Discovery of SARS-CoV-2 RNA-dependent-RNA-polymerase (RdRp) Inhibitor from Sambiloto (Andrographis paniculata) Based on Molecular Docking and ADMET Prediction Approach

Dina Ahsana*, Rizki Rahmadi Pratama, Alfisyahriatunnida Meily, Andika
Faculty of Pharmacy, Universitas Muhammadiyah Banjarmasin, South Kalimantan, Indonesia

ABSTRACT

The rapid spread of the coronavirus disease 2019 (COVID-19) has led to the development of therapeutic inhibitor drug of SARS-CoV-2, which can inhibit the viral enzyme RNA-dependent-RNA-polymerase (RdRp), thereby preventing the replication, transcription, and synthesis of RNA virus in the host cells. Previous in-vitro studies revealed that Andrographis paniculata has the potential to inhibit the virus. Therefore, this study aims to isolate the specific compounds of Andrographis paniculata, which play a role in inhibiting SARS-CoV-2 RdRp using molecular docking. A total of 19 compounds were identified in previous literature studies, while remdesivir and favipiravir were used as the positive control. All compounds and proteins were applied to minimize and optimize energy. Furthermore, the docking method was carried out using Autodock 4.2.6 software with a specific grid box containing the active site of RdRp (ID: 6M71), and the Lamarckian Genetic Algorithm was used to determine the conformation. The best docking was screened on ADMET prediction and the binding energy was evaluated. There are 18 compounds of Andrographis paniculata including the top three, namely andrographolactone (ΔG = -8.86 kcal/mol), andrographolide (ΔG = -7.74 kcal/mol), and andrographidine-A (ΔG = -7.68 kcal/mol), which showed the strongest binding affinity to the SARS-CoV-2 RdRp protein compared to other compounds and the positive control remdesivir (ΔG = -5.73 kcal/mol) and favipiravir (ΔG = -5.20 kcal/mol). Furthermore, active amino acids play a role in this interaction by forming strong hydrogen bonds, such as TYR 619, LYS 621, ASP 760, and ASP 623. Andrographolactone has the highest potential as SARS-CoV-2 RdRp inhibitor, hence, it can be used as a novel therapeutic candidate.

Keywords: Andrographis paniculata; andrographolactone; RNA-dependent-RNA-polymerase (RdRp); SARS-CoV-2

INTRODUCTION

A new pandemic known as Corona Virus Disease 2019 (COVID-19) was first reported in December 2019 in Wuhan, China, and caused by the Severe Acute Respiratory Syndrome Corona Virus-2 (SARS-CoV-2) (Gorbalenya, et al., 2020). Furthermore, the disease is spreading rapidly around the world with millions of victims, and it also has a major social and economic impact (da Silva, et al., 2020; Nimgampalle, et al., 2021). To overcome the infection and viral replication, it is important to understand the proteins involved in the process. The viral spike protein binds to the human receptor within a metallopeptidase, namely Angiotensin-Converting Enzyme 2 (ACE2) (Borse, et al., 2021; Dong, et al., 2020). After the virus enters the host cell, its positive genomic RNA attaches to the ribosome to translate two large terminal polyproteins, which are processed by proteolysis into components for packaging new virions. The 3CLpro and PLpro provide components for packaging new virions of enormous viral polyproteins translated on the host ribosomes, after which RNA-dependent-RNA-polymerase (RdRp) replicates the SARS-CoV-2 RNA genome (Morse, et al., 2020).

Previous studies revealed that RdRp plays a vital role in SARS-CoV-2 replication as a potential drug target (Lung, et al., 2020; Parvez, et al., 2020). It has also been highlighted as a fundamental target in computational strategies, such as molecular docking due to its importance in the viral replication stage. Molecular docking is a robust, rational, and inexpensive method, which provides an understanding of how the critical NSP interacts with ligands at the active site. Therefore, it supports the design and screening of novel antiviral agents against COVID-19 (Wu, et al., 2020; Yu, et al., 2020). Another literature predicted the binding of andrographolide and its derivatives to RdRp using molecular docking and simulation, and the results showed that the analogs oxo-andrographolide have strong binding energy compared to andrographolide (Sharma, et al., 2020). Neoandrographolide (AGP3) had significant binding of andrographolide and its derivatives to RdRp, thereby inhibiting the target therapeutically (Murugan, et al., 2021). Herbal medicines play an important role in the control and prevention of infectious diseases (Kwon, et al., 2019). Several clinical studies revealed that they have beneficial effects in treating and preventing epidemic
diseases, such as the SARS Coronavirus (SARS-CoV) (Yang, et al., 2020). COVID-19, caused by SARS-CoV-2, is a new type of coronavirus, which has 80% similarity to SARS-CoV and belongs to the Betacoronavirus subgenus of the Betacoronavirus genus (J. F.-W. Chan et al., 2020; Lipsitch et al., 2020; Lung et al., 2020; Wrapp et al., 2020). Due to these similarities, several studies showed that the use of herbal medicines in the treatment of COVID-19 has a beneficial effect (K. W. Chan, et al., 2020). Z. Wang & Yang, (2021) revealed the potential activity of Chinese Herbal Medicine against SARS-CoV-2 in China Clinical Trial, including Herba andrographii (Xiyanping Injection), Sophora flavescens Ait. (Matrine-sodium chloride injection) and diammonium glycyrrhizinate enteric-coated capsules. In China’s experience of fighting the COVID-19 pandemic, the Chinese Herbal Medicine (CHM) therapy schedule was included in the treatment guidelines because it has played an indispensable role (Du, et al., 2021).

Sambiloto (Andrographis paniculata) also known as the “King of Bitters”, belongs to the Acanthaceae family, and has been used for centuries in Asia for medicine. In the official book of Indonesian medicinal plants, bitter herbs are used as diuretics and antipyretics agents (Patin et al., 2018; Ratmani et al., 2012). The in-silico study by Murugan et al., (2020) reported that several compounds from A. paniculata have promising binding affinity to RdRp with proper binding to the catalytic site, which helps to inhibit the target using SARS-CoV-1 PDB ID: 6NUR. Thailand declared its pilot project openly in January 2021 for administering and investigating the effectiveness of A. paniculata extract in patients diagnosed with COVID-19 (Lim, et al., 2021). An RCT study also recommended that the regimen of the extract therapy, namely oral 60 mg, t.i.d., for five days can be given to adult patients diagnosed with the disease. Furthermore, the adverse effects caused by the therapy are limited and benign (Wanarattana, et al., 2021).

Indonesia has several traditional medicinal compounds that can be used to treat SARS-CoV and SARS-CoV-2, but the mechanism of their activity and efficiency remains unclear. Therefore, a comprehensive computational approach with molecular docking was used to predict the activity of A. paniculata compounds against RdRp as one of the mechanisms against SARS-CoV-2 infection. In Rafi et al., (2020)’s study, the compounds extracted from the plant were obtained from the presumed results using LC-MS/MS and classified based on the plant part, namely stems and leaves using sonication with 70% ethanol. Principal Component Analysis (PCA) was also used to separate and classify the compounds in its leaves, such as andrographanine, 14-deoxyandrographolide, andrographolactone, dehydandrographolide as well as the stem extracts, including andrographolide, apigenin-7,40-dimethylether, 5-hydroxy-7,8-dimethoxy flavanone, and andrografidin A by observing values of the peak area. Andrographolactone was found in A. paniculata leaves, hence, it can be a target plant part for the isolation of compounds. A total of 31 metabolites were identified in the stem and leaf extracts with different intensities and they were divided into groups of diterpene lactones, flavonoids, and phenolic acids.

METHODS

Materials
A laptop ASUS ROG GL-552VX with a specification of CoreTM i7-6700HQ Processor Intel®, 12 Gb RAM, CPU @2.60 GHz ~ 2.59 GHz, and Microsoft Windows 10 as the operating system was used to perform molecular docking. The software used in this study includes Avogadro for ligand energy minimization and Swiss PDB Viewer for protein optimization. Molecular docking was carried out using Autodock 4.2.6, after which Discovery Studio Visualizer and PyMOL were used for visualizing protein-ligand interactions. The Lipinski screening and ADMET prediction were carried out using SCFBio and pkCSM web tools.

Methods
Detailed docking studies are necessary to predict the candidate SARS-CoV-2 RdRp inhibitor of A. paniculata compounds, and the schematic molecular docking study process is presented in Figure 1.

Ligand and Protein Preparation
The crystal structure of SARS-CoV-2 RdRp (ID: 6M71) was downloaded from the RCSB Protein Data Bank. The target protein was prepared by removing water molecules and adding all hydrogen using the Discovery Studio Visualizer. Its structure was optimized with the Swiss PDB Viewer software using a GROMOS96 force field, and then saved as a .pdb file. A total of 19 compounds of A. paniculata were generated as ligands from the previously collected data, while Rafi et al., (2020)’s study was used for screening to find potential anti-SARS-CoV-2. The compounds were downloaded at PubChem and they passed the Lipinski screening using the SCFBio webservice. Remdesivir and favipiravir were used as a positive control, after which hydrogen was added to all ligands, followed by energy minimization energy using the MMFF94 force field of Avogadro software.

Molecular Docking
Molecular docking studies of A. paniculata compounds and positive control to RdRp (6M71) were carried out using AutoDock. The protein and ligand were uploaded to AutoDock Tools, and detected torsions, which allow the rotation of all rotatable bonds were used for the
ligands. The Gasteiger partial and Kollman charges were automatically added to the test compounds. The suitable grid box on the side of active RdRp (ID:6M71) from Parvez et al. (2020) was used after evaluating another study. The semi-flexible docking method is ligand flexibly setting where protein is still in rigid conformation. The molecular docking was performed using the Lamarckian Genetic Algorithm with a population size of 150 people and a maximum evals number of 2,500,000 for every 100 independent runs. The best pose was evaluated using the lowest binding energy score (∆G) with inhibition constant (Ki) value as well as the functional crucial amino acid, which was detected to have a role in docking interaction by Discovery Studio Visualizer.

**ADMET Prediction**

The Absorption, Distribution, Metabolism, Excretion, and Toxicity (ADMET) filtering can be used to predict the pharmacological properties, metabolizing system, and toxicity of oral drug discovery candidate. The Predicting Small Molecule Pharmacokinetic Properties Using Graph-Based Signatures (pkCSM) online server was used to reduce academic costs as well as increase the potential of a highly accurate prediction. All the parameters model prediction of ADMET on pkCSM were obtained from Pires et al. (2015). The 19 compounds that demonstrated good binding affinity were screened for ADMET by changing the format PDB into SMILES and then uploading them to the pkCSM online server.

**RESULTS AND DISCUSSION**

The viral polymerase RdRp, also known as nsp12, can be a crucial target for inhibiting the replication, transcription, and synthesis of RNA virus in host cells (Gao et al., 2020). RdRp protein can cause a high mutation rate, thereby leading to the emergence of a new virus that affects the disease profile, such as escaping the host immunity or increasing resistance to antiviral therapeutics (Wabalo et al., 2021). Cheminformatics and computational drug repurposing are tangible strategies for developing antiviral SARS-CoV-2 in a shorter period compared to the new drug development techniques. Furthermore, several computational drug methods, such as molecular docking have identified potential drug inhibitors for SARS-CoV-2 infection. The success of the docking process was evaluated with the scoring function of free binding energy (∆G) and the main role of amino acid interaction. Several in-silico studies of *A. paniculata* molecules on RdRp SARS-CoV-2 were carried out using homologous modelings, such as Srikanth et al., (2021), which docked andrographolide compounds to the target protein with autodock and MOE software. Murugan et al., (2021) used the Molecular docking and Molecular dynamic (MM-GBSA) approach on five compounds of *A. paniculata*, and the best results were obtained in neoandrographolide (AGP3). Sharma et al., (2020) showed that oxo-andrographolide had the best binding to the SARS-CoV-2 RdRp protein (ID: 6M71) through molecular docking, molecular dynamic (MM-GBSA), Clustering of conformations, PCA, and drug likeness properties-ADME prediction. This study’s results are expected to improve previous findings through more comprehensive compounds of *A. paniculata* samples using a molecular docking study approach.

**Lipinski Rules Screening**

Lipinski’s rule was used to predict a compound’s bioavailability or ability to be absorbed and circulated in the body when administered orally. The results showed that the 19 compounds of *A. paniculata* passed the criteria for Lipinski’s rule, as shown in Supplementary Table S1.
Furthermore, 19 compounds surpassed the Log P value screening, which indicates that the drugs can easily be absorbed and they have an ideal lipophilicity to pass through the lipid bilayer of intestine, thereby making it easy for them to reach the target protein and interact. The ideal molecular weight given by *A. paniculata* compounds indicates that the drug can diffuse through the cell membrane, thereby increasing its oral bioavailability in the body. The ligands result was consistent with the good evaluation results of hydrogen donors, and the acceptors showed that the compound can quickly be absorbed due to the presence of weak hydrogen bonds with cells. Minimal energy is also required for the drugs to enter the blood vessels. Therefore, the 19 compounds of *A. paniculata* can become a safe orally active drug for humans based on the good absorption and bioavailability of the predicted Lipinski’s rule.

**Validation of Positive Control (Remdesivir and Favipiravir) as RdRp (6M71) Inhibitor**

RdRp protein (ID: 6M71) without a native ligand was used in this study, hence, the existing antiviral drugs have highly selective inhibitors for the virus’s RNA polymerase (Loza-Mejía & Salazar, 2020). Some of them are still in a clinical trial stage, such as remdesivir and favipiravir, which were used as a positive control for native ligand alternatives. They also served as inhibitors in Gao et al. (2020)’s studies about RdRp 6M71 protein, hence, the analysis of remdesivir amino acid interaction is very crucial for docking active sites.

The best grid box containing an active site of 6M71 with the lowest energy binding score of remdesivir was obtained from Parvez et al. (2020). The grid box size was $X = 80$, $Y = 94$, $Z = 94$, and can accommodate the ligand with 0.375 Å spacing and coordinates of central grid points of 121.253 Å, 121.376 Å, 120.149 Å, which suit the protein cavity. The interaction for conventional hydrogen bond (LYS 621, ASP 618, ASP 761, CYS 813, GLU 811, SER 814), carbon-hydrogen bond (PRO 620, ASP 618), pi-anion (ASP 760), and alkyl (LYS 798) were found in the amino acid interaction with remdesivir, as shown in Figure 2 and Supplementary Table S2.

![Figure 2. Visualizing protein-ligand interactions of positive control (a) remdesivir and (b) favipiravir](image-url)
This interaction of amino acids was also observed in other studies on 6M71 RdRp, which referenced Gao et al. (2020). The results showed that the residue ASP 618 found in Motif A active site protein is a classic divalent-cation–binding residue. Jang et al. (2021) also revealed that remdesivir-RdRp (ID: 6M71) binding involved a significant contribution of ASP 618 amino acid on hydrogen bonding. Another hydrogen interaction was formed between remdesivir and the RdRp enzyme 6M71, namely LYS 621. This interaction was reported in Eweas et al. (2021) and Gao et al. (2020)’s studies on Motif A (fingers subdomain), where it appeared in the prediction of the active site of 6M71. Jang et al., (2021) and Pintilie et al., (2020) revealed that LYS 621 is one of the framers of hydrogen interactions on 6M71 protein. Furthermore, Gao et al. (2020) reported that PRO 620 is responsible for stabilizing the protein’s active site in the palm subdomain, and its interaction with inhibitor activity can destabilize protein expression. Other amino acids of CYS 813, GLU 811, SER 814, and LYS 798 were found in a palm subdomain, which contributes to the polymerase activity of the enzyme (Gao et al., 2020).

The critical amino acids that played a role in the primary interaction of positive control-RdRp were also relevant to other studies. This indicates that the grid box area used contains several active amino acids, which play an important role in the inhibitory activity of SARS-CoV-2 RdRp (6M71). Subsequently, a docking study of 19 *A. paniculata* compounds to RdRp was carried out using the validated grid box.

**Evaluation of RdRp (6M71) Inhibitor Activity on Chemical Constituents *A. paniculata***

Based on the docking results, some potential antiviral drugs were detected against RdRp, which has the best conformation with the lowest energy (most enormous negative Gibbs’ free energy of binding (∆G) score), as shown in Table 1. The top three docking scores of bioactive *A. paniculata* compound were ∆G = -8.86 kcal/mol as the lowest binding energy of andrographolactone, followed by andrographolide ∆G = -7.74 kcal/mol and andrographidine-A ∆G = -7.68 kcal/mol. Furthermore, these binding energies were lower compared to the positive control of remdesivir and favipiravir, namely -5.73 kcal/mol and -5.20 kcal/mol, respectively. The high negative free energy of binding (∆G) scores indicate the presence of spontaneous protein-ligand binding that affects and stabilizes protein-ligand interaction. They are also a better inhibitor of the molecular docking prediction (Xing Du et al., 2016; Nusantoro & Fadlan, 2021). The lowest binding score correlated with the lowest inhibition constant (Ki) value. Based on the results, the lowest Ki value was obtained from andrographolactone, namely 321.37 nM (nanomolar), followed by andrographolide 2.10 μM (micromolar) and andrographidine-A of 2.34 μM (micromolar). These results indicate the concentration required to produce a half-maximum inhibition value and show a stronger ligand affinity for macromolecules (Yasin et al., 2020).

The docking results showed that andrographolactone can stabilize and has a stronger affinity to bind to the active side of RdRp (ID: 6M71) compared andrographolide and andrographidine-A. This indicates that it has the potential to be a candidate anti-SARS-CoV-2 drug by blocking RdRp as a template anti-Genom of positive-sense RNA to prevent viral replication (Ahmad et al., 2020).

The top three compounds, which had good binding energy, namely andrographolactone, andrographolide and andrographidine-A, were identified as terpenoids. Andrographolactone is another compound that showed high binding energy with RdRp protein. Furthermore, it is a novel diterpene with an unprecedented skeleton that was isolated from the aerial part of *A. paniculata* (G. C. Wang et al., 2009). Andrographolactone has the potential to act as an anti-inflammatory agent by inhibiting TNF-a (Firdayani & Srijanto, 2012), which can prevent cytokine storms. This is the first study to show its activity as an anti-SARS-CoV-2, specifically as a RdRp inhibitor.

Andrographolide is a bioactive and major constituent in the leaves extract of *A. paniculate*, and it has anti-inflammatory, antiviral, antitumor, and hepatoprotective activities (Jayakumar et al., 2013). Sa-Ngiamsuntorn et al. (2021) revealed that *A. paniculata* extract and andrographolide have the same IC$_{50}$ as remdesivir against SARS-CoV-2 infection. This evidence can be used for future antiviral development. Furthermore, previous in-vitro studies on the anti-SARS-CoV-2 activity of *A. paniculata* extract and andrographolide on Calu-3 cells showed high inhibition at the late phases of the viral life cycle including viral assembly and maturation with IC$_{50}$ of 0.036 μg/mL and 0.034 μM. Another study using Vero E6 cells reported that andrographolide had a stronger effect compared to the extract. The chemical structure of andrographolide and remdesivir have a common functional group-containing naphthalene ring against SARS-CoV-2 infection. Srikanth et al., (2021) showed a very strong affinity of andrographolide to RdRp (6M71) compared to RBD SARS-CoV-2, which indicates its potential activity as a RdRp inhibitor. These compounds have anti-inflammatory properties by inhibiting the th1/th17 response and stabilizing cytokine expression. Nie et al. (2017) showed that derivatize andrographolide can inhibit TNF-α/ NF-κB and TLR4/NF-κB signaling pathways to suppress cytokine pro-inflammation, and this prevents the occurrence of cytokine storm that is often observed in COVID-19 patients.
Swaminathan et al. (2021) revealed the potential activity of *A. paniculata* phytocompounds against ten structural and non-structural SARS-CoV-2 proteins using molecular docking and dynamic simulation. The result showed that andrographidine-A have good binding energy with membrane protein, NSP15, and spike protein of SARS-CoV-2. The limitation of these studies was that there was no test on the RdRp target. The top three compounds with the best docking score, namely andrographolactone, andrographolide, and andrographidine-A have relatively higher chemical potential to cause increased reactivity compared to remdesivir and favipiravir, which indicate that they are a strong inhibitor of SARS-CoV-2 RdRp based on molecular docking. They also have anti-SARS-CoV-2 potential based on previous wet and dry lab studies.

### Analysis of Amino Acid Interaction

This study identified a critical amino acid that plays a role in hydrogen interaction and active side of RdRp, such as TYR 619, LYS 621, ASP 760, and ASP 623, as shown in Figure 3 and Supplementary Table S2. The hydrogen bond is one of the non-covalent binders, which play a significant role in the docking score, complex formation, and strength binding modes (Fikrika et al., 2016). Andrographolactone have strong binding energy to bind with RdRp protein, and the amino acid interaction of the compounds did not show hydrogen bond interaction. However, it formed two pi-alkyl with ARG 349 and PRO 461 in finger domain RdRp, as shown in Figure 3a. This study showed that TYR 619 had the highest number of hydrogen bonds, namely 11, which contribute to the second rank of docking compound andrographolide, as shown in Figure 3b. Previous studies reported that it also formed a hydrogen bond from remdesivir-RdRp (ID: 6M71) complex using natural bioactive compounds through molecular docking (Abd El-Aziz et al., 2021). Eweas et al. (2021) showed that TYR 619 was present at the predicted active site of the protein, which formed a complex with remdesivir and hydroxychloroquine. TYR 619 (Y 619) is an essential amino acid in the active site of Motif A RdRp (ID: 6M71) (Gao et al., 2020).
LYS 621 in the active site also contributes to the formation of three hydrogen interactions in andrographidine-A, which was ranked third based on the docking score, as shown in Figure 3c. This study’s results showed that the amino acid have eight former hydrogen bonds. Previous studies on remdesivir-RdRp (ID: 6M71) interaction revealed that LYS 621 is one of the amino acids responsible for the formation of hydrogen (Eweas et al., 2021; Jang et al., 2021; Pintilie et al., 2020). Gao et al. (2020) reported that it was present on Motif A (fingers subdomain), and appeared in the prediction of the active side of RdRp protein. The active amino acid residues of ASP 760 and ASP 623 have 8 and 4 former hydrogen bonds, respectively. This is comparable with previous studies, such as Pintilie et al. (2020) which studied remdesivir-RdRp (ID: 6M71), and revealed the presence of the hydrogen interaction of ASP 760 or D760 and ASP 623. Pirzada et al., (2021) identified potential inhibitors of RdRp (ID: 6M71) using FDA-approved antiviral drugs, such as remdesivir, ledipasvir, and paritaprevir. The results showed that ASP 760 and ASP 623 formed the bonds in the catalytic binding site of RdRp and this finding was strengthened by Gao et al., (2020).

This study’s result showed that the four amino acids formed hydrogen bond that were responsible for the energy binding score and they also stabilized the ligand-protein RdRp inhibition. The results of amino acid interaction were comparable with the positive control–bound protein complex. This finding showed that the docking position of ligand on RdRp were ideal for the active site containing enzyme catalytic area, which is important for viral replication and transcription.

**ADMET Prediction**

The ADMET prediction of the top three *A. paniculata* compounds using pkCSM is presented in Table 2, while that of all compound is shown in Supplementary Table S3 with parameters models from Pires et al., (2015). The absorption prediction showed that andrographidine-A had low permeability on Caco-2 cell, and the three compounds showed good absorption on the intestine, hence, they have the potential to be absorbed on the intestinal membrane. However, andrographidine-A can be considered in the design of drug delivery systems to increase its ability to be well absorbed and penetrated, which helps to obtain an ideal therapeutic bioavailability. Andrographolactone have low skin permeability compared to andrographidine-A and andrografolide, indicating that it is bad for topical delivery design, but can be used for oral delivery.

The distribution process of andrographidine-A determined the prediction of Low VDss, which is poorly distributed to the brain and unable to penetrate the CNS. The compound can also have low lipophilicity to cross the blood-brain barrier and central nervous system. However, andrographolactone and andrografolide can penetrate with high distribution in this high lipophilicity area, and this helped to decrease the side effects and toxicity or improve the pharmacological activity of drugs.

Most of the metabolic reactions primarily found in the liver are Cytochrome P450, which has some major enzymes promoting prodrug activation or detoxification by converting high polarity drug to excreted molecules.
| Process               | Models                     | Unit                                      | Andrographolactone | Andrographolide | Andrographidine A |
|----------------------|----------------------------|-------------------------------------------|--------------------|-----------------|-------------------|
| Absorption           | Caco-2 cell permeability   | Numeric (log Papp in 10-6 cm/s)           | 1.798              | 1.075           | 0.379             |
|                      | Intestinal absorption (human) | Numeric (% Absorbed)                    | 96.48              | 92.432          | 67.146            |
|                      | Skin permeability          | Numeric (log Kp)                          | -2.229             | -3.539          | -2.736            |
| Distribution         | VDss (human)               | Numeric (log L/kg)                        | 0.818              | -0.097          | -0.659            |
|                      | BBB permeability           | Numeric (log BB)                          | 0.239              | -0.615          | -1.35             |
|                      | CNS permeability           | Numeric (log PS)                          | -2.223             | -2.629          | -4.023            |
| Metabolism           | CYP2D6 substrate          | Categorical (Yes/No)                      | No                 | No              | No                |
|                      | CYP3A4 substrate          | Yes                                       | Yes                | Yes             | No                |
|                      | CYP1A2 inhibitor          | Yes                                       | Yes                | No              | No                |
|                      | CYP2C19 inhibitor         | Yes                                       | No                 | No              | No                |
|                      | CYP2C9 inhibitor          | No                                        | No                 | No              | No                |
|                      | CYP2D6 inhibitor          | No                                        | No                 | No              | No                |
|                      | CYP3A4 inhibitor          | No                                        | No                 | No              | No                |
| Excretion            | Total clearance           | Numeric (log ml/min/kg)                   | 1.288              | 1.179           | 0.74              |
|                      | Renal OCT2 substrate      | Categorical (Yes/No)                      | No                 | No              | No                |
| Toxicity             | AMES toxicity             | Categorical (Yes/No)                      | No                 | No              | No                |
|                      | hERG I inhibitor          | No                                        | No                 | No              | No                |
|                      | hERG II inhibitor         | No                                        | No                 | No              | No                |
|                      | Hepatotoxicity           | No                                        | Yes                | No              | No                |
|                      | Skin sensitisation       | Yes                                       | No                 | No              | No                |
|                      | T. pyriformis toxicity    | Numeric (log ug/L)                        | 2.611              | 0.636           | 0.285             |
|                      | Minnow toxicity          | Numeric (log mM)                          | -0.35              | 1.155           | 4.074             |
The results showed that andrographolactone and andrografolide were predicted as substrate of the CYP3A4 enzyme. They are easy to metabolize and excrete from the body to prevent toxicity. Cytochrome P450 3A4 (CYP3A4) is the major cytochrome because CYP3A4 can metabolize most types of drug. The prediction results revealed that andrographolactone can serve as a CYP3A4 substrate, CYP1A2 inhibitor, and CYP2C19 inhibitor.

Based on the excretion prediction, andrographolactone, andrographolide, and andrographidine-A are not renal OTC2 substrate that cause interactions when administered concurrently with OCT2 Inhibitors. The result of toxicity prediction shows that these compound do not indicate the mutagenicity on Ames toxicity prediction and hERG inhibitor, which can lead to heart problems. Based on T.Pyriformis toxicity, all compounds can cause toxicity to the protozoan. The hepatotoxicity test revealed that andrographolactone and andrographidine-A are not toxic, while the Skin Sensitisation prediction showed that andrographolactone have an effect on the skin.

The ADMET prediction using the pkCSM of the top three docking scores showed that andrographolactone have more good values in ADMET studies. This result can be proved by comparing in-vitro and in-vivo studies.

CONCLUSION

The results showed the lower binding energy (ΔG) of the top three bioactive A. paniculata compounds, namely andrographolactone, andrographolide, and andrographidine-A, respectively, which can be prevented by the replication, transcription, and synthesis of SARS-CoV-2 RdRP (ID: 6M71) were creates hydrogen interaction with TYR 619, LYS 621, ASP 760, and ASP 623. TYR 619, LYS 621, ASP 760, and ASP 623. The ADMET prediction revealed that andrographolactone has low toxicity, which is ideal for an orally active drug for humans. However, there is a need for further studies on andrographolactone as a novel therapeutic candidate by Molecular Dynamic or QSAR, followed by pre-clinical in-vitro and in-vivo SARS-CoV-2 studies.

CONFLICT OF INTEREST

The authors declared that there is no conflict of interest.
Eweas, A. F., Alhossary, A. A., & Abdel-moneim, A. S. (2021). Molecular docking reveals ivermectin and remdesivir as potential repurposed drugs against SARS-CoV-2. *Frontiers in Microbiology, 11*(January), 1–15. https://doi.org/10.3389/fmicb.2020.592908

Fikrika, H., Ambarsari, L., & Sumaryada, T. (2016). Molecular docking studies of catechin and its derivatives as anti-bacterial inhibitor for glucosamine-6-phosphate synthase. *IOP Conference Series: Earth and Environmental Science, 31*(1). https://doi.org/10.1088/1755-1315/31/1/012009

Firdayani, & Srijanto, B. (2012). Bioactive Constituents from *Andrographis paniculata* ness with hepatoprotective potentials through in silico method. *Proceeding of International Conference on Drug Development of Natural Resources, 223–228.*

Gao, Y., Yan, L., Huang, Y., Liu, F., Zhao, Y., Cao, L., Wang, T., Sun, Q., Ming, Z., Zhang, L., Ge, J., Zheng, L., Zhang, Y., Wang, H., Zhu, Y., Zhu, C., Hu, T., Hua, T., Zhang, B., ... Rao, Z. (2020). Structure of the RNA-dependent RNA polymerase from COVID-19 virus. *Science, 368*(6492), 779–782. https://doi.org/10.1126/science.abb7498

Gorbalenya, A. E., Baker, S. C., Baric, R. S., de Groot, R. J., Drosten, C., Gulyaeva, A. A., Haagmans, B. L., Lauber, C., Leontovich, A. M., Neuman, B. W., Penzar, D., Perlman, S., Poon, L. L. M., Samborskiy, D., Sidorov, I. A., Sola, I., & Ziebuhr, J. (2020). Severe acute respiratory syndrome-related coronavirus: The species and its viruses – a statement of the Coronavirus Study Group. *BioRxiv.* https://doi.org/10.1101/2020.02.07.937862

Jang, W. D., Jeon, S., Kim, S., & Lee, S. Y. (2021). Drugs repurposed for COVID-19 by virtual screening of 6,218 drugs and cell-based assay. *Proceedings of the National Academy of Sciences of the United States of America, 118*(30), 1–9. https://doi.org/10.1073/pnas.2024302118

Jayakumar, T., Hsieh, C. Y., Lee, J. J., & Sheu, J. R. (2013). Experimental and clinical pharmacology of *Andrographis paniculata* and its major bioactive phytoconstituent andrographolide. *Evidence-Based Complementary and Alternative Medicine, 2013* (Figure 1). https://doi.org/10.1155/2013/846740

Kwon, C. Y., Lee, B., Chung, S. Y., & Kim, J. W. (2019). Herbal medicine for post-stroke anxiety: A systematic review and meta-analysis of randomized controlled trials. *Complementary Therapies in Clinical Practice, 35*(March 2020), 237–252. https://doi.org/10.1016/j.ctcp.2019.02.015

Lim, X. Y., Sue, J., Chan, W., Yew, T., Tan, C., Teh, B. P., Ridzuan, M., Abd, M., Mohamad, S., Fazlin, A., & Mohamed, S. (2021). *Andrographis paniculata* (Burm. F.) Wall. andrographolide analogues as SARS-antivirals? A rapid review. *Natural Product Communications, 16*(5), 1–15. https://doi.org/10.1177/1934578X211016610

Lipsitch, M., Swerdlow, D. L., & Lyn Finelli. (2020). Defining the epidemiology of COVID-19 - Studies needed. *The New England Journal of Medicine, 328,* 1194–1196. https://doi.org/10.1056/NEJMp2002125

Loza-Mejia, M. A., & Salazar, J. R. (2020). In silico exploration through molecular docking and molecular dynamics of some cinnamoyl substituted compounds on targets related to SARS-CoV-2. *Revista Del Centro de Investigación de La Universidad La Salle, 14*(53), 67–88. http://52.226.65.210/index.php/recein/article/view/2653/2664%0Ahttp://52.226.65.210/index.php/recein/article/view/2653

Lung, J., Lin, Y. S., Yang, Y. H., Chou, Y. L., Shu, L. H., Cheng, Y. C., Liu, H. Te, & Wu, C. Y. (2020). The potential chemical structure of anti-SARS-CoV-2 RNA-dependent RNA polymerase. *Journal of Medical Virology, 92*(6), 693–697. https://doi.org/10.1002/jmv.25761

Morse, J. S., Lalonde, T., Xu, S., & Liu, W. R. (2020). Learning from the past: Possible urgent prevention and treatment options for severe acute respiratory infections caused by 2019-nCoV. *ChemBioChem, 21*(5), 730–738. https://doi.org/10.1002/cbic.202000047

Murugan, N. A., Pandian, C. J., & Jeyakanthan, J. (2021). Computational investigation on *Andrographis paniculata* phytochemicals to evaluate their potency against SARS-CoV-2 in comparison to known antiviral compounds in drug trials. *Journal of Biomolecular Structure and Dynamics, 39*(12), 4415–4426. https://doi.org/10.1080/07391102.2020.1777901

Nie, X., Chen, S. R., Wang, K., Peng, Y., Wang, Y. T., Wang, D., Wang, Y., & Zhou, G. C. (2017). Attenuation of innate immunity by andrographolide derivatives through NF-κB signaling pathway. *Scientific Reports, 7*(1), 1–10. https://doi.org/10.1038/s41598-017-04673-x

Ningampalle, M., Devanathan, V., & Saxena, A. (2021). Screening of chloroquine, hydroxychloroquine and its derivatives for their binding affinity to multiple SARS-CoV-2 protein drug targets. *Journal of Biomolecular Structure and Dynamics, 39*(14), 4949–4961. https://doi.org/10.1080/07391102.2020.1782265
Nusantoro, Y. R., & Fadlan, A. (2021). The effect of energy minimization on the molecular docking of acetone-based oxindole. *Jurnal Kimia dan Pendidikan Kimia*, 6(1), 69–77. https://doi.org/10.20961/jkpk.v6i1.45467

Parvez, M. S. A., Karim, M. A., Hasan, M., Jaman, J., Karim, Z., Tahsin, T., Hasan, M. N., & Hosen, M. J. (2020). Prediction of potential inhibitors for RNA-dependent RNA polymerase of SARS-CoV-2 using comprehensive drug repurposing and molecular docking approach. *International Journal of Biological Macromolecules*, 163, 1787–1797. https://doi.org/10.1016/j.ijbiomac.2020.09.098

Patin, E. W., Zaini, M. A., & Sulastr, Y. (2018). Pengaruh variasi suhu pengegerangan terhadap sifat fisiko kimia teh daun sambiloto (*Andrographis paniculata*). *Pro Food (Jurnal Ilmu dan Teknologi Pangan)*, 4(1), 251–258. https://doi.org/10.29303/profood.v4i1.72

Pintilie, L., Tanase, C., & Mohapatra, R. K. (2020). Molecular docking studies on synthetic therapeutic agents for COVID-19. *Chemistry Proceedings*, 3(1), 46. https://doi.org/10.3390/ccsocc-24-08352

Pires, D. E. V., Blundell, T. L., & Ascher, D. B. (2015). pKCSM: Predicting small-molecule pharmacokinetic and toxicity properties using graph-based signatures. *Journal of Medicinal Chemistry*, 58(9), 4066–4072. https://doi.org/10.1021/acs.jmedchem.5b00104

Pirzada, R. H., Haseeb, M., Batool, M., Kim, M. S., & Choi, S. (2021). Remdesivir and ledipasvir among the fda-approved antiviral drugs have potential to inhibit SARS-CoV-2 replication. *Cells*, 10(5). https://doi.org/10.3390/cells10050152

Rafi, M., Karomah, A. H., Heryanto, R., Septaningsih, D. A., Kusuma, W. A., Amran, M. B., Rohman, A., & Prajogo, B. (2020). Metabolite profiling of *Andrographis paniculata* leaves and stem extract using UHPLC-Orbitrap-MS/MS. *Natural Product Research*, 0(0), 1–5. https://doi.org/10.1080/14786419.2020.1789637

Ratnani, R. D., Hartati, I., & Kurniasari, L. (2012). Potensi produksi andrographolide dari sambiloto (*Andrographis paniculata* Nees) melalui proses ekstraksi hidrotropi. *Momumentum*, 8(1), 6–10. https://doi.org/10.36499/jim.v8i1.279

Sa-Ngiamsuntorn, K., Sukatsa, A., Pewkliang, Y., Thongsri, P., Kanjanasirirat, P., Manopwisedjaroen, S., Charoensuthivarakan, S., Wongtrakoonnate, P., Pitiporn, S., Chaopreecha, J., Kongsomros, S., Jearawuttanakul, K., Wannalo, W., Khemawoot, P., Chutipongtanate, S., Borwornpinyo, S., Thitithanyanont, A., & Hongeng, S. (2021). Anti-SARS-CoV-2 activity of *Andrographis paniculata* extract and its major component andrographolide in human lung epithelial cells and cytotoxicity evaluation in major organ cell representatives. *Journal of Natural Products*, 84(4), 1261–1270. https://doi.org/10.1021/acs.jnatprod.0c01324

Sharbidre, A., Dhage, P., Duggal, H., & Meshram, R. (2021). In silico investigation of tridax procumbens phytoconstituents against SARS-CoV-2 infection. *Biointerface Research in Applied Chemistry*, 11(4), 12120–12148. https://doi.org/10.33263/BRIAC114.1212012148

Sharma, A., Vora, J., Patel, D., Sinha, S., Jha, P. C., & Shrivastava, N. (2020). Identification of natural inhibitors against prime targets of SARS-CoV-2 using molecular docking, molecular dynamics simulation and MM-PBSA approaches. *Journal of Biomolecular Structure and Dynamics*, 0(0), 1–16. https://doi.org/10.1080/07391102.2020.1846624

Srikanth, L., Venkata, P., & Krishna, G. (2021). Andrographolide binds to spike glycoprotein and RNA-dependent RNA polymerase (NSP12 ) of SARS-CoV-2 by in silico approach : a probable molecule in the development of anti-coronaviral drug. *Journal of Genetic Engineering and Biotechnology*, 19(101), 1–7. https://doi.org/10.1186/s43141-021-00201-7

Swaminathan, K., Karunakaran, K. N., Manoharan, J. P., & Vidyalakshmi, S. (2021). SARS-CoV-2 multiple target inhibitors from *Andrographis paniculata*: An in-silico report. *European Journal of Molecular and Clinical Medicine*, 8(3), 1653–1685. https://www.embase.com/search/s?subaction=viewrecord&id=L2011572564&from=export

Wabalo, E. K., Dubiwak, A. D., Senbetu, M. W., & Gizaw, T. S. (2021). Effect of genomic and amino acid sequence mutation on virulence and therapeutic target of severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2). *Infection and Drug Resistance*, 14, 2187–2192. https://doi.org/10.2147/IDR.S307374

Wanaratna, K., Leethong, P., Inchai, N., Chueawiang, W., Tabmee, A., Sirinavin, S., Medicine, A., Hospital, S., Wanaratna, K., & Medicine, A. (2021). Efficacy and safety of *Andrographis paniculata* extract in patients with mild COVID-19: A randomized controlled trial. *Medrxiv*, 7(8), 21259912. https://doi.org/10.1101/2021.07.08.21259912

Wang, G. C., Wang, Y., Williams, I. D., Sung, H. H. Y., Zhang, X. Q., Zhang, D. M., Jiang, R. W., Yao, X. S., & Ye, W. C. (2009). Andrographolactone, a unique...
diterpene from *Andrographis paniculata*. *Tetrahedron Letters*, 50(34), 4824–4826. https://doi.org/10.1016/j.tetlet.2009.05.097

Wang, Z., & Yang, L. (2021). Chinese herbal medicine: Fighting SARS-CoV-2 infection on all fronts. *Journal of Ethnopharmacology*, 270(113869), 1–18. https://doi.org/10.1016/j.jep.2021.113869

Wrapp, D., Wang, N., Corbett, K. S., Goldsmith, J. A., Hsieh, C. L., Abiona, O., Graham, B. S., & McLellan, J. S. (2020). Cryo-EM structure of the 2019-nCoV spike in the prefusion conformation. *Science (New York, N.Y.*), 367(6483), 1260–1263. https://doi.org/10.1126/science.abb2507

Wu, C., Liu, Y., Yang, Y., Zhang, P., Zhong, W., Wang, Y., Wang, Q., Xu, Y., Li, M., Li, X., Zheng, M., Chen, L., & Li, H. (2020). Analysis of therapeutic targets for SARS-CoV-2 and discovery of potential drugs by computational methods. *Acta Pharmaceutica Sinica B*, 10(5), 766–788. https://doi.org/10.1016/j.apsb.2020.02.008

Yang, Y., Lu, Q.-B., Liu, M.-J., Wang, Y.-X., Zhang, A.-R., Jalali, N., Dean, N. E., Longini, I., Halloran, M. E., Xu, B., Zhang, X.-A., Wang, L.-P., Liu, W., & Fang, L.-Q. (2020). Epidemiological and clinical features of the 2019 novel coronavirus outbreak in China. *Medrxiv*. https://doi.org/10.1101/2020.02.10.20021675

Yasin, S. A., Azzahra, A., Ramadhan, N. E., & Mylanda, V. (2020). Studi penambatan molekuler dan prediksi admet senyawa bioaktif beberapa jamu Indonesia terhadap SARS-CoV-2 main protease (Mpro). *Berkala Ilmiah Mahasiswa Farmasi Indonesia (BIMFI)*, 7(2), 24–41. https://doi.org/10.48177/bimfi.v7i2.45

Yu, R., Chen, L., Lan, R., Shen, R., & Li, P. (2020). Computational screening of antagonists against the SARS-CoV-2 (COVID-19) coronavirus by molecular docking. *International Journal of Antimicrobial Agents*, 56(January). https://doi.org/10.1016/j.ijantimicag.2020.106012