed to determine the potential of rhubarb (*R. officinale* Baill.) emodin as an inhibitor of SARS-CoV-2 $3CL^{pro}$ activity.

2. Method

2.1 Sample preparation

One of the bioactive compounds found in *Rheum officinale* Baill. is emodin. This compound is believed to own antiviral potential supported previously *in vitro* studies [21], [23]. Emodin 2D, 3D, and canonical SMILES structures were obtained from PubChem (https://pubchem.ncbi.nlm.nih.gov/) in *.sdf* and canonical SMILES formats. The sterilization of protein targets from impurities was carried out using AutoDock and Notepad++ software. Ligand minimization was carried out using PyRx 1.5.7. In addition, a 3-chymoptrypsin-like protease ($3CL^{pro}$) protein database was obtained from RSCB PDB (https://www.rscb.org/) and air removal molecules with PyMol 1.7.4 (Schrödinger, Inc., USA) [24] (Figure 1).

Table 1. Results of ligand sample preparation

Ligand	ID	Formula	Canonical SMILE	3D Structure

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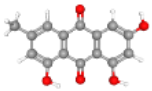
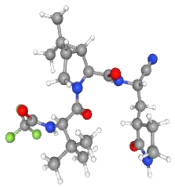
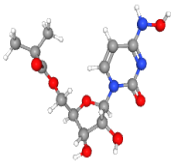
Emodin	3220	$C_{15}H_{10}O_5$	CC1=CC2=C(C(=C1)O)C(=O)C3=C(C2=O)C=C(C=C3O)O	
Paxlovid	155903259	$C_{23}H_{32}F_3N_5O_4$	COC1COCCC1NC2CC3C(C2)(CCO3)C(=O)N4CCN(CC4)C5=NC=CC(=N5)C(F)(F)F	
Molnupiravir	145996610	$C_{13}H_{19}N_3O_7$	CC(C)C(=O)OCC1C(C(C(O1)N2C=CC(=NC2=O)NO)O)O	

2.2 Biological activity probability

Biological activity between ligand and the target protein was carried out by predicting the target by visiting the Swiss Target Prediction at www.swisstargetprediction.ch/ and the PubChem database (https://pubchem.ncbi.nlm.nih.gov/). The information obtained from this procedure is in the form of canonical SMILES data. Data is saved in *.pdb* format and displayed in Autodock and Notepad++. Then docking is done to determine the energy that occurs when the molecule is bound to other molecules. Molecular docking can be done with the PyRx software because it has high accuracy [25]. This is used to determine the activity between emodin and the $3CL^{pro}$ receptor that plays a role within the formation of the COVID-19 protein.

2.3 Molecular interaction and visualization

The visualization aims to describe the docking results in detail. 3D visualization is conducted using PyMOL software and 2D using LigPlot+ 1.4.5. The visualization result using PyMol is in the type of a protein-ligand molecular complex structure [26]. Meanwhile, hydrogen interactions were visualized using LigPlot+. The results were analyzed by comparing the binding affinity, RMSD value, the type of ligand and receptor, the amino acid that is the binding site for the active compound at the binding site, and amino acid residues around the binding site [25], [27]. The conclusion of the data is the potency of emodin from the comparison of ligand-receptor interactions.

3. Results and Discussion

The predicted target results from three compounds, namely: emodin, paxlovid, and molnupiravir against 3CLpro showed the subsequent results.

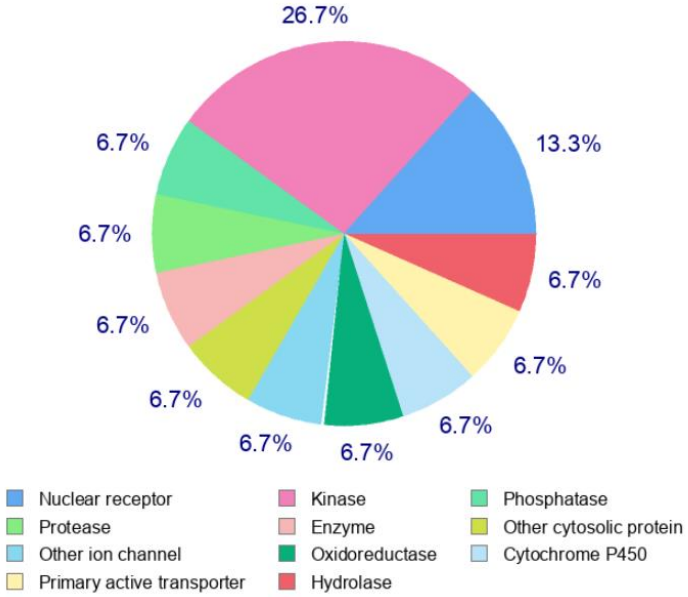


Figure 1. Prediction target of emodin

The target prediction results showed that emodin incorporates a target category in the sort of proteases as much as 6.7% of the entire target class (Figure 1). Meanwhile, the total protease target class expected with paxlovid amounted to 50%. However, the potential of molnupiravir as a predictive target is unknown. This result indicates that the potential for emodin to be targeted at proteases is little but specific, creating it appropriate to be used as a ligand substance of $3CL^{pro}$ (Figure 2).

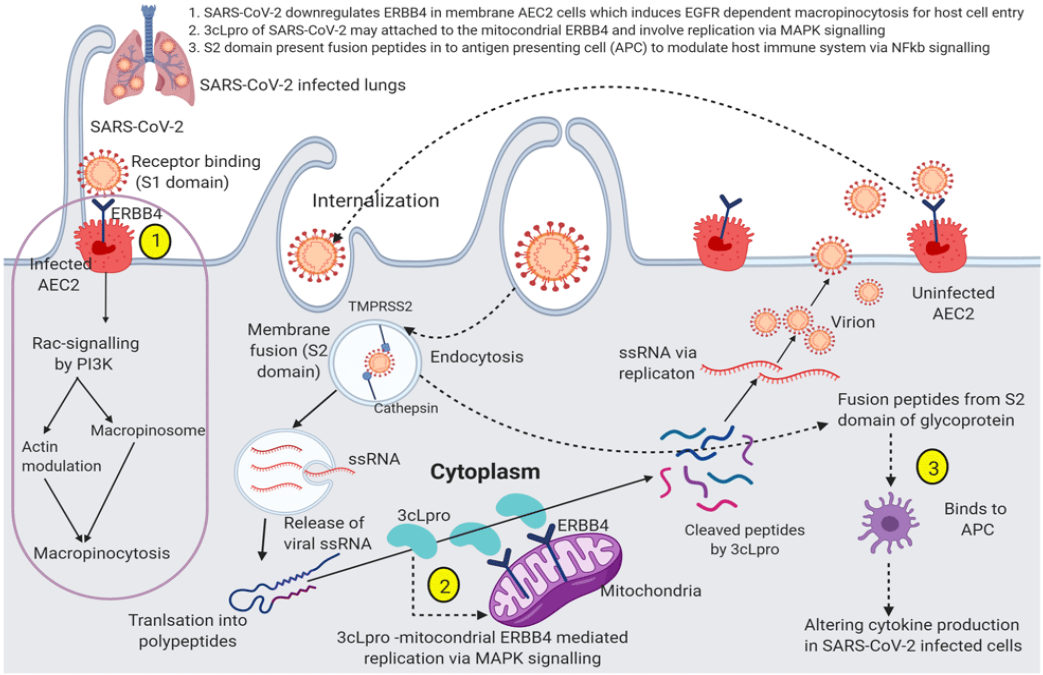


Figure 2. Molecular docking target on SARS-CoV-2 $3CL^{pro}$ [28].

Molecular docking was carried out with PyRx software to generate selected potential ligand parameters for the $3CL^{pro}$ receptor. Parameters used include binding affinity (kcal/mol), RMSD (Å), and bond interactions. The predictive value of binding affinity used is that the smallest because it shows the position of the ligand-

receptor interaction is obtaining additional stability [29]. The energy required for the binding affinity of the receptor ligand is determined by the type of bond, the amino acid group at the active site, and the amino acid residues around the ligand [30]. The more hydrogen bonds, the more stable the ligand and protein interaction [31].

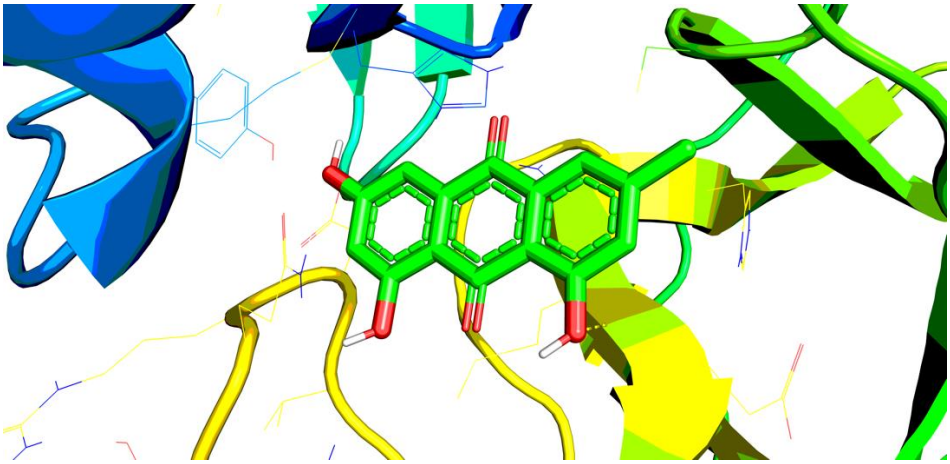


Figure 3. Molecular docking result of emodin using PyMOL software.

In addition, the RMSD (root mean square deviation) value was calculated from the best ligand-protein conformation of the atomic distance of one conformation with atoms of the same type in another conformation. Two types of RMSD will be used as parameters, particularly RMSD u.b. (upper bond) and RMSD l.b. (lower bound). RMSD u.b. describes the fit of each atom in one conformation to itself in another conformation and the symmetry is neglected. Meanwhile, RMSD l.b. is the result of the calculation of max rmsd'(c1, c2) and rmsd'(c2,c1). RMSD' is obtained from the match of each atom in one conformation with the nearest atom of the same type of component in another conformation. This RMSD value cannot be used directly because it is not symmetrical [32]. The RMSD value is claimed to be valid if it is lower than 2 [33]. After molecular docking, data within the style of ligand-protein interactions are often visualized in 3D using PyMOL (Figure 3) and 2D using LigPlot+ to show the type of bond, amino acid as the receptor active site, and ligand-receptor binding distance (Figure 4).

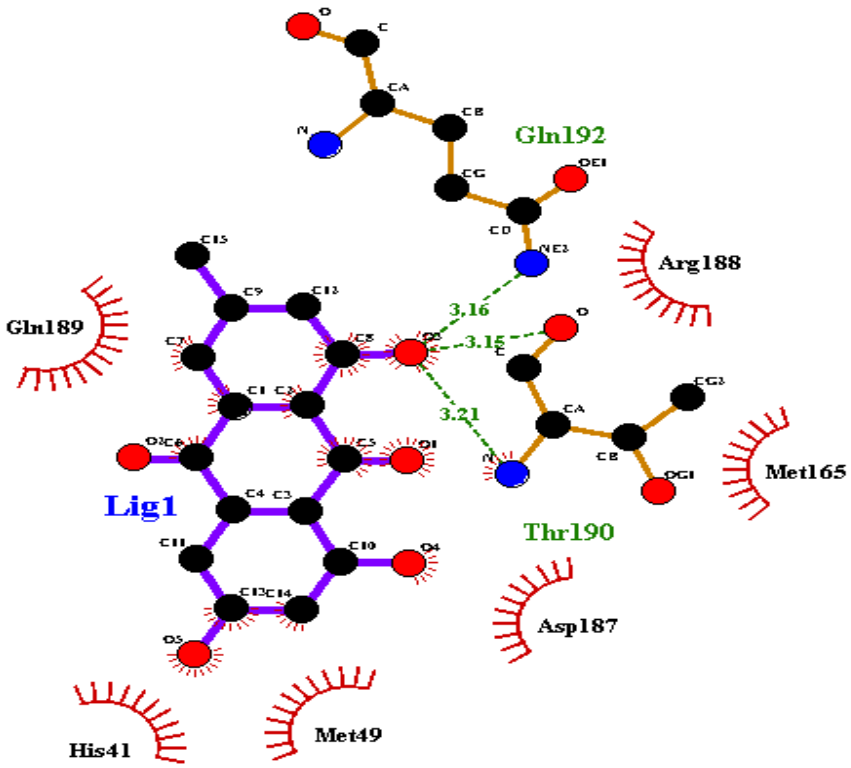


Figure 4. Emodin visualization using LigPlot^{+}

After carrying out the previous procedure, it is important to record the value of the molecular docking gain to facilitate the analysis and determination of drug candidates from natural secondary compounds. The subsequent are the results of molecular docking of three ligands against 3CL^{pro}.

Table 2. Molecular docking results of three tested ligands toward SARS-CoV-2 3CL^{pro}.

Ligand	***Binding affinity* (kkal/mol)**	**RMSD (Å)**	**Jarak atomic ikatan hidrogen (Å)**	**Residu asam amino ikatan hidrogen**	**Residu asam amino interaksi hidrofobik**
Ligand THR280	-7,6	0	3.21	Gly143	Cys145, His163, His41, Met49, Met165, Gln192, Thr190, Arg188, Pro168, Gln189, Phe140, Asn142, Leu141, and Ser144
			2.98	Glu166	
			2.90	Glu166	
			3.02	Glu166	
Emodin	-7,6	0	3.16	Gln192	Arg188, Met165, Asp187, Met49, His41, and Gln189
			3.15	Thr190	
			3.21	Thr190	
Paxlovid	-6,9	0	3.22	Gln69	Gly120, Gln19, Asn119, Gly15, Pro122, Glu14, Asn72, Ala70, and Ser121
			3.21	Gly71	
			3.21	Lys97	
Molnupiravir	-6,9	0	2.95	His163	Arg188, Gln189, Met165, Glu166, Asn142, Phe140, Leu141, Cys145,

					His164, His41, Met49, and Asp187.

Based on the table, it is known that the RMSD value of all ligand-compound interactions is zero. This value is lower than the maximum value of RMSD in order that the ligand-receptor conformation formed is stable. This stable conformation is indicated by the amino acid position of docking results not abundant, totally different from the crystallographic results [34]. Meanwhile, the bond that produces the lowest energy is the interaction between emodin and the 3CLpro receptor of -7.6 kcal/mol (Table 2). The binding affinity value is the same as the interaction energy between the THR280 ligand and its receptor protein. Meanwhile, paxlovid and molnupiravir showed higher binding affinity than the native ligand. In molecular docking, the lower binding activity energy value, the more stable the ligand-receptor conformation is [25], [29]. The type of bond found in the tested ligand is hydrogen bond. Hydrogen bond is a weak bond and the most preferred in the docking process. As a result, the presence of hydrogen bonds is very important in reducing binding affinity values throughout docking [35].

Amino acids on the receptor's active site for ligand binding are polar, uncharged, positively charged, and negatively charged amino acids. Because of the presence of polar hydrogen bonds, they can form bonds between hydrophilic amino acids. Additionally, amino acid residues around the ligand and receptor binding sites also influence the ligand and receptor bond [35].

The character of amino acid residues around the binding site is varied. The highest number of residues was found within the molpinavir-$3CL^{pro}$ interaction, namely twelve residues. That impact affects the binding energy and stability of the secondary and the tertiary structure of the tested compound on the active site of the target enzyme [36], [37]. Suppose the amino acid residue of a test ligand is analog to the native ligand. In that case, it is doubtless that the interactions that occur are similar. The activity between the tested ligand and its receptor is analogous to the interaction between the native first ligand and its receptor [38]. The number of residues within the interaction of emodin with $3CL^{pro}$ is sort of equivalent to the interaction between the original ligand and $3CL^{pro}$. This caused the binding affinity of the 2 ligands to be the same. Therefore, emodin has high potential as a drug candidate for inhibiting SARS-CoV-2 proteins formation.

Rhubarb (*R. officinale* Baill.) has been tested *in silico* as a $3CL^{pro}$ inhibitor in SARS-CoV-2. However, the results obtained by this study are theoretical and should be explored further through in vivo and in vitro tests to validate the potential advantages of the bioactive compounds present in rhubarb (*R. officinale* Baill.).

4. Conclusion

Rhubarb (*R. officinale* Baill.) was predicted to have antiviral activity because of the presence of emodin. Emodin encompasses a binding affinity value of -7.6 kcal/mol and an RMSD value of 0 so that it binds strongly to the active site of SARS-CoV-2 $3CL^{pro}$. However, further research is needed to support the results of this study.

5. Acknowledgement

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