Inhibition of SARS-Cov-2 proteases by medicinal plant bioactive constituents: Molecular docking simulation

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Abstract. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a virus that has caused the corona pandemic since 2019 or known as Covid-19. The cleavage of its polyprotein started this viral replication into functional viral proteins by two proteases: 3-chymotrypsin-like protease (3CL protease), also known as main protease (Mpro), and Papain-like protease (PLpro). Medicinal plant bioactive constituents could potentially become protease inhibitor agents of this virus and prevent viral replication. Thus, further might be developed into drug candidates for diseases with no specific drug currently available. The first step of discovering the medicine is virtual screening with a molecular docking simulation approach. The stable conformation structure of the bioactive compounds was docked into the enzymes SARS-CoV-2 Main Protease (PDB ID: 6XMK) and SARS-CoV-2 Papain-Like Protease (PDB ID: 7CMD). Molecular docking simulations were operated using Molegro Virtual Docker (MVD) program after the validation process. In this study, analysis of the docking simulation was carried out of compounds in Andrographis paniculata, Phyllanthus niruri L., Aloe vera, and Sonchus arvensis. They are medicinal plants that have been used as a medicine for generations and may have potential as antivirals. A docking score with a more negative presentation binding energy value has a more significant potential to be a lead compound. Several potential compounds were evaluated for their absorption, distribution, metabolism, excretion, and toxicity (ADMET) properties. This method can reduce the trial and error factor in the drug discovery stage, although it needs further proof by experimentation in a wet laboratory.

Keywords: molecular docking, SARS-CoV-2 protease, medicinal plant.

1. Introduction
The coronavirus disease 2019 (COVID-19) pandemic as a result of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) attack has been a global public health problem until now [1]. More than 235 million people have been infected, and more than 4.8 million people have died worldwide due to the COVID-19 outbreak in early October 2021 [2]. It is crucial to develop an effective vaccine and discovers drugs and therapeutics to prevent and treat COVID-19. Nonetheless, the therapeutic choice for COVID-19 is currently limited [1]. Multiple layers of prevention and treatment against COVID-19 are needed, including identifying therapeutic drugs that can interfere with viral entry or viral propagation.
Stages in a typical drug discovery organization include target selection, hit identification, lead optimization, preclinical and clinical studies that take a long time and high cost. An approach that has been done is by repurposing drugs. Remdesivir, an antiviral developed to treat Ebola, is a few repurposed medicines authorized by different regulatory agencies to treat COVID-19 [3,4]. The repurposing of approved or investigational drugs could provide a practical approach. There are existing detailed information on drug chemistry together with human pharmacology and toxicology, allowing rapid clinical trials and regulatory review [5].

Another approach could be made to drug and treatment discovery by using botanicals and dietary supplements derived from natural substances, whether it is offered for use in a conventional food or traditional remedies or its use is as a dietary supplement or as a dietary ingredient in a dietary supplement product [6]. Medicinal plants are essential sources of structurally novel compounds that function as a lead for the development of novel drugs. Some plants known have potential properties as herbal medicine in Indonesia, which are empirically used and have proven their effectiveness as antivirals. Andrographis paniculata [7], Aloe vera [8,9], and Phyllanthus niruri [10-12] have been reported to have inhibitory effects against some viruses.

Viral proteases have been developed as drug targets because these enzymes play a critical role in viral protein maturation. Drug discovery and design that directly act on conserved enzymes like the main protease or 3C-like protease (Mpro or 3CLpro) and papain-like protease (PLpro) inhibition have potential clinical use [13].

Virtual screening has been a routine assay in drug discovery with docking simulation as a computational technique. Parameters for virtual screening may change, but the overall protocol is very straightforward [14,15]. In this study, we evaluate in silico the potential bioactive compounds from Andrographis paniculata, Phyllanthus niruri L., Aloe vera, and Sonchus arvensis as SARS-CoV-2 replication inhibitor candidates, especially viral proteases activity. It was performed with a molecular docking approach and ADMET analysis of bioactive constituents.

2. Materials and Methods

2.1. Validation and Docking Simulation

The tools used are HP laptops with Intel Core i7 processors that have been installed with several licensed and open-source programs to draw 2D and 3D structures such as Marvin Sketch 6.2, the docking simulation program Molegro Virtual Docker (MVD) 6.0, and Molegro Molecular Viewer (MMV) 7.0.

The materials used are the 2D and 3D structures of compounds in herbs as ligands. The 3D structure of SARS-CoV-2 Mpro and Plpro was downloaded from Protein Data Bank (www.rcsb.org) with PDB ID: 6XMK and 7CMD, respectively. The ligands were the structural compounds in the herbs that have been reported. The design of the ligand compound was carried out using Marvin Sketch. The ligands that have been drawn in the 2D structure are then converted to 3D, and the most stable conformation is taken and saved in .mol2 format.

The protein structure was inserted into the MVD program, excluding water molecules and cofactors. In the 3D structure of this protein, there is already a bound ligand so that the binding region is used as a reference ligand. Furthermore, docking analysis was carried out to see the minimum energy and interactions that occurred, such as the presence of hydrogen bonds resulting from the complex. Validation of the method is carried out by extracting the reference ligand then the redocking process. The docking method is declared valid if the RMSD value is less than 2 [16]. The bioactive compounds from herbs are imported into the program and aligned with the reference ligand. Docking simulations were carried out for each of these compounds by converting them into an active ligand (set as an active ligand), and the results were analyzed.

2.2. ADMET Analysis

Physicochemical properties such as absorption, distribution, metabolism, excretion, and toxicity (ADMET) profiling of compounds were determined using the pkCSM ADMET descriptors algorithm
The absorption of drugs is influenced by factors including membrane permeability that is indicated by colon cancer cell line (Caco-2), intestinal absorption, skin permeability levels, and P-glycoprotein substrate or inhibitor. The distribution of drugs depends on the blood-brain barrier (logBB), CNS permeability, and the volume of distribution (VDss). Metabolism is presumed based on the CYP models for substrate or inhibition (CYP2D6, CYP3A4, CYP1A2, CYP2C19, CYP2C9, CYP2D6, and CYP3A4). Excretion is presumed based on the total clearance model and renal OCT2 substrate. The toxicity of drugs is presumed based on AMES toxicity, hERG inhibition, hepatotoxicity, and skin sensitization. These parameters were calculated and checked for compliance with their standard ranges [17].

3. Results and Discussion
Molecular docking simulation has been one of the most fundamental and essential approaches for virtual screening in drug discovery. It allows the prediction of molecular interactions between a protein and a ligand in the cavity sites [18]. The pharmacological properties of plants are the contribution of their bioactive compounds. These compounds interact with specific functional proteins (as receptors) that could have the effect of activating or inhibiting them. In the case of searching for drugs to overcome the attack or infection of the SARS-CoV-2 virus, the viral proteases were used as target proteins, which if there is an interaction with stable binding energy. It will inhibit the activity or function of the protease so that virus replication is disrupted and its growth is inhibited. Because no human proteases with a similar cleavage specificity are known, such inhibitors are unlikely to be toxic [19].

**Figure 1.** Molecular docking reference ligand into 6XMK. (a) ligand dock position; (b) Redocking reference ligand QYS_401 and (c) interaction reference ligand QYS with amino acid residue in SARS-CoV-2 main protease.
Molecular docking simulation for bioactive compound into SARS-CoV-2 Main Protease (Mpro) 6XMK has performed in docking position coordinate: x = -20.15; y = 4.28; z = 2.28 with radius 11 Å. Redocking of Compound (1S,2S)-2-[(N-[(4,4-difluorocyclohexyl) methoxy]carbonyl]-L-leucyl]amino]-1-hydroxy-3-[(3S)-2-oxopyrrolidin-3-yl]propane-1-sulfonic acid (QYS_401) as reference ligand with protein chain B showed rerank score as -108.188 and RMSD as 2.0.

Figure 2. Molecular docking reference ligand into 7CMD. (a) ligand dock position; (b) Redocking reference ligand TTT_502 and (c) interaction reference ligand TTT with amino acid residue in SARS-CoV-2 papain-like protease.

Molecular docking simulation for bioactive compound into SARS-CoV-2 papain-like protease (PLpro) 7CMD has performed in docking position coordinate: x = 3.97; y = -11.26; z = 48.62 with radius 15 Å. Re docking of Compound 5-amino-2-methyl-N-[(1R)-1-naphthalen-1-ylethyl]benzamide (TTT_502) as reference ligand showed rerank score as -130.178 and RMSD as 0.37.

The docking results of compounds in plants *Phyllanthus niruri* L., *Andrographis paniculata*, *Aloe vera*, and *Sonchus arvensis* L. into SARS-CoV-2 main and papain-like proteases were presented in Table Supplementary 1.

According to rerank score docking, there are several compounds in *Phyllanthus niruri* L. and *Sonchus arvensis* L that have the most significant negative score. So, it could be stated that both plants have sufficient potency to inhibit the action of viral proteases. However, *Andrographis paniculata* and *Aloe vera* showed moderate potency to be used as drug candidates or lead compounds to inhibit SARS-CoV-2 virus replication. Some compounds with a more negative score indicate more stable bond energy and higher potency. Table 1 and Table 2 show the ten best compounds of each viral proteases inhibitor potential.
Tabel 1. Ten best compounds as SARS-CoV-2 Mpro inhibitor potential.

| No | Compounds | Origin Plants | Score Docking |
|----|------------|---------------|---------------|
| 1  | Quercetin 3-O-â-D-glucopyranosyl-(2-1)-O-â-D-xylopyranoside | Phyllanthus niruri L. | -156.84 |
| 2  | Rutin      | Phyllanthus niruri L., Sochus arvensisi L. | -153.27 |
| 3  | Nirurin    | Phyllanthus niruri L. | -139.63 |
| 4  | Quercitrin | Phyllanthus niruri L. | -131.02 |
| 5  | Cubebin dimethyl ether | Phyllanthus niruri L. | -123.38 |
| 6  | Hyperoside | Sochus arvensisi L. | -121.76 |
| 7  | Phylentralin | Phyllanthus niruri L. | -121.62 |
| 8  | Linnanthin | Phyllanthus niruri L. | -120.51 |
| 9  | Urinatetralin | Phyllanthus niruri L. | -120.05 |
| 10 | Corilagin  | Phyllanthus niruri L. | -117.24 |

Tabel 2. Ten best compounds as SARS-CoV-2 PLpro inhibitor potential

| No | Compounds                | Origin Plants | Score Docking |
|----|--------------------------|---------------|---------------|
| 1  | Seco-4-hydroxylintetralin | Phyllanthus niruri L. | -121.95 |
| 2  | Hypophyllanthin          | Phyllanthus niruri L. | -118.50 |
| 3  | Isolintetralin           | Phyllanthus niruri L. | -117.35 |
| 4  | Cubebin dimethyl ether   | Phyllanthus niruri L. | -115.58 |
| 5  | Phyllanthin              | Phyllanthus niruri L. | -115.15 |
| 6  | Nirphyllin               | Phyllanthus niruri L. | -114.14 |
| 7  | 2,3-Desmethoxy seco-isolintetralin | Phyllanthus niruri L. | -114.01 |
| 8  | Niranthin                | Phyllanthus niruri L. | -112.73 |
| 9  | Niruriflavone            | Phyllanthus niruri L., Sochus arvensisi L. | -111.39 |
| 10 | Rutin                    | Phyllanthus niruri L., Sochus arvensisi L. | -111.07 |

Although some of these compounds are predicted to have efficacy to inhibit proteases, it is crucial to carry out a safe and optimal use of herbal medicines requires a full understanding of their bioavailabilities and ADMET profiles. One way to filter out compounds with probable absorption problems is known as Lipinski's ‘rule of five’ [20,21]. The rule states that poor absorption or permeation of a drug is more feasible when the chemical structure meets two or more of the following criteria:

1. Molecular weight (MW) is greater than 500.
2. The calculated log P-value is more than five.
3. There are more than five hydrogen bond donors (HBD)
4. The number of hydrogen bond acceptors (HBA) is greater than ten.
5. Molar refractivity (MR) should be between 40-130

Analysis of 18 potential compounds above (Rutin and Cubebin dimethyl ether are both protease inhibitors, so only one compound is counted), only 11 compounds meet Lipinski’s rule or are included in drug-likeness. The ADMET properties of the eleven compounds can be seen in Table Supplementary 2.
Based on the results of virtual screening using docking techniques and ADMET properties, the *Phyllanhus niruri* has the potential to develop as a Covid19 virus inhibitor plant with Cubebin dimethyl ether being the strongest candidate to inhibit viral Mpro and Seco-4-hydroxylintetralin as PLpro inhibitors. The prediction of mechanism action Cubebin dimethyl ether with SARS-CoV-2 Mpro such as oxygen atoms formed a hydrogen bond with residue Gln 189, Cys 44, and Tyr 54 as total energy hydrogen bonds was -1.72 kcal/mol. Seco-4-hydroxylintetralin interacts with SARS-CoV-2 PLpro by oxygen atoms forming a hydrogen bond with amino acid residue Tyr 273, Gly 163, Thr 301, and Arg 166. H-bond as -5.68 kcal/mol.

![Figure 3. Interaction (a) Cubebin dimethyl ether with Mpro and (b) Seco-4-hydroxylintetralin with PLpro](image)

**4. Conclusion**

Based on the results of virtual screening using docking techniques and ADMET properties, the *Phyllanhus niruri* has the potential to develop as a Covid19 virus inhibitor plant with Cubebin dimethyl ether being the strongest candidate to inhibit Mpro and Seco-4-hydroxylintetralin as PLpro inhibitors. This method can reduce the trial and error factor in the drug discovery stage, although it needs further proof by experimentation in a wet laboratory.

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