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# In Silico Analysis of Catechin, Galangin, and Hesperidin as Competitors of the SARS-CoV-2 Spike Protein

**Astuti Kusomorini[*1], Nisrina Hasna[2], Yani Suryani[3], Opik Taufiqurrahman[4]**

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[1,2,3]Department of Biology, Faculty of Science and Technology UIN Sunan Gunung Djati, Bandung 40614

*e-mail*:
[1*]*astutiAuinsgd.ac.id*
[2]*nisriniahasna14@gmail.com*
[3]*yani.suryani@uinsgd.ac.id*
[4]*otaupiqurrohman@gmail.com*

**Corresponding author*

***Abstract.*** *Currently, Covid-19 has become endemic. However, the development of Covid-19 drugs continues to be carried out to suppress the growth of the Sars-Cov-2 virus. Some compounds with antiviral activity are catechin, galangin, and hesperidin. Angiotensin-converting enzyme-2 (ACE-2) is a protein that enters viruses into the cell. Based on that, ACE-2 can be used as a primary target to suppress the development of the Sars-Cov-2 virus. This study aimed to test the catechin, galangin, and hesperidin compounds in inhibiting the SARS CoV-2 virus from attaching to ACE-2 by trying the interactions of catechin, galangin, and hesperidin compounds with ACE-2 using the in-silico method. The material used was the three-dimensional structure of the compounds catechin, galangin hesperidin, and ACE-2. The tools used were FAF-Drugs4, Discovery Studio, and Pyrex software. Low-affinity energy values (kcal/mol) indicate promising results. The results showed that the energy affinity value of catechin was -6.2 kcal/mol, galangin was -6.3 kcal/mol, and hesperidin was -8.3 kcal/mol. This value is lower than the control affinity energy (chloroquine and favipiravir), which is -5.2 kcal/mol and -4.8 kcal/mol, respectively. Based on this, catechin, galangin, and hesperidin can be used as inhibitors/competitors for the Sars-Cov-2 to attach to ACE-2.*

***Keywords:*** *ACE-2, COVID-19, flavonoid, molecular docking, SARS-CoV-2*

**Citation**

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## INTRODUCTION

SARS-CoV-2 is a zoonotic virus that spreads from animals to humans via the Angiotensin-converting Enzyme 2 (ACE-2) receptor (Guo et al., 2020). This virus has mutated into several variants, including Alpha (B.1.17), Beta (B.1.351), Delta (B.1.617.2), Omicron (B.1.1.529), and others (Indonesian Ministry of Health, 2020). Patients with this disease exhibit mild to moderate symptoms such as fever, headache, dry cough, sore throat, and blown or breathlessness (Sohrabi et al., 2020; Su et al., 2020). Angiotensin-converting enzyme-2 (ACE-2) is a type 1 integral membrane protein expressed in some tissues, including the heart, respiratory tract, and digestive tract. This protein is the virus's first contact point with the human body's cells (Basu et al., 2020).

Coronavirus disease-2019 (COVID-19) patients are treated medically by reducing symptoms, such as administering antivirals. Favipiravir is an antiviral compound used

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to treat COVID-19 (Serap & Serhat, 2020). Aside from favipiravir, chloroquine is an antiviral compound for malaria that has been shown in vitro to have inhibitory activity against the COVID-19 variant Beta (B.1.351) (Wang et al., 2020).

Drug development based on natural bioactive compounds is the best option because they have fewer side effects than chemical medicines (Atanas et al., 2021). Flavonoids are plant-bioactive compounds that have been shown to reduce the inflammation caused by various infections, including viral infections (Tapas, 2008). The flavonoid has been tested in vitro that it can inhibit the active center of the ACE-2 receptor (Muchtaridi et al., 2020). These compounds are known to have a medical effect, such as catechin as hepatoprotective (Tapas, 2008), galangin as antibacterial (Jin et al., 2021), and hesperidin as an anti-inflammatory (Widiasari, 2018 ).

In silico study using a molecular docking approach is an alternative method for screening the new COVID-19 drug candidates. This method has advantages such as low cost, short time, greater efficacy than monotherapy in combination with other drugs, and the mechanism of action of new drugs is easily identified (Galea et al., 2020). This study used an in silico molecular docking test on flavonoid compounds, namely catechin, galangin, and hesperidin, to predict new COVID-19-specific drug candidates and to identify the potential of these flavonoid compounds to inhibit the ACE-2 receptors as competitors for the SARS-CoV-2 Spike protein.

## MATERIALS AND METHODS

### Materials and Tool

The materials used consist of three-dimensional (3D) structures of receptors and ligands. The receptor is ACE-2, and the Ligand consists of catechins, galangin, hesperidin, and control compounds (chloroquine and favipiravir). The 3D structure of the receptor is downloaded from the protein database (https://www.rcsb.org/). At the same time, the 3D ligand structure is downloaded at PubChem (https://pubchem.ncbi.nlm.nih.gov/). The PDB code for ACE-2 is 1R42. PubChem code for catechin is 9064, galangin 5281616, hesperidin 10621, chloroquine 27190, and favipiravir 492405.

The tools used are hardware and software. The hardware consists of a personal computer with the Windows 10 operating system. The software consists of FAF-Drugs4 program, PyRx, and Discovery Studio.

### Procedures

#### *Pharmacological Analysis*

This analysis was carried out to determine the reaction of the body's test compound (catechins, galangin, and hesperidin) (drug-likeness). The examination consists of Absorption, Distribution, Metabolism, Excretion, and Toxicity (ADMET). Pharmacological analysis followed Lipinski's rules using the FAF-Drugs4 program on the RPBS Web Portal site (https://mobyle.rpbs.univ-paris-diderot.fr/cgi-bin/portal.py?form=FAF-Drugs3#forms::FAF -Medicine4).

#### *Preparation of Receptor*

The 3D structure of the ACE-2 protein was prepared by removing water molecules and natural ligands using the tools available in the Discovery Studio Visualizer application. The crafted structure file was exported in .pdb format.

#### *Preparation of Ligands*

Ligand preparation was carried out using tools in the PyRx software. Catechin, galangin, and hesperidin were converted into.

pdb format. Afterward, energy minimization was performed on all 3D beams of previously changed compounds. The compound file was then saved in .pdb format.

### *Molecular Docking*

Molecular docking was carried out using the PyRx software application with the Autodock Vina system, Auto Dock 4.0, and Auto Dock Tools. The prepared ACE-2 protein files were entered into the application. Afterward, the A chain was selected to be tethered with a ligand. The ACE-2 protein file was converted into .pdbqt format using Autodock> Make Macromolecule, while the prepared Ligand was converted. pdbqt format using Autodock > Make Ligand. Molecular docking was done by pressing Vina Wizard> Autodock> Autodock. TBindingnsions of the docking grid were set according to the dimensions formed by chloroquine and favipiravir (control), which bind to the active site (binding site) on the ACE-2 receptor and make it the center point. The analysis results were saved in. pdb format once the tethering was complete.

### *Visualization*

The interaction of the Ligand with the ACE-2 was visualized in 2D and 3D diagrams using the Discover Studio Visualizer software to determine the amino acid residues involved in the binding that occurs to the ligand compound with the target protein (Listyani et al., 2019).

## RESULTS AND DISCUSSION

### Pharmacological Analysis

The analysis process follows the Lipinski rule of five. Lipinski's rule of 5 helps distinguish between drug-like and non-drug-like molecules. It predicts a high probability of success or failure due to drug-likeness for molecules complying with 2 or more of the following rules: 1) Molecular mass less than 500 Dalton, 2) High lipophilicity (expressed as LogP less than 5), 3 Less than 5 hydrogen bond donors, 4 Less than 10 hydrogen bond acceptors, and 5) Molar refractivity should be between 40-130. Rasool et al. (2019) stated that the desired criterion for Lipinski's rules is zero rule violations. The prediction results of the Lipinski and ADME-Tox profiles for the compounds catechin, galangin, and hesperidin are presented in Table 1.

Table 1. Analysis results of ADME-Tox characteristics

| **ADME-Tox** | **Compound** | | | | |
|---|---|---|---|---|---|
| **Characteristics** | **Catechin** | **Galangin** | **Hesperidin** | **Klorokuin[o]** | **Favipiravir[o]** |
| MW (<500 Dalton) | 290.27 | 270.24 | 610.56* | 319.87 | 157.1 |
| logP (<5) | 0.51 | 2.25 | -1.02 | 4.63 | -0.56 |
| HBD (<5) | 5 | 3 | 8* | 1 | 3 |
| HBA (<10) | 6 | 5 | 15* | 3 | 5 |
| Solubility | Good | Good | Good | Good | Good |
| OB | Good | Good | Good | Good | Good |
| 4/400 | Good | Good | Good | Good | Good |
| 3/75 | Good | Good | Good | Bad | Good |
| Lipinski's Violation | 0 | 0 | 3 | 0 | 0 |

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The Lipinski rule analysis revealed that hesperidin was the only compound that violated the Lipinski rule. This compound violates the Lipinski rule three times on molecular weight (MW), hydrogen bond donor (HBD), and halogen bond (HB). The MW of each compound is 290.27 Dalton for catechin, 270.24 Dalton for galangin, and 610.56 Dalton for hesperidin. According to Syahputra (2014), molecules with a molecular weight of more than 500 Daltons will be complex for the body to absorb because the compounds cannot pass through the cell membrane. The lipophilicity analysis showed that the drug candidate compounds have an expected value of less than five (5). This value determines the compound's ability to dissolve in fat in the body (Lipinski, 2004). However, a too-negative value is undesirable because it may prevent the molecule from passing through the cell membrane (Adriani, 2018). In drug-candidate compounds, hydrogen bonds play a critical role in receptor interactions. According to Lipinski's rules, the number of suitable hydrogen bonds donor (HBD) is less than five (5). Catechin and galangin had an HBD of less than five, indicating that they met the expected criteria, whereas hesperidin had an HBD greater than five. According to Pratama (2020), the amount of HBD greater than 5 causes the compound's interaction with the receptor to be disrupted. The hydrogen bond acceptor (HBA) analysis revealed that catechin and galangin have 6 and 5 HBA values, while hesperidin had more than 10 HBAs. The number of good HBAs is less than 10 (Lipinski, 2004). According to Adriani (2018), the higher the hydrogen bonds, the higher the energy required for the absorption process in the body (Syahputra, 2014).

The solubility level, bioavailability (OB), the relationship between molecular weight and logP (4/400), and the relationship between logP and solubility (3/75) were all examined in the ADME-Tox properties of drug candidate compounds. The analysis result in Table 1 shows that all compounds were predicted to havegood solubility, allowing them to be absorbed by the body properly. Bioavailability (OB) is the percentage of drug that enters the circulatory system and distributes throughout the body. According to Novian (2019), good drug candidates have an OB percentage greater than 30%. The analysis showed that all the compound has an OB value greater than 30%, indicating a good OB value. The analysis result of toxicity properties based on the relations between molecular weight and logP (4/400) also showed promising results, and the relationship between logP and solubility (3/75), except for chloroquine which had a value of 3/75 that, is categorized as less toxicity.

**Molecular Docking Analysis**

The results of the molecular docking of catechin, galangin, and hesperidin with ACE-2 are shown in Table 2. Based on Table 2. the binding affinity value between hesperidin and ACE-2 had the lowest value (-8.3 kcal/mol). Syahputra (2014) exposed that the more inferior the affinity value, the more stable the interaction between the ligand and the receptor. The binding affinities of catechin and galangin to ACE-2 were not much different, -6.2 kcal/mol and -6.3 kcal/mol, respectively, which were lower than the control (chloroquine is -5,2 Kcal/mol and favipiravir is -4,8 Kcal/mol).

Based on Table 3, the interaction between catechin and ACE-2 forms three hydrogen bonds, two hydrophobic bonds, and eight Vander wall bonds (Figure 1). Interaction between galangin and ACE-2 forms three hydrogen bonds, two hydrophobic bonds, and seven Vander wall bonds (Figure 2). Mean-

while, the interaction between hesperidin and ACE-2 forms six hydrogen bonds, two electrostatic bonds, four hydrophobic bonds, and eleven Vander wall bonds (Figure 3). As for the control compound, the interaction between chloroquine and ACE-2 forms one hydrogen bond, two electrostatic bonds, three hydrophobic bonds, and eight Vander wall bonds (Figure 4). The interaction between Favirapir and ACE-2 forms four hydrogen bonds, two halogen bonds, one hydrophobic bond, and two Vander wall bonds (Figure 5). Comparing the number of bonds between the test compounds (catechin, galangin and hesperidin) with the control compounds (chloroquine and Favirapir), the interaction of the test compound with ACE-2 is very stable. Figure 1-5 is a visualization of the test and control compounds bound to the active site of ACE-2. All compounds are in the same active site. This means that all the compounds are attached at the right location on the receptor, while Figure 6 shows the types of amino acids that comprise ACE-2 that attach to the test and control compounds.

Table 2. Results of molecular docking of compounds with ACE2 receptors

| Compound | Binding affinity (kcal/mol) | RMSD (Å) |
|---|---|---|
| Catechin | -6.2 | 2.2 |
| Galangin | -6.3 | 2.1 |
| Hesperidin | -8.3 | 3.0 |
| Klorokuin* | -5.2 | 1.4 |
| Favipiravir* | -4.8 | 1.8 |

Table 3. Comparison of the number of interactions of compounds with ACE2 receptors

| Ligands | Interaction Type | | | | | Amount |
|---|---|---|---|---|---|---|
| | Hydrogen | Electrostatic | Halogen | Hydrophobic | Van der Waals | |
| Catechin | 3 | - | - | 2 | 8 | 15 |
| Galangin | 3 | - | - | 3 | 7 | 13 |
| Hesperidin | 6 | 2 | - | 4 | 11 | 23 |
| Klorokuin* | 1 | - | - | 3 | 8 | 12 |
| Favipiravir* | 4 | - | 2 | 1 | 2 | 9 |

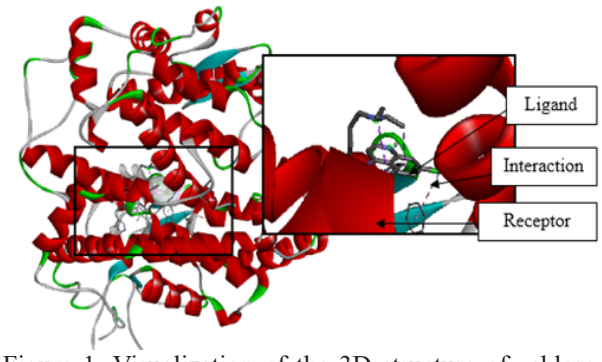


Figure 1. Visualization of the 3D structure of chloroquine with ACE2 receptor

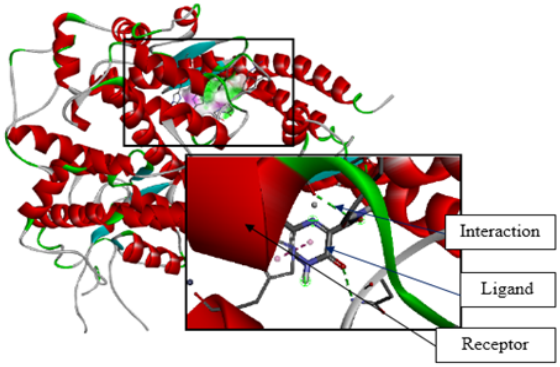


Figure 2. Visualization of the 3D structure of favipiravir with ACE2 receptor

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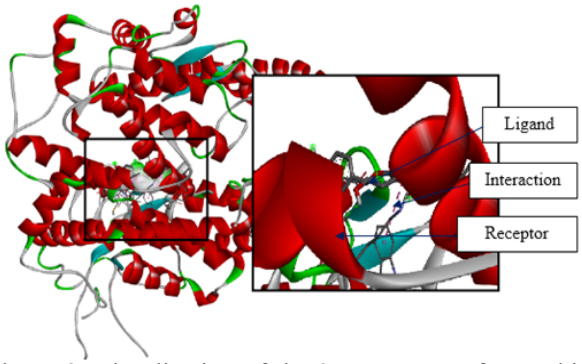



Figure 3. Visualization of the 3D structure of catechin with ACE-2 receptor

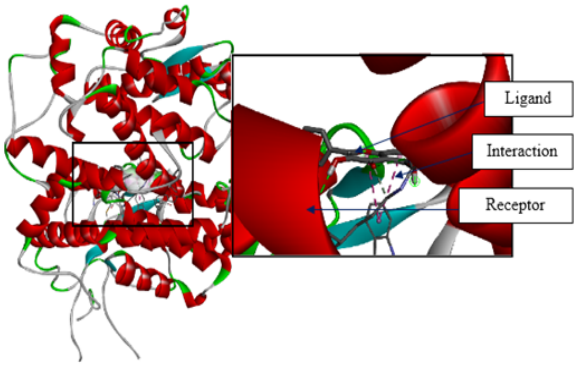
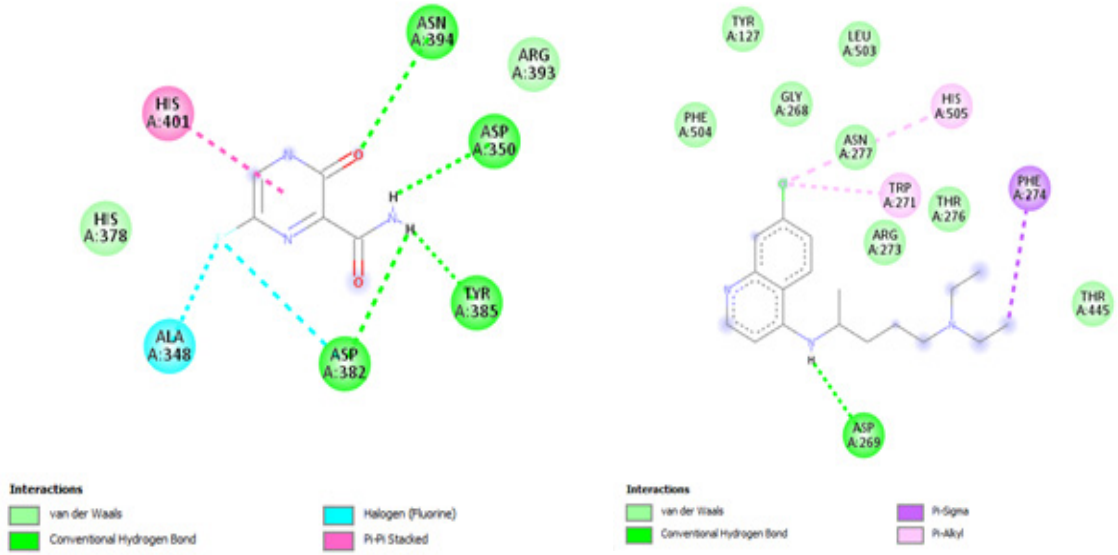



Figure 4. Visualization of the 3D structure of galangin with ACE-2 receptor


Figure 5. Visualization of the 3D structure of hesperidin with ACE-2 receptor



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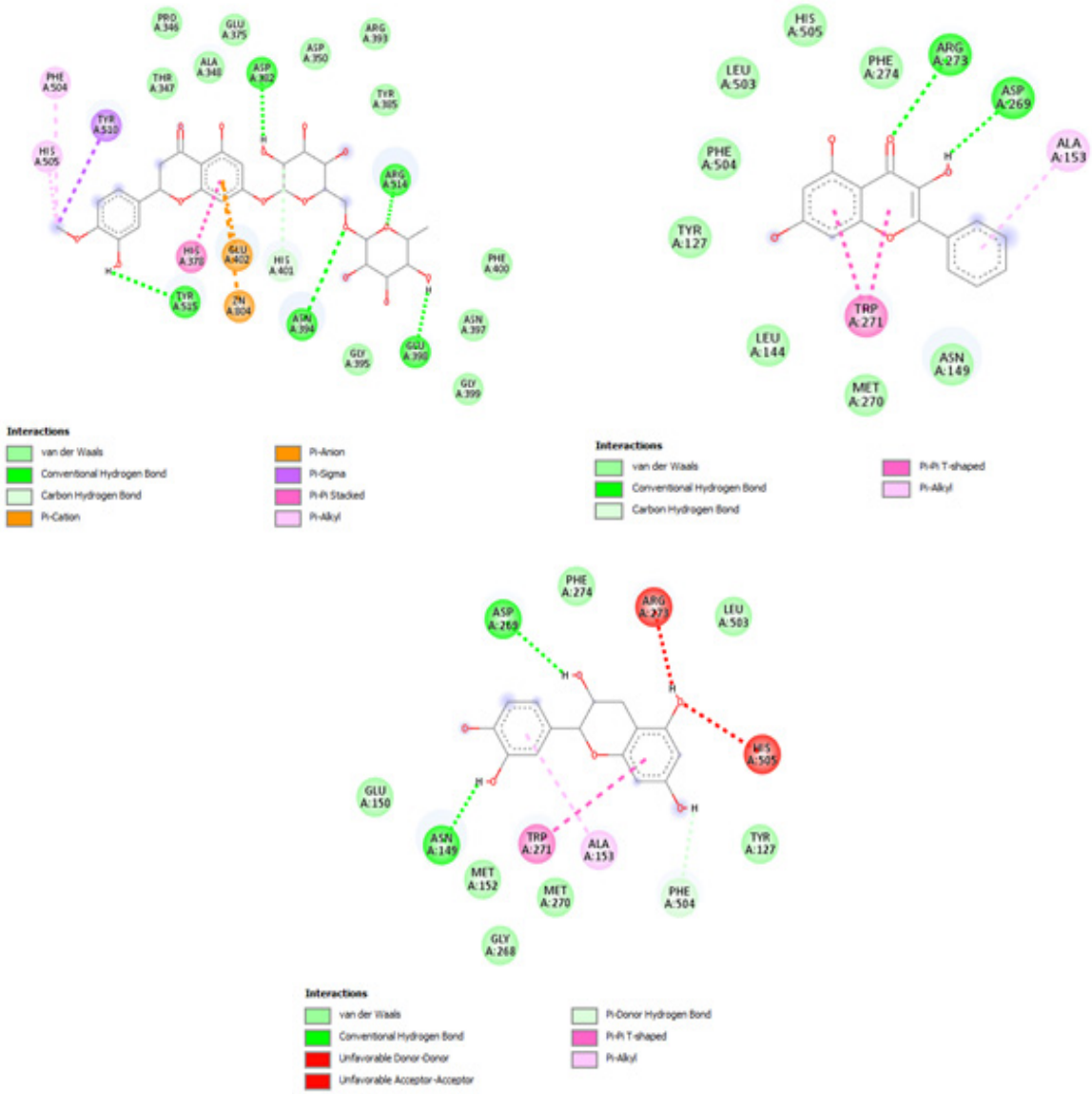


Figure 6. Visualization of the chemical interactions between the ligand and the ACE-2 protein

## CONCLUSION

The catechin, galangin, and hesperidin compounds have stable interactions with ACE-2, which is indicated by the lower affinity values of the three compounds compared to the control. Apart from that, the three compounds are safe for consumption, and this is shown by fulfilling the Lipinski pharmacological test requirements.

## AUTHOR CONTRIBUTION

A.K. wrote the manuscript and supervised all the study processes; Y.S. and O.T. designed the research and analyzed the data, and N.H. collected and conducted the investigation.

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## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest in this research.

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