Herbal plants from Riau Province as inhibitors of COVID-19 binding to ACE2 receptor by computer aided molecular design an in-silico method

R Novianty*, S Ananta1, and M A Karim1
1Department of Chemistry, Faculty of Mathematical and Natural Science, Universitas Riau, Pekanbaru 28293, Indonesia
*rirynnovianty@lecturer.unri.ac.id

Abstract. Corona Virus Disease (COVID-19) is announced as pandemic by World Health Organization (WHO) since 11th March 2020. Riau Province has many herbal plants e.g. Cheilocostus specious, Euphorbia hirta l, Cassia torra, Bryophyllum pinnatum, Daniella ensifolia, and Ziziphus mauritiana that can cure many diseases and there is not report yet focusing on in-silico method using SARS-CoV-2 protease (PDB ID:6LU7). This study aims to reveal the potential of compounds contained in Riau Herbal Plants as anti SARS-CoV-2 through its binding to protein receptors. The study was conducted by molecular docking using Autodock Vina 1.5.6 and drug ability studies using Swiss ADME. The docking results of six active compounds including diosgenin, tannin, triterpenoid, chrysophanol, flavone and phytosterol were -8.3; -7.5; -7.5; -7.2; -6.9; -6.9 respectively against the affinity result for natural ligand of COVID-19 (-6.1). This result indicates the stronger bond between ACE2 and inhibitors because the affinity value of active compounds are higher than natural ligand of COVID-19. The SwissADME results show that triterpenoid and tannin violate some Lipinski Rules that make their drug-likeness is low. In general, all the active compounds were potential as candidates of SARS-CoV-2 antiviral but the most potential one is diosgenin in Cheilocostus specious.

1. Introduction
Since December 2019, a new virus that attacks the respiratory tract has been discovered, namely SARS-CoV-2 or better known as the corona virus. The virus has now been declared a pandemic on March 11, 2020 by the World Health Organization (WHO)[1]. This corona virus pandemic has caused at least 213 countries affected by the corona virus outbreak with the number of cases of around 16 million people worldwide[2]. This virus has been researched and it has been found that there are similarities between the genome of this corona virus and the genome that belongs to a bat. Even after further investigation, this virus has some similarities with the existing Sever Acute Respiratory System (SARS) viruses so that this virus is grouped into the SARS virus family[3]. The mechanism of corona virus infection in the human body begins with the attachment of a spike protein from the corona virus to a receptor protein in human cells, namely Angiotensin Converting Enzyme Receptor 2 (ACE2)[4].

Usually, the mucosal cells of the respiratory tract will be infected first and then will spread into the alveoli epithelial cells in the lungs. The process of transferring genetic material by viruses to cells will be carried out in a process called endocytosis[5]. The pandemic situation is still ongoing...
and no one knows how long this situation will continue because no effective vaccine has yet been found. Preventive agents or drugs to prevent SARS-CoV-2 virus must be found immediately so that this situation does not continue for too long. However, referring to the existing experience, handling this virus outbreak will take quite a long time, maybe it could be years until an antiviral or corona virus prevention drug is found[6]. Thus, what can be done now to prevent the wider spread of the corona virus, it is necessary to find preventive drugs that can inhibit the corona virus before the virus attaches to the ACE2 binding site. Referring to the current situation where governments around the world are asking their citizens not to do too much activity outside the home, research for ligands as SARS-CoV-2 inhibitors is carried out using in silico (computer assisted) methods such as using molecular docking software. Molecular docking may be a study that studies how two or more molecular structures can bind together which are described through 3-dimensional visualization. Molecular docking is employed to predict the shape of intermolecular bonds between two or more molecules, but it also can be wont to see interactions between ligands and proteins, possible places where interactions occur between ligands and proteins and study the standard and quantity of energy between interacting particles[7]. Molecular docking studies have made a really important contribution to the method of drug discovery for several years. One of the most motivations in drug discovery is to spot the position of small innovative molecules, show high binding affinity, and selectivity at the target alongside a feasible ADME profile (adsorption, distribution, metabolism, excretion). The drug design process requires techniques to work out and predict the geometry, conformation, and electronic properties of small molecules (drugs with molecular weights but 500) and macromolecules (protein receptors), during this case, molecular docking can display the info needed to drug design[8].

Herbal plants have a very big role, especially in dealing with easily transmitted diseases in the past. From various clinical studies that have been conducted, it is found that herbal plants have great potential in handling the SARS-CoV-2 virus, especially during a pandemic like this time[9]. H1N1 virus infection can reduce the level of infection by using herbal plants that have been tested through a meta-analysis[10]. Referring to previous experience, the use of herbal plants is one of the steps that needs to be tried and developed to deal with COVID-19. The herbal plant has been reported to have several advantages in its use as a treatment for COVID-19 until now [11]. Riau Province has many herbal plants e.g. Cheilocostus specious, Euphorbia hirta L, Cassia torra, Bryophyllum pinnatum, Daniella ensifollia, and Ziziphus mauritiana which will cure many diseases and there’s not reporting yet that specialize in in-silico method using SARS-CoV-2 protease (PDB ID:6LU7). This study aims to reveal the potential of compounds contained in Riau Herbal Plants as anti SARS-CoV-2 through its binding to protein receptors. The study was conducted by molecular docking using Autodock Vina 1.5.6 and drug ability studies using SwissADME.

2. Materials and Methods
2.1 Software and program
The research was carried out with computer assistance (an in silico method) such as using several software such as Discovery Studio 2020, Autodock Vina 1.5.4, PyMol, and SwissADME. Discovery Studio 2020 is used to visualize protein and ligand structures, separate native ligands from protein structures, remove water molecules from protein and ligand structures, and add polar hydrogen to the structures. PyMol is used to visualize the interaction between protein and ligand and SwissADME is used to see the adsorption and permeation properties of a compound that is used as a drug candidate according to the Lipinski Rule.
2.2 Preparation of ligand structures for chrysophanol, diosgenin, flavone, phytosterol, tannin, and triterpenoid

The six compounds that we have been tested in this research is founded from Riau’s herbal plant such as given in Table 1.

Table 1. The origin of the compounds tested in this study

| No | Traditional Name | Scientific Name | Chemical Compound | Tested Compound |
|----|------------------|-----------------|-------------------|-----------------|
| 1  | Sitawa           | Cheilocostus speciosus | Diosgenin, Tannin, Steroid, Alkaloid, Phenol, etc [12] | Diosgenin |
| 2  | Daun Sikiliar    | Euphorbia hirta | Alkane, Triterpenoid, Phytosterol, Tannin, Polyphenol, and Flavonol[13,14] | Phytosterol |
| 3  | Galinggiang      | Cassia torra | Tannin, Chrysophanol, Emodin, Anthroquinones, etc [15] | Tannin |
| 4  | Sidingin         | Bryophylium pinnatum | Oleic Acid, Hexadecanoid Acid, Octadecanoid Acid, Benzaldehyde, 23-dihydro-3,5-dihydroxy-6-metil-4H-Pyran-4-one, Flavone [16] | Flavone |
| 5  | Siak-siak        | Daniella ensifolia | Dihidronaftaquinon, Chrysophanol, Isoeugentol [17] | Chrysophanol |
| 6  | Bidara           | Ziziphus spina-christi L. | Spinanin A, Tannin, β-Sitosterol, Rutin, Quarsetine, Triterpenoid, Betulinic Acid [18] | Triterpenoid |

The structures of the compounds used in this study can be downloaded at PubChem (https://pubchem.ncbi.nlm.nih.gov) with a 3-dimensional structure in the Spatial Information File (.SDF) file format. The structures used in this study were then studied for their physicochemical properties in the human body with the help of SwissADME (https://www.swissadme.ch) to determine the activity of these compounds in the human body[19,21]. These physicochemical properties can be used to analyze the best drug candidates based on molecular weight, H-donor, H-acceptor, and log P according to Lipinski's Rule[20,21]. The physicochemical properties that have been tested by SwissADME can be seen in Table 2 below.

Table 2. Physicochemical properties of the present ligands

| Physicochemical Properties | Chrysophanol | Diosgenin | Flavone | Phytosterol | Tannin | Triterpenoid |
|----------------------------|--------------|-----------|---------|-------------|--------|-------------|
| PubChem CID                | 10208        | 99474     | 10680   | 12303662    | 250395 | 451674      |
| Molecular Formula          | C_{15}H_{10}O_{4} | C_{27}H_{22}O_{3} | C_{15}H_{10}O_{2} | C_{20}H_{9}O | C_{27}H_{26}O_{18} | C_{30}H_{18}O_{8}S |
| Molecular Weight (g mol^{-1}) | 254.24      | 414.62    | 222.24  | 414.71      | 636.47 | 552.76      |
| Hydrogen-binding donors    | 2            | 1         | 0       | 1           | 11     | 3           |
| Hydrogen-binding acceptors | 4            | 3         | 2       | 1           | 18     | 7           |
| Rotatable bond count       | 0            | 0         | 1       | 6           | 10     | 4           |
| XLogP3                     | 0.92         | 4.94      | 2.27    | 6.73        | -2.42  | 4.37        |
The compounds that have been downloaded in the form of SDF are then opened in the "Discovery Studio 2020" software to optimize the structure of the compound that will be coded as a candidate ligand that will become the target protein inhibitor. After the structure is optimized, it will be saved in the form of a PDB (Protein Data Bank) extension and will be opened in Autodock Vina on the Ligand menu and then determined the torque on the ligand and finally the ligand is saved in the form of a PDBQT extension[22].

2.3 Preparation of macromolecule structures of the protein SARS-CoV-2
The structure of the corona virus protein used in this study can be downloaded on the https://www.rcsb.org page coded 6LU7 with the PDB file extension. The downloaded file is then opened at Discovery Studio 2020 to remove all water molecules present in the 6LU7 protein structure and add the existing polar hydrogen to the structure. Then the original ligand of the 6LU7 protein is separated and stored into two different files. The original ligand of the 6LU7 protein is called an N3 inhibitor which is a positive control in this study. After the optimization of the structure was carried out in Discovery Studio, the protein structure was then opened in Autodock Vina to set up grid boxes which were useful for determining where the binding could occur and the file was saved in PDBQT format[23-25].

2.4 Docking methodology
The docking process is carried out with the help of Autodock Vina 1.5.4 software. Previously the target protein (6LU7) had a grid box set to determine the location for the possible binding site to occur, in this experiment the grid box size used was 40 x 40 x 40 (x, y and z) with the grid center set at position -26,283, 12,599, and 58,569 (x, y and z) and grid spacing of 1000 Å. After the ligand and target protein preparations are ready, then a set of codes (commands) is created in a notepad and will be operated via the command prompt to start the docking analysis. The results that appear in this documenting analysis are the value of affinity binding / gibbs free energy (∆G) in kcal / mol units which is the result of the interaction between the ligand and the target protein[22].

3. Results
The results of the docking that has been carried out for the six ligand candidate compounds and the native ligand which is a positive control in this experiment can be seen in Table 3.

Table 3. Binding affinities of chrysophanol, diosgenin, flavone, phytosterol, tannin, and triterpenoid at the active site of SARS-CoV-2

| Ligands       | 1st     | 2nd     | 3rd     | 4th     | 5th     | 6th     | 7th     | 8th     | 9th     |
|---------------|---------|---------|---------|---------|---------|---------|---------|---------|---------|
| Chrysophanol  | -7.2    | -7.1    | -7.1    | -7.0    | -7.0    | -6.9    | -6.7    | -6.4    | -6.3    |
| Diosgenin     | -8.3    | -7.9    | -7.7    | -7.6    | -7.5    | -7.5    | -7.4    | -7.3    | -7.3    |
| Flavone       | -6.9    | -6.8    | -6.5    | -6.2    | -5.9    | -5.8    | -5.7    | -5.6    | -5.6    |
| Phytosterol   | -6.9    | -6.5    | -6.4    | -6.1    | -6.1    | -6.1    | -6.0    | -5.9    | -5.9    |
| Tannin        | -7.5    | -7.4    | -7.4    | -7.4    | -7.3    | -7.3    | -7.3    | -7.3    | -7.2    |
| Triterpenoid  | -7.5    | -7.4    | -7.4    | -7.2    | -7.1    | -7.0    | -6.8    | -6.8    | -6.7    |
| Natural ligand| -6.1    | -6.0    | -5.9    | -5.8    | -5.8    | -5.7    | -5.7    | -5.6    | -5.5    |
From Table 3 it can be seen from the seven docking compounds that diosgenin has the lowest binding affinity value compared to other compounds and a positive control with a value of -8.3 kcal / mol. The binding affinity value above is influenced by the presence of several interactions that arise between the target protein and the compounds that act as ligands. These interactions include hydrophobic interactions, hydrogen bonds and electrostatic interactions. This interaction can be seen in Figure 1 and Table 4 below.

![Figure 1](image)

**Figure 1.** Binding conformation of the compounds in the SARS-CoV-2 binding site. (A) Ligand-binding interactions between Flavone and 6LU7. (B) Ligand-binding interactions between Triterpenoid and 6LU7. (C) Ligand-binding interactions between Phytosterol and 6LU7. (D) Ligand-binding interactions between Chrysophanol and 6LU7. (E) Ligand-binding interactions between Diosgenin and 6LU7. (F) Ligand-binding interactions between Tannin and 6LU7.
Table 4. Interactions of SARS-CoV-2 amino acid residues with ligands at receptor sites.

| Ligands     | Binding Affinity, ΔG (kcal/mol) | Hydrogen-binding interaction | Amino acids involved | Hydrophobic interaction | Electrostatic interaction |
|-------------|---------------------------------|------------------------------|----------------------|-------------------------|-------------------------|
| Chrysophanol| -7.2                            | Gln110 and Ser158           | Val104 dan Ile106    | -                       |                         |
| Diosgenin   | -8.3                            | Ser144, Cys 145, and Gln189 | His163, Pro168, Thr190, and Ala191 |                         |                         |
| Flavone     | -6.9                            |                              | Val104 and Ile106    |                         |                         |
| Phytosterol | -6.9                            |                              | Val104 and Phe294    |                         |                         |
| Tannin      | -7.5                            | Gln110, Thr111, Asn151, Thr292, and Asp295 | Val202 and Phe294 | His246                  |                         |
| Triterpenoid| -7.5                            | Gln110                        | Val104, Arg105, and Ile106 |                         |                         |

4. Discussion

Recently, research using the in silico method (with the help of computers) is becoming increasingly used, especially in pandemic situations like this. Researchers have done a lot of research in silico to find the best drug candidates for the current coronavirus outbreak caused by SARS-CoV-2. In this study, research was also carried out using the in silico method using local wisdom in Riau Province. Local wisdom used is herbal plants that have been frequently used by local people as traditional medicine in healing various diseases. The plants used in this study can be seen in Table 1. The compounds used in this docking analysis are also obtained from herbal plants in Table 1 and these compounds can be isolated from the extracts of these herbal plants. The compounds used were chrysophanol, diosgenin, flavones, phytosterols, tannins, triterpenoids, and N3 inhibitor (native ligand of 6LU7) which were the positive controls for this study. Docking was carried out on ligand candidates with the target protein (6LU7) and the results showed that diosgenin compounds from the Cheilocostus speciosus plant had the lowest affinity binding / ΔG value, which was -8.3 kcal / mol. The lower ΔG value indicates that the interaction between the target protein and the ligand occurs very spontaneously which is also influenced by the interactions that are present between the two[26, 27]. A negative ΔG value is an indication of the formation of a stable complex structure between the target protein and the ligand[28]. From Figure 1 and Table 4, we can see some of the interactions between the amino acid residues of the SARS-CoV-2 protein and the ligand candidates, including hydrophobic interactions, hydrogen bonds, and electrostatic interactions. Hydrophobic interaction is the main supporting factor for the stability of the protein-ligand complex structure. Hydrogen bonding also affects the structural stability of the protein-ligand complexes formed but with a lesser role on structural stability [29]. Binding affinity, structure, chemical properties, structural stability of protein-ligand complexes, and the biological reactivity of proteins and nucleic acids can be affected by the presence of electrostatic interactions[30]. SwissADME is used to analyze the activity of drug candidates in the human body according to the Lipinskii Rule. Lipinski's Rule of Five helps to determine the level of absorption or permeability of the lipid bilayer in the human body developed by Christopher A. Lipinski. The Lipinski rule governs a molecular weight (BM) of not more than 500 g / mol, a partition coefficient value (logP that binds to lipophilicity or hydrophobicity) less than 5, has a number of hydrogen bond donors less than 5 and has a number of H-acceptors less than 10[31]. The log P value indicates the solubility of a compound in lipids which is useful for seeing its polarity which is one of the criteria for a good drug because it can increase adsorption and permeation. If log P is negative then the compound tends to be polar (hydrophilic) like Tannin. So tannins which have a log P value of -2.42 are not recommended because they will have difficulty passing through the lipid bilayer. If the log P value> 5, such as Phytosterol (6.73), it tends to last longer in the lipid bilayer.
so that it can be spread throughout for quite a long time and this can cause toxicity in the body. Meanwhile, chrysophanol, diosgenin, flavone, and triterpenoid which have a log P value of 0 < P < 5 are still classified as hydrophobic but can still dissolve quite well in polar solvents so that their adsorption and permeation in the body is quite good. Compounds that have a molecular weight of less than 500 g / mol such as chrysophanol, diosgenin, flavones, and phytosterols will be easier to digest in the body, so they don't take that long. H-donor less than 5 and H-acceptor less than 10 affect the rate of adsorption and permeation of a compound in the human body. Compounds such as chrysophanol, diosgenin, flavones, phytosterols and triterpenoids that meet the above rules will have a faster adsorption and permeation rate in the body than tannins that violate the above rules.

5. Conclusion
The results of this study found that the six docking compounds had potential as inhibitors for Angiotensin Converting Enzyme 2 Receptor (ACE2) because they had less affinity binding values than positive controls (N3 inhibitor). However, from the results of the ADME analysis study it was found that compounds that could be used as good drug candidates were chrysophanol, diosgenin, and flavones and the results of docking analysis with Autodock Vina found that diosgenin with an affinity binding of -8.2 kcal / mol had the smallest value. So it can be concluded that diosgenin derived from Cheilocostus speciosus is the best drug candidate and inhibitor in this study.

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