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

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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.

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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 to the catalytic site of 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 Sarbecovirus 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 andrographiti* (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; Ratnani 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 (Wanaratna 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, dehydroandrograpolide 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 webserver. 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

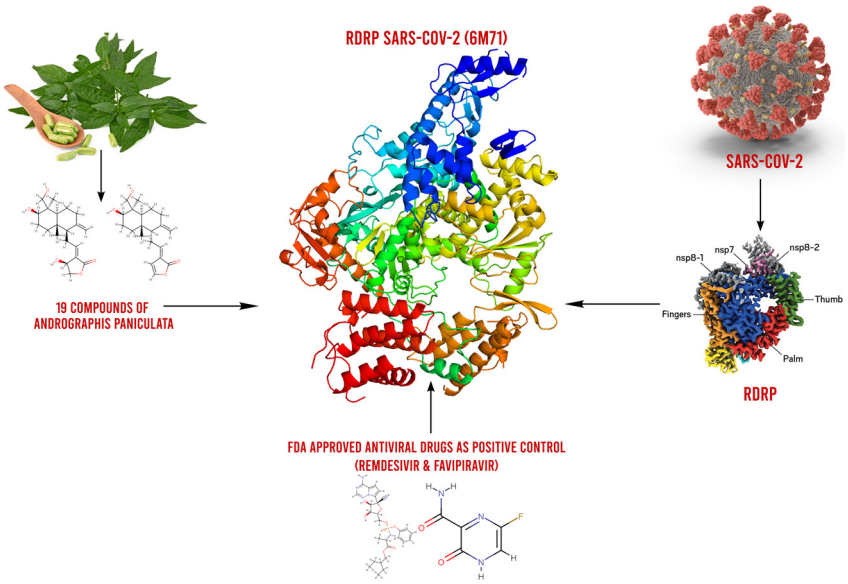

Figure 1. Schematic molecular docking study of inhibitor SARS-CoV-2 RdRp

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 (ΔG) 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 (ΔG) 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 grid box size preferences in some previous studies (Borse et al., 2021; da Silva et al., 2020; Nimgampalle et al., 2021; Parvez et al., 2020) that use the same protein code 6M71 with remdesivir as a positive control of docking were tested. These studies referred to Gao et al. (2020) of 6M71 polymerase RdRp, which had a complex structure of nsp12 (RdRp) with its cofactors, namely nsp7 and nsp8. These two cofactors can catalyze the synthesis of viral RNA, which plays a central role in the replication and transcription cycle of COVID-19 virus. This protein has three subdomains that are close to the active site. Furthermore, its active site in motif A contains residues 611 to 626, which have a classic divalent-cation–binding residue, while motif C consists of 753 to 767, including catalytic 759 to 761 (Gao et al., 2020). The catalytic residues are important for the Enzyme-Inhibitor complex's stability and viral replication (Sharbidre et al., 2021).

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.

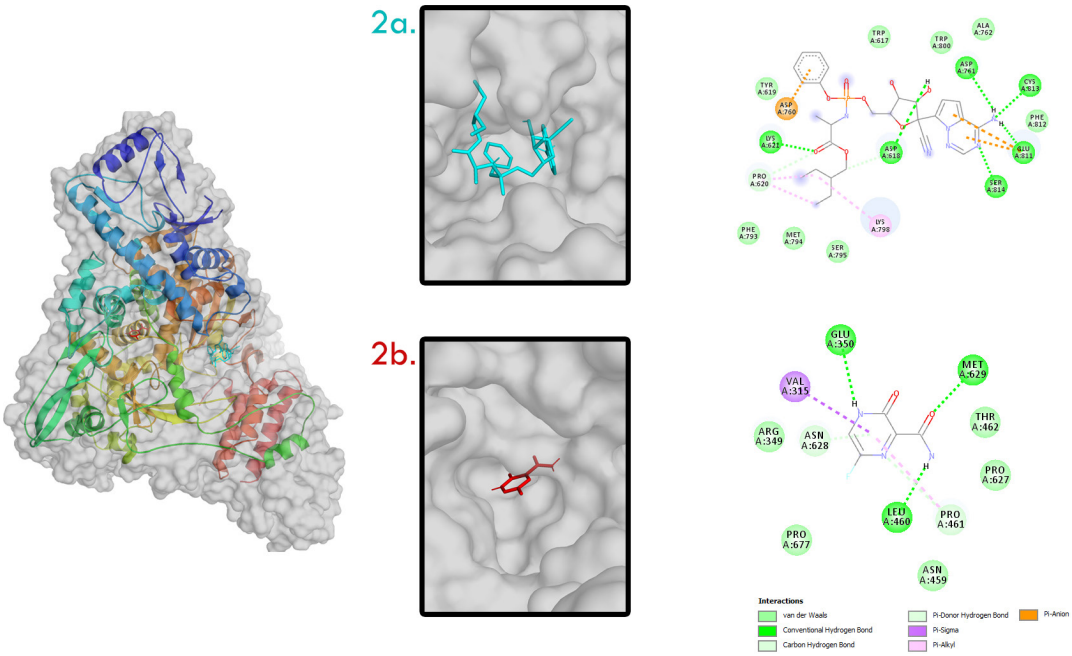

Figure 2. Visualizing protein-ligand interactions of positive control (a) remdesivir and (b) favipiravir

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 (ΔG) score), as shown in Table 1. The top three docking scores of bioactive *A. Paniculata* compound were ΔG = -8.86 kcal/mol as the lowest binding energy of andrographolactone, followed by andrographolide ΔG = -7.74 kcal/mol and andrographidine-A ΔG = -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 (ΔG) 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 uM (micromolar) and andrographidine-A of 2.34 uM (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 indicate 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 derivate 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.

Table 1. Binding energy values (docking score) of 19 *A. Paniculata* and positive control against SARS-CoV-2 RdRp (ID: 6M71)

No	Ligands	Binding Energy (ΔG)	Inhibition Constant (Ki)
1	Andrographolactone	-8.86 kcal/mol	321.37 nM (nanomolar)
2	Andrographolide	-7.74 kcal/mol	2.10 uM (micromolar)
3	Andrographidine A	-7.68 kcal/mol	2.34 uM (micromolar)
4	Andrographanine	-7.58 kcal/mol	2.77 uM (micromolar)
5	Neoandrographolide	-7.55 kcal/mol	2.94 uM (micromolar)
6	14-deoxy-11-oxoandrographolide	-7.54 kcal/mol	2.97 uM (micromolar)
7	3-O-caffeoylquinic acid	-7.29 kcal/mol	4.53 uM (micromolar)
8	Apigenin	-7.13 kcal/mol	5.90 uM (micromolar)
9	12S-hydroxyandrographolide	-7.08 kcal/mol	6.44 uM (micromolar)
10	14-deoxyandrographolide	-7.04 kcal/mol	6.96 uM (micromolar)
11	Dehydroandrographolide	-6.98 kcal/mol	7.66 uM (micromolar)
12	Deoxyandrographolide	-6.95 kcal/mol	8.02 uM (micromolar)
13	5,2'-dihydroxy-7,8-dimethoxyflavanone	-6.79 kcal/mol	10.61 uM (micromolar)
14	14-deoxy-17-hydroxyandrographolide	-6.77 kcal/mol	10.90 uM (micromolar)
15	5,7-dihydroxy-8-methoxyflavone	-6.77 kcal/mol	10.95 uM (micromolar)
16	Paniculide C	-6.42 kcal/mol	19.70 uM (micromolar)
17	Caffeic acid	-6.42 kcal/mol	19.75 uM (micromolar)
18	Paniculide B	-6.15 kcal/mol	31.25 uM (micromolar)
19	Remdesivir	-5.73 kcal/mol	62.84 uM (micromolar)
20	Cinaminic acid	-5.72 kcal/mol	63.63 uM (micromolar)
21	Favipiravir	-5.20 kcal/mol	155.21 uM (micromolar)

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).

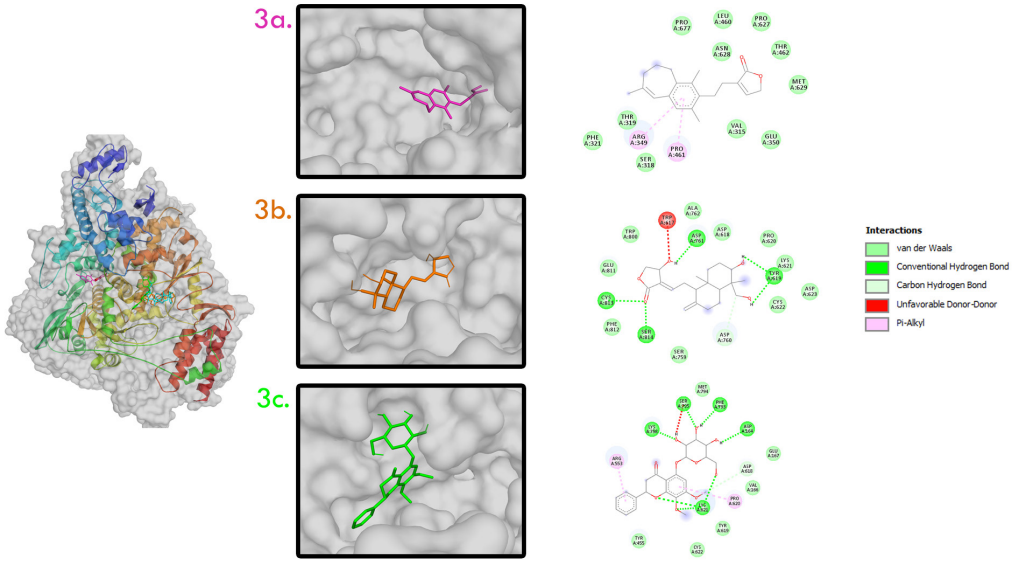

Figure 3. Visualizing protein-ligand interactions of top three best docking scores (a) andrographolactone, (b) andrographolide, and (c) andrographidine-A

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.

Table 2. ADMET prediction of top three docking score *A. paniculata* compounds using pkCSM online server

Process	Models	Unit	Compounds		
			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	No
	CYP1A2 inhibitor		Yes	No	No
	CYP2C19 inhibitor		Yes	No	No
	CYP2C9 inhibitor		No	No	No
	CYP2D6 inhibitor		No	No	No
	CYP3A4 inhibitor		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
	hERG II inhibitor		No	No	No
	Hepatotoxicity		No	Yes	No
	Skin sensitisation		Yes	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.

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