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Computational exploration of *Andrographis paniculata* herb compounds as potential antiviral agents targeting NSP3 (6W02) and NSP5 (7AR6) of SARS-COV-2

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Abstract

The ongoing COVID-19 pandemic, caused by SARS-CoV-2, requires an urgent search for effective antiviral agents. NSP3 and NSP5, play critical roles in the replication and transcription. This study aimed to screening the physicochemical properties, toxicity, and antiviral potential of herbal compounds from *Andrographis paniculata* against NSP3 (6W02) and NSP5 (7AR6) of SARS-CoV-2. The herb compounds were obtained from the KNApSAcK Family and PubChem databases. Their interactions with *Remdesivir*, an FDA-approved antiviral for SARS-CoV-2, NSP3 and NSP5 were analyzed using molecular docking through Molegro Virtual Docker 6.0. There are 27 compounds out of a total of 41 herb compounds that meet the Veber Rule and have predicted good physicochemical properties. Moreover, 15 herb compounds predicted to be non-toxic based on GHS, not-mutagenic, not-carcinogenic, and not-allergenic to the skin. The screening results using PASS Online showed 5 compounds highly potential to be candidates for SARS-CoV-2 antivirals, namely: *12S-Hydroxyandrographolide, 14-Deoxy-11,12-didehydroandrographolide, Andrographolide, Andropanolide,* and *Isoandrographolide.* Based on molecular docking results, it was found both *Remdesivir* and the herb compounds still required more energy than the native ligand on the 6W02 target protein. In contrast, for the 7AR6 target protein, both *Remdesivir* and herb compounds required less energy than the native ligand. In conclusion, *A. paniculata* herb compounds showed inhibitory activity on 6W02 and 7AR6 receptors, suggesting their potential as herbal treatments for SARS-CoV-2 antivirals by targeting the NSP3 and NSP5 proteins. Further validation through in vitro and in vivo studies is needed.

Keywords: *Andrographis paniculata;* NSP3 (6W02); NSP5 (7AR6); SARS-CoV-2; Molegro Virtual Docker; Herb compounds

1. Introduction

COVID-19 has been declared a global pandemic by WHO since 2020 until this day. COVID-19 is caused by *severe acute respiratory syndrome coronavirus 2* (SARS-CoV-2), with the respiratory system as its target. Symptoms caused by COVID-19 can be variably ranging as mildly as fever, sore throat, cough, to shortness of breath until respiratory failure [1] There are two categories of therapy for COVID-19: one targeting the virus and the other targeting the immune system [2].

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Many studies have discovered that SARS-CoV-2 has four main structural proteins, namely the spike protein (S), membrane protein (M), envelope (E), and nucleocapsid protein (N) which contribute to viral host entry and viral replication [3]. Furthermore, SARS-CoV-2 has 16 other -n-structural proteins (NSP), which encode essential enzymes for RNA processing and virus replication [4]. NSP3 (also called papain-like protease) has a role in producing NSP1, NSP2, and NSP3 in the early viral replication process. NSP3 also has been known to have a role in suppressing the immune response [5]. The Mpro (nsp5), which is encoded by the primary open reading frame 1ab (ORF1ab), plays a crucial role in the cleavage of two overlapping polyproteins (pp1a and pp1ab) into 16 non-structural proteins. These non-structural proteins are vital for viral replication and maturation processes [6].

Until this day, the FDA has only fully approved Veklury (*Remdesivir*) as SARS-CoV-2 antiviral drug [7]. In drug discovery process, a drug repurposing strategy can significantly reduce time and laboratory cost of the process [8]. In this article, we used virtual screening process to screen potential compounds derived from *Andrographis paniculata*. *A. paniculata* has been known to be source of several potent compounds which have medicinal applications [9,10]. This study aimed to screening the physicochemical properties, toxicity, and antiviral potential of herbal compounds from *A. paniculata* against NSP3 (6W02) and NSP5 (7AR6) of SARS-CoV-2.

2. Material and methods

2.1. Software

Avogadro, SwissADME Online Tool, pkCSM Online Tool, ProTox-II Online Tool, PASS Online Tool, and Molegro Virtual Docker (MVD) version 6.0 were used.

2.2. Ligand and Protein Preparation

This study used herb compounds from the *A. paniculata* with the antiviral drug *Remdesivir.* A total of 41 herb compounds derived from *A. paniculata* were obtained from the KNApSAcK Family databse (http://www.knapsackfamily.com). The ligands used in this study were active herb compounds downloaded as SMILE files from PubChem (http://pubchem.ncbi.nlm.nih.gov/). The protein data bank website (https://www.rcsb.org/) provided the 3D structure protein NSP3 SARS CoV-2 with PDB ID: 6W02 and NSP5 SARS CoV-2 with PDB ID: 7AR6. Furthermore, the Avogadro application and the MMFF94 force field of the Steepest Descent algorithm were used to minimize energy for three replications, and the results were saved in the form *mol2 (SYBYL2.*Mol2). The Molegro Virtual Docker 6.0 software is used to validate receptors by removing water molecules and ligand references and adding hydrogen atoms. Validation was performed three times and a value of 2 Å was chosen for the Root Mean Square Deviation (RMSD). The native ligand for 6W02 was APR_201 [A] and for 7AR6 was DMS_406 [A].

2.3. Prediction of Biological Activity

The ligand herb compounds from *A. paniculata* were evaluated physicochemical and ADME properties as drug candidates using the SwissADME online tool (http://swissadme.ch) and classified using Veber Rules. Then, using pkCSM online tool (https://www.biosig.unimelb.edu.au/pkcsm/prediction) and ProTox-II Online Tool (https://tox-new.charite.de/protox_II/) predict the toxicity properties of the herb compounds and observe the results based on (LD50) per oral in rodents with the standard classification of compound toxicity based on the Globally Harmonized System (GHS) and other compound toxicity based on Hepatoxicity, Ames-toxicity, and Skin sensitization. Then, the prediction test for the antiviral and immunomodulator activity properties of herb compounds *A. paniculata* used PASS Online tools (http://www.way2drug.com/) which had results Pa>Pi (Potential activity>Potential inhibitor) or Pa>0.7. The selected compounds were then carried out by molecular docking using the Molegro Virtual Docker 6.0 software.

2.4. Molecular Docking

Molecular docking was utilized through the Molegro Virtual Docker version 6.0 application to establish the correlation between the test herb compound and the receptor. Validation of molecular docking was conducted by ensuring that the root mean square deviation (RMSD) parameter value was below 2Å. The molecular docking results were assessed based on the Rerank Score, Moldock Score, and H-Bond, which were replicated thrice. The interactions between the amino acid residues were identified through the formation of hydrogen and steric bonds.

2.5. Protein-Ligand Interaction Analysis

The Molegro Virtual Docker 6.0 software was utilized for molecular docking visualization. The research focused on investigating the bonding between proteins and ligands, specifically the herb compounds of *A. paniculata*, and their respective target proteins based on the type of interaction and bond formed. The rerank score, which measures bond

energy, was utilized to assess the amount of energy needed for the ligand and receptor to form a bond. Subsequently, the Molegro Virtual Docker 6.0 software was utilized to generate a realistic 3D diagram of the complex interaction between the ligand and the receptor following the docking process.

3. Results and discussion

3.1. Prediction of Biological Activity

In this article, we perform screening the physicochemical properties, toxicity, and antiviral potential of herbal compounds from *A. paniculata*.

Table 1 Prediction of physicochemical properties herb compound in *A. paniculata*

Compound	Veber Rules						Result
	MW	LogP	HBA	HBD	TPSA	Torsion	
12S-Hydroxyandrographolide	368.46	1.65	6	4	107.22	4	Good
14-Acetyl-3,19-isopropylideneandrographolide	432.55	3.97	6	0	71.06	4	Good
14-Acetylandrographolide	392.49	2.80	6	2	93.06	5	Good
14-Deoxy-11,12-didehydroandrographolide	332.43	3.02	4	2	66.76	3	Good
14-Deoxy-11-oxoandrographolide	348.43	2.29	5	2	83.83	4	Good
14-Deoxy-17-hydroxyandrographolide	352.47	2.49	5	3	86.99	5	Good
14-Deoxyandrographolide	334.45	3.17	4	2	66.76	4	Good
3-O-beta-D-Glucopyra-sylandrographolide	512.59*	0.57	10	6*	166.14*	6	-
Chlorogenic acid	354.31	-0.38	9	6*	164.75*	5	-
Andrographidin B	492.43	0.47	12*	6*	188.51*	6	-
5,4'-Dihidroxy-7,8,2',3'-tetramethoxy flavone 5-glucoside	536.48*	0.55	13*	5	186.74*	8	-
5,4'-Dihidroxy-7,8,2',3'-tetramethoxyflavone	374.34	2.30	8	2	107.59	5	Good
5,7,2',3'-Tetramethoxyflava-ne	344.36	2.79	6	0	63.22	5	Good
5-Hydroxy-3,7,8,2'-tetramethoxyflavone	358.34	2.83	7	1	87.36	5	Good
5-Hydroxy-7,2',6'-trimethoxyflavone	328.32	2.93	6	1	78.13	4	Good
5-Hydroxy-7,8,2',3'-tetramethoxyflavone 5-glucoside	520.48*	0.87	12*	4	166.51*	8	-
Andrographidine E	490.46	1.09	11*	4	157.28*	7	-
5-Hydroxy-7,8-dimethoxyflava-ne	300.31	2.61	5	1	64.99	3	Good
Andrographidine C	460.43	0.78	10	4	148.05*	6	-
7-O-Methylwogonin	298.29	2.81	5	1	68.9	3	Good
Andrograpanin	318.45	3.97	3	1	46.53	4	Good
Andrographic acid	364.43	2.18	6	4	115.06	5	Good
Andrographidin A	462.45	0.61	10	4	144.15*	6	-
Andrographiside	512.59*	0.62	10	6*	166.14*	6	-
Andrographolide	350.45	2.30	5	3	86.99	3	Good
Andropa-lide	350.45	2.30	5	3	86.99	3	Good
Apigenin 7,4'-dimethyl ether	298.29	2.79	5	1	68.9	3	Good

beta-Sitosterol	414.71	7.19*	1	1	20.23	6	-
Bisandrographolide C	664.87*	5.27*	8	4	133.52	8	-
Caffeic acid	180.16	0.93	4	3	77.76	2	Good
Cinnamic acid	148.16	1.79	2	1	37.3	2	Good
Dihydroskullcap flavone I	316.31	2.18	6	2	85.22	3	Good
Ferulic acid	194.18	1.36	4	2	66.76	3	Good
Isoandrographolide	350.45	2.41	5	2	75.99	2	Good
Neoandrographolide	480.59	2.31	8	4	125.68	7	Good
Ninandrographolide	496.59	1.52	9	5	145.91*	7	-
Paniculide A	264.32	2.09	4	1	59.06	3	Good
Paniculide B	280.32	1.30	5	2	79.29	4	Good
Paniculide C	278.30	1.50	5	1	76.13	4	Good
Skullcapflavone 1,2'-O-beta-D-glucopyra-side	476.43	0.82	11*	5	168.28*	6	-
Wogonin 5-glucoside	446.40	0.53	10	5	159.05*	5	-
Remdesivir	602.58*	0.18	12*	4	213.36*	14*	-

* Does not meet Veber's Rule parameters.

In this article we used Veber rule to screen 41 herb compound of *A. paniculata* to determine of its ADME properties. Veber rule is a variation to Lipinski rule of five (molecular weight, LogP, Hydrogen Bond Acceptors [HBA], and Hydrogen Bond Donor [HBD]), where Veber more criteria added which is topological surface area (TPSA) and number rotatable bonds (Torsion) [11,12]. Based on table 1, only 27 herb compounds that consistent with Veber rule. These compounds are predicted to have similar ADME properties with initial drugs. However, *Remdesivir* as the comparator drug didn't meet the good Veber rule.

The molecular weight of drugs or compounds plays a vital role in their distribution within the body. Smaller molecules with fewer atoms have lower molecular weights and can easily pass through cell membranes [13]. The LogP value, reflecting the hydrophobicity of a compound, is critical in drug development as it influences pharmacokinetics and oral absorption through lipid bilayer membranes. High LogP values (>5) result in prolonged membrane retention and reduced selectivity for target enzymes, while negative values prevent passage through lipid bilayers [14]. Ligand HBA and HBD values determine their flexibility in binding to protein enzymes or targets and are related to their biological activity. Hydrogen bonding affects chemical and physical properties, such as solubility, boiling, and melting points, influencing compound biological activity [15]. The TPSA value serves as a parameter for assessing compound absorption, permeability, bioavailability, and penetration, calculated based on the hydrogen bonding surface area between N and O atoms [16]. Additionally, torsion, representing the number of rotatable atoms, affects a compound's flexibility in binding to protein enzymes or targets [17].

The next step is to predict the toxicity of the compounds that passed the physicochemical property screening, which includes 27 herb compounds. Toxicity prediction parameters used in this study are ames toxicity, hepatoxicity, skin sensitization, and LD50 based on GHS classification.

Table 2 shows that out of the 27 herb compounds that passed the toxicity prediction screening, 15 of them are marker compounds, such as *Andrographolide*; diterpenoid compounds like *12S-Hydroxyandrographolide, 14-Deoxy-11, 12-didehydroandrographolide, Isoandrographolide, Andropanolide*; and flavonoid compounds like *5,4'-Dihydroxy-7,8,2',3'-tetramethoxyflavone, 5-Hydroxy-3,7,8,2'-tetramethoxy flavone, 5-Hydroxy-7,2',6'-trimethoxyflavone, 5-Hydroxy-7,8-dimethoxy flavanone, Dihydroskullcap flavone I*; as well as other compounds including *7-O-Methylwogonin, Apigenin 7,4'-dimethyl ether, Caffeic acid, Cinnamic acid*, and *Ferulic acid*. These 15 compounds are predicted to have low toxicity levels, are non-hepatotoxic, and well-tolerated by the body. However, *Remdesivir*, as a comparative drug, has predicted hepatotoxicity.

Table 2 Prediction of toxicity compounds in *A. paniculata*

Compound	pkCSM			Protox II		Result
	Ames Tox	Hepatox	Skin	LD50 (mol/kg)	Class	
12S-Hydroxyandrographolide	-	-	-	33600	VI	-
14-Acetyl-3,19-isopropylideneandrographolide	-	-	-	100	III*	Out
14-Acetylandrographolide	-	-	-	300	III*	Out
14-Deoxy-11,12-didehydroandrographolide	-	-	-	6060	VI	-
14-Deoxy-11-oxoandrographolide	Yes*	-	-	34	II*	Out
14-Deoxy-17-hydroxyandrographolide	-	-	-	34	II*	Out
14-Deoxyandrographolide	-	-	-	34	II*	Out
5,4'-Dihidroxy-7,8,2',3'-tetramethoxyflavone	-	-	-	5000	VI	-
5,7,2',3'-Tetramethoxyflavanone	Yes*	-	-	2000	IV	Out
5-Hydroxy-3,7,8,2'-tetramethoxyflavone	-	-	-	5000	VI	-
5-Hydroxy-7,2',6'-trimethoxyflavone	-	-	-	4000	V	-
5-Hydroxy-7,8-dimethoxyflava-ne	-	-	-	1310	IV	-
7-O-Methylwogonin	-	-	-	3919	V	-
Andrograpanin	-	-	-	34	II*	Out
Andrographic acid	-	Yes*	-	4800	V	Out
Andrographolide	-	-	-	1890	IV	-
Andropa-lide	-	-	-	1890	IV	-
Apigenin 7,4'-dimethyl ether	-	-	-	3919	V	-
Caffeic acid	-	-	-	2980	V	-
Cinnamic acid	-	-	-	2500	V	-
Dihydroskullcap flavone I	-	-	-	2000	IV	-
Ferulic acid	-	-	-	1772	IV	-
Isoandrographolide	-	-	-	1130	IV	-
Neoandrographolide	-	Yes*	-	5	I*	Out
Paniculide A	Yes*	-	-	7	II*	Out
Paniculide B	Yes*	-	-	7	II*	Out
Paniculide C	Yes*	-	-	400	IV	Out
Remdesivir	-	Yes*	-	1000	IV	-

* Does not meet toxicity prediction test parameters

The results of LD50 values classified according to the Globally Harmonized System (GHS) indicate that compounds falling into classes IV, V, and VI passed this test and are predicted to have low toxicity, making them safe for use [18]. Skin sensitization tests indicate that all herb compounds from *A. paniculata* and Remdesivir do not exhibit toxicity to the skin, making them safe for use. Based on the Ames Toxicity parameter results, all herb compounds from *A. paniculata* are not carcinogenic except for five compounds: *14-Deoxy-11-oxoandrographolide, 5,7,2',3'-Tetramethoxyflavanone, and Paniculide A to C.* In terms of hepatoxicity, all herb compounds are considered safe for oral use, with the exception of *Andrograhic acid* and *Neoandrographolide*. However, the comparator drug Remdesivir has shown liver toxicity in clinical trials. Patients treated with Remdesivir reported an increase in liver enzymes, with hepatic enzyme elevation

being the most frequent adverse drug reaction (114 cases, 88%). The liver transaminases (aspartate transaminase and alanine transaminase) were involved in 79 cases (61%), while bilirubin was implicated in 4 cases (3%). Other cases were reported as hepatic failure or hepatitis [19].

The next step involved predicting the potential antiviral and immunomodulator activity of the secondary metabolite compounds that passed the toxicity prediction screening. Then, the top five compounds with higher antiviral activity predictions than others were selected. The antiviral activity prediction was carried out using the PASS Online website. The parameters used in the antiviral activity prediction were Pa > Pi or Pa > 0.7.

Table 3 Prediction of antiviral and immunomodulator activity compound in *A. paniculata*

Compound	**Antiviral**			**Immunomodulator**			**Result**
	Pa	**Pi**	**Rank**	**Pa**	**Pi**	**Rank**	
12S-Hydroxyandrographolide	0.517	0.020	2	0.677	0.020	3	Yes
14-Deoxy-11,12-didehydroandrographolide	0.471	0.034	3	0.676	0.020	4	Yes
5,4'-Dihidroxy-7,8,2',3'-tetramethoxyflavone	0.447	0.008	9	-	-	-	-
5-Hydroxy-3,7,8,2'-tetramethoxyflavone	0.433	0.023	12	0.498	0.043	6	-
5-Hydroxy-7,2',6'-trimethoxyflavone	0.447	0.019	10	-	-	-	-
5-Hydroxy-7,8-dimethoxyflavanone	0.449	0.014	7	0.395	0.067	8	-
7-O-Methylwogonin	0.437	0.022	11	-	-	-	-
Andrographolide	0.454	0.012	4	0.751	0.011	1	Yes
Andropanolide	0.454	0.012	5	0.751	0.011	2	Yes
Apigenin 7,4'-dimethyl ether	0.450	0.018	6	-	-	-	-
Caffeic acid	0.449	0.017	8	-	-	-	-
Cinnamic acid	0.421	0.022	13	0.443	0.055	7	-
Dihydroskullcap flavone I	0.407	0.022	15	-	-	-	-
Ferulic acid	0.412	0.100	14	0.379	0.071	9	-
Isoandrographolide	0.638	0.010	1	0.674	0.020	5	Yes
Remdesivir	0.814	0.004	-	0.312	0.074	-	Yes

Based on the above Table 3, the top 5 selected compounds, namely: *12S-Hydroxyandrographolide, 14-Deoxy-11, 12-didehydroandrographolide, Andrographolide, Andropanolide,* and *Isoandrographolide.* These five compounds show higher potential for antiviral and immunomodulatory activities compared to other herb compounds. Remdesivir, as a comparative drug, has a potential antiviral activity of 0.814 (Pa>0.7), indicating that Remdesivir has been proven as an effective antiviral drug. Higher Pa (Probability "to be active") values indicate a potential for activity, aligning with the results of laboratory tests, while Pi (Probability "to be inactive") values suggest the opposite or lack of activity [20].

3.2. Molecular Docking

The molecular docking objective is to compare the ability to bind herb compounds *A. paniculata* with remdesivir, a drug approved by the FDA as an antiviral for SARS-CoV-2. The target proteins in this article are NSP3 (6W02) and NSP5 (7AR6) from SARS CoV-2. The target protein used for molecular docking has an RMSD value of ≤2 Å [21]. We obtained the smallest RMSD values of 1,207 Å for 6W02 and 0,717 Å for 7AR6. Protein 6W02 has a native ligand, namely *adenosine-5-diphosphoribose* (APR), while protein 7AR6 has *dimethyl sulfoxide* (DMS) as its native ligand, are shown in Figure 1 and 2.

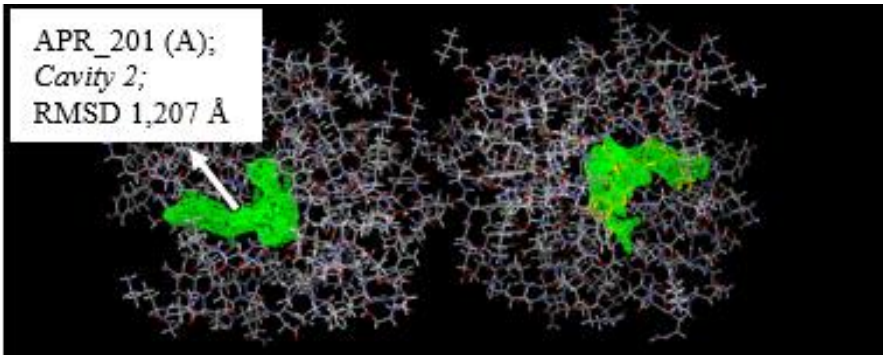


Figure 1 Receptor 6W02 preparation and validation cavites

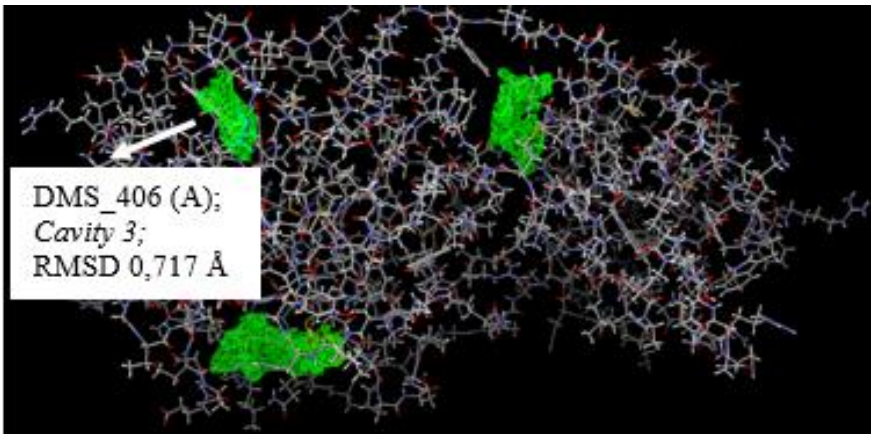


Figure 2 Receptor 7AR6 preparation and validation cavites

At the protein preparation stage, we found that the 6W02 and 7AR6 proteins have several cavities. We validated all cavities, took one cavity with the smallest RMSD value, and re analyzed it three times. Docking of herb compound ligands carried out in the cavity with the smallest RMSD value. Each compound was docked for three replications on the target protein, and ligands with the lowest energy were taken. The results of molecular docking are in the form of pose scoring functions such as MoldDockSore, Rerank Score, and H-bond score [22]. Molecular docking results are listed in Tables 4 and 5

Table 4 Molecular docking result with receptor 6W02

Compound	Rerank Score	MolDock Score	H-Bond
Ligand Native APR_201 (A)	-160.705	-219.050	-21.361
12S-Hydroxyandrographolide	-98.687	-130.154	-9.909
14-Deoxy-11,12-didehydroandrographolide	-95.816	-120.704	-9.162
Andrographolide	-100.057	-127.702	-8.208
Andropanolide	-100.890	-129.562	-8.461
Isoandrographolide	-103.950	-131.457	-7.897
Remdesivir	-125.628	-198.826	-8.529

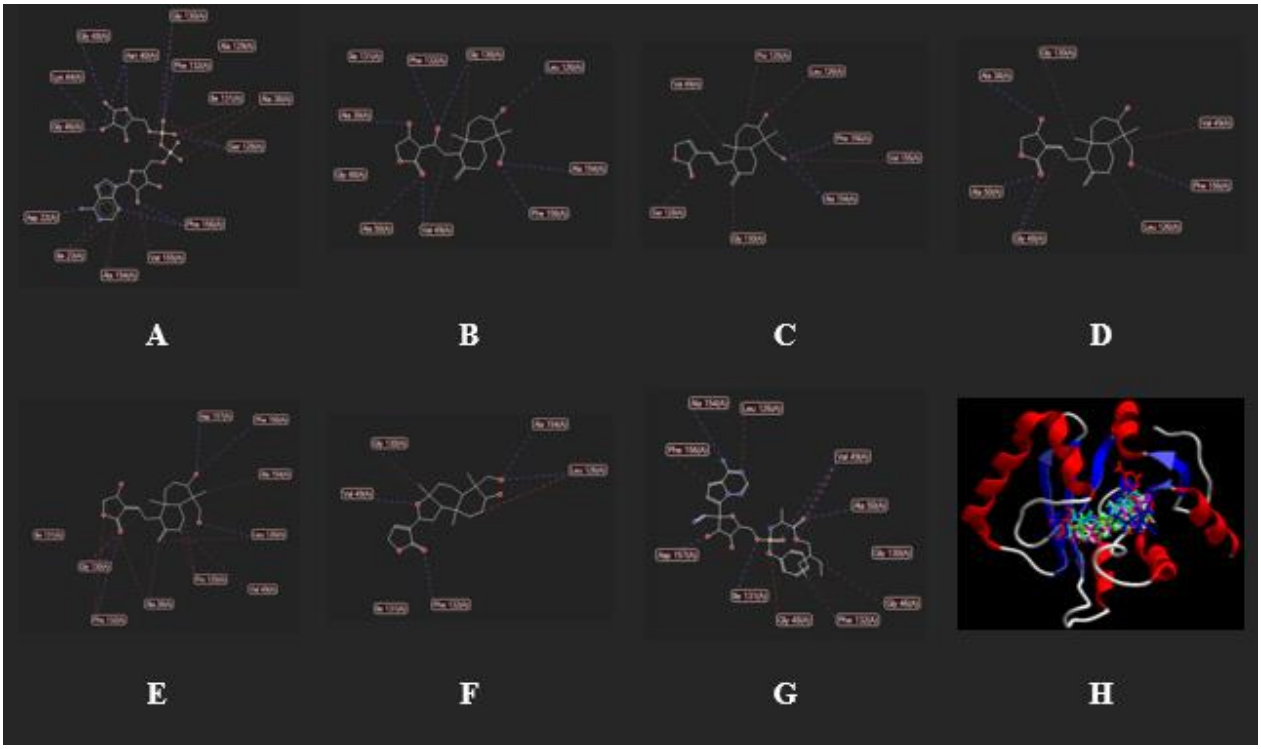
Table 5 Molecular docking result with receptor 7AR6

Compound	Rerank Score	MolDock Score	H-Bond
Ligand Native DMS_406 (A)	-23.958	-27.524	-0.675
12S-Hydroxyandrographolide	-47.358	-71.686	-0.541
14-Deoxy-11,12-didehydroandrographolide	-69.760	-87.150	-3.957
Andrographolide	-53.112	-81.527	-2.259
Andropanolide	-66.764	-90.367	-4.207
Isoandrographolide	-77.035	-94.040	-2.671
Remdesivir	-102.196	-165.828	-10.909

The Rerank Score assessment was utilized to predict ligand binding affinity, where a lower value signifies higher affinity. A lower Rerank Score indicates a stronger and more active ligand-receptor bond [23]. Based on the results of molecular docking, it was found that remdesivir requires the least energy to bind to 6W02 or 7AR6 compared to the herb compounds *A. paniculata*. However, we found that molecular docking results of remdesivir and herb compounds still require greater energy than the native ligand on the target protein 6W02. In contrast, the target protein 7AR6, remdesivir and herb compounds require less energy than the native ligand.

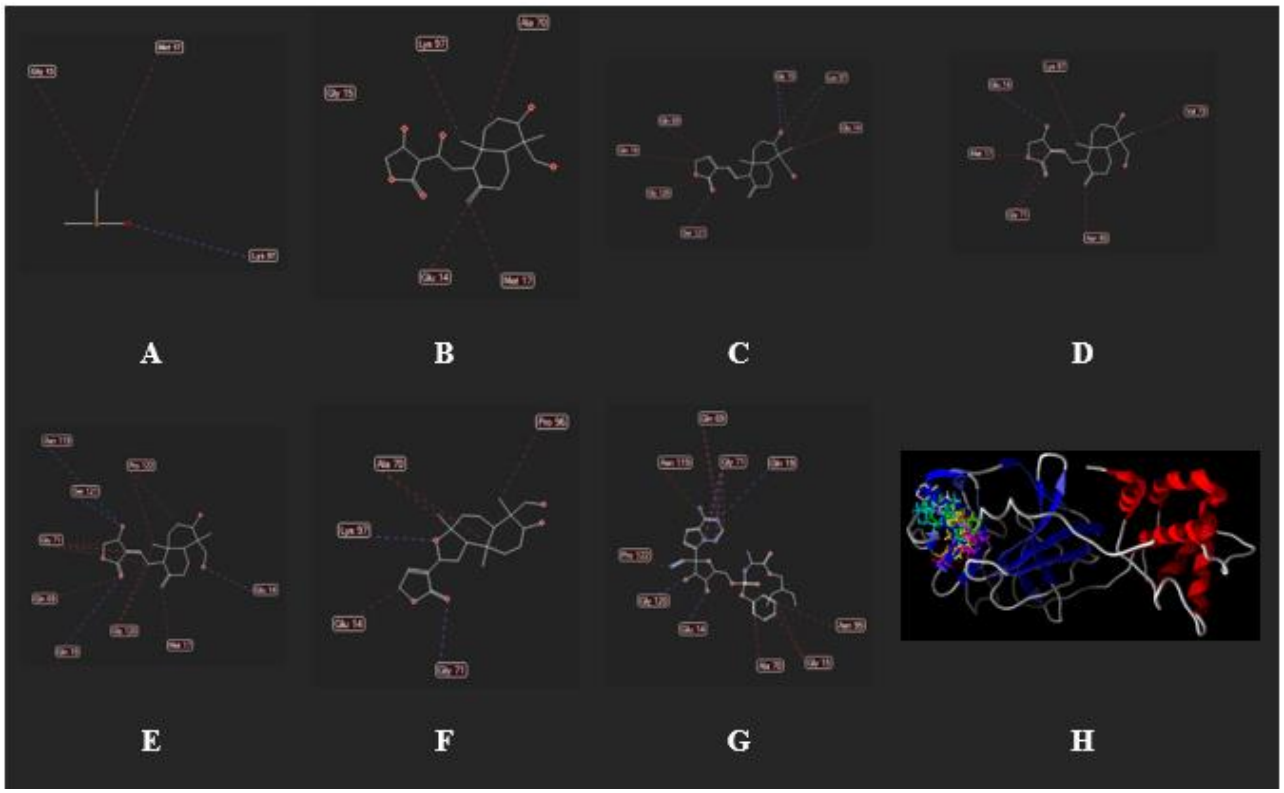
3.3. Protein-Ligand Interaction Analysis

In this research, two types of interactions are studied: hydrogen bonds and steric bonds. Hydrogen bonds are represented by a blue dashed line in Figures 3 and 4, indicating the atomic distance (Å), while steric bonds are shown with a red dashed line, reflecting the bond distance (Å). A decrease in bond distance signifies a stronger and more stable bond, whereas a greater distance implies a weaker bond that can be easily broken. The energy associated with hydrogen bonds between proteins and ligands is known as hydrogen bond energy, whereas steric bonds refer to the energies of steric interactions between proteins and ligands [24].



Hydrogen bonds (blue dotted line) and steric bonds (red dotted line) of native ligand APR (A), 12S-Hydroxyandrographolide (B), 14-Deoxy-11, 12-didehydroandrographolide (C), Andrographolide (D), Andropanolide (E), Isoandrographolide (F), Remdesivir (G), and 3D view results of docking with 6W02 (H).

Figure 3 2D forms of hydrogen bonds and steric bonds 6W02 and 3D view of docking



Hydrogen bonds (blue dotted line) and steric bonds (red dotted line) of native ligand APR (A), 12S-Hydroxyandrographolide (B), 14-Deoxy-11, 12-didehydroandrographolide (C), Andrographolide (D), Andropanolide (E), Isoandrographolide (F), Remdesivir (G), and 3D view results of docking with 7AR6 (H).

Figure 4 2D forms of hydrogen bonds and steric bonds 7AR6 and 3D view of docking

Table 6 Hydrogen bonds and steric bonds interaction of 6W02 receptor

Compound	Hydrogen bonds	Steric bonds
Ligand Native APR_201 (A)	Asn 40 (3.08); Asp 22 (2.85); Gly 46 (3.28); Gly 48 (3.08); Gly 130 (2.78); Ile 23 (2.60); Phe 132 (2.82); Phe 156 (3.29 & 3.13); Ser 128 (3.29)	Ala 38 (3.02 & 3.01); Gly 130 (3.05); Ile 23 (3.07); Ser 128 (3.11); Val 155 (2.98)
12S-Hydroxyandrographolide	Ala 38 (3.11); Ala 50** (3.36); Ala 154** (3.22); Gly 130* (3.19); Leu 126 (2.77); Phe 132* (3.40); Phe 156* (3.27); Val 49** (2.68)	Gly 130* (3.00); Val 49** (3.07)
14-Deoxy-11,12-didehydroandrographolide	Ala 154** (3.30 & 3.09); Leu 126 (2.98); Phe 156* (2.83); Ser 128* (3.10)	Gly 130* (3.11); Pro 125 (3.01); Val 49** (2.85); Val 155* (3.00)
Andrographolide	Ala 38 (2.98); Ala 50** (3.33); Gly 48* (3.03); Phe 156* (3.10)	Gly 48** (3.05); Gly 130* (3.01); Leu 126** (2.97); Val 49 (2.98)
Andropanolide	Asp 157** (3.30); Gly 130* (3.10); Leu 126 (3.42); Phe 132* (2.91); Phe 156* (3.02)	Ala 38* (3.13 & 3.17); Ala 154 (2.93); Gly 130* (3.15); Leu 126** (3.16); Phe 132** (3.10); Pro 125 (2.87 & 2.75); Val 49** (3.13)
Isoandrographolide	Ala 154** (2.63); Leu 126 (3.27); Phe 132* (2.93) Val 49** (2.95)	Gly 130* (3.19); Leu 126** (2.87 & 2.80)
Remdesivir	Ala 50 (3.09); Ala 154 (3.05); Asp 157 (3.12); Ile 131 (3.34); Val 49 (2.94 & 2.65)	Gly 46 (2.97); Gly 48 (2.87); Leu 126 (3.17); Phe 132 (3.08); Phe 156 (3.09); Val 49 (3.11)

* The same amino acid as the native ligand; ** The same amino acid as the compartor drug Remdesivir.

The docking process involved determining the ligand-amino acid interaction and bond energy. Amino acid residues in the receptor played a crucial role in these interactions, specifically through hydrogen bonds and steric bonds. Table 6 and 7 display the involved amino acids and functional groups in hydrogen bonding and steric bonding for receptors 6W02 and 7AR6.

Table 5.6 shows the amino acids involved in hydrogen bonding in *A. paniculata* compound, similar to the native ligand APR_201 (A), include Gly 48 (Glycine 48), Gly 130 (Glycine 130), Phe 132 (Phenylalanine 132), Phe 156 (Phenylalanine 156), and Ser 128 (Serine 128). On the other hand, the amino acids involved in hydrogen bonding in *A. paniculata* compound, similar to the reference drug Remdesivir, include Ala 50 (Alanine 50), Ala 154 (Alanine 154), Asp 157 Aspartic acid 157), and Val 49 (Valine 49).

Table 5.7 shows the amino acid interactions involved in hydrogen bonding in *A. paniculata* compound, similar to the native ligand DMS_406 (A), which includes Lys 97 (Lysine 97). On the other hand, the amino acids involved in hydrogen bonding in *A. paniculata* compound, similar to the reference drug Remdesivir, are Asn 119 (Asparagine 119), Gln 19 (Glutamine 19), Glu 14 (Glutamic acid 14), and Gly 71 (Glycine 71).

Hydrogen bond distances that potentially exhibit similar activity to the native ligand and Remdesivir fall within the range of 2.5-3.5Å [25]. Hydrogen bonding plays a crucial role in the docking mechanism and in forming binding affinities. All ligands exhibit low binding affinities, which are associated with the number of hydrogen bonds formed, as hydrogen bonds have higher energy compared to electrostatic and steric bonds [26]. Additionally, hydrogen bonding can influence the activity and physicochemical properties of compounds, such as solubility, boiling point, and melting point [27].

Table 7 Hydrogen bonds and steric bonds interaction of 7AR6 receptor

Compound	**Hydrogen bonds**	**Steric bonds**
Ligand Native DMS_406 (A)	Lys 97 (3.01)	Gly 15 (3.00); Met 17 (2.99)
12S-Hydroxyandrographolide	-	Ala 70** (3.06); Glu 14 (2.96); Lys 97 (2.64); Met 17* (3.07)
14-Deoxy-11,12-didehydroandrographolide	Gly 15(3.16); Lys 97* (3.01); Ser 121 (3.10)	Gln 69** (2.80); Glu 14 (3.19); Gly 15* (3.13); Gly 120 (3.03); Lys 97 (2.68)
Andrographolide	Glu 14** (2.60); Gly 71** (2.69)	Asn 95** (3.03); Gly 71 (3.14); Lys 97 (2.42); Met 17* (3.14); Val 73 (3.10)
Andropanolide	Asn 119** (2.60); Gln 19** (2.75); Glu 14 (2.60); Ser 121 (2.60)	Gln 69** (2.44); Gly 71** (2.91, 3.08, & 3.07); Gly 120 (2.87 & 3.19); Met 17* (2.45); Pro 122 (2.89 & 3.14)
Isoandrographolide	Gly 71** (3.01); Lys 97* (3.02)	Ala 70** (3.15 & 2.91); Glu 14 (3.15); Pro 96 (3.19)
Remdesivir	Asn 119 (2.71); Gln 19 (3.10); Glu 14 (3.25); Gly 71 (2.93 & 2.77); Gly 120 (3.25)	Ala 70 (3.00); Asn 95 (3.03); Asn 119 (3.03); Gln 69 (2.78 & 2.70); Gly 15* (3.06 & 2.38); Gly 71 (3.13 & 3.15); Pro 122 (2.56)

* The same amino acid as the native ligand; ** The same amino acid as the compartor drug Remdesivir.

Table 5.6 shows the amino acids involved in steric bonding in *A. paniculata* compound, similar to the native ligand APR_201 (A), include Ala 38 (Alanine 38), Gly 130 (Glycine 130), and Val 155 (Valine 155). On the other hand, the amino acids involved in steric bonding in *A. paniculata* compound, similar to the reference drug Remdesivir, include Gly 48 (Glycine 48), Leu 126 (Leucine 126), Phe 132 (Phenylalanine 132), and Val 49 (Valine 49).

Table 5.7 shows the amino acid interactions involved in steric bonding in *A. paniculata* compound, similar to the native ligand DMS_406 (A), which includes Gly 15 (Glycine 15) and Met 17 (Methionine 17). On the other hand, the amino acids involved in steric bonding in *A. paniculata* compound, similar to the reference drug Remdesivir, are Ala 70 (Alanine 70), Asn 95 (Asparagine 95), Gln 69 (Glutamine 69), and Gly 71 (Glycine 71).

Steric bond, also known as Van der Waals bond, has the ability to stabilize bonds between atoms. This occurs when two nearby atoms experience weak and non-specific attractive forces. It is important to note that the strength of this interaction significantly decreases as the distance between molecules increases. In the context of amino acids, steric

bonding can provide space for hydrogen interactions with active amino acids. Additionally, steric bonding also exerts a significant influence on the hydrogen bonds formed within the molecule [28].

4. Conclusion

Screening results showed that 27 of 41 herb compounds complied with Veber rules. Based on the GHS, the 15 compounds belong to class IV-VI, are not mutagenic, not hepatotoxic, not carcinogenic, and do not cause allergic skin reactions. The screening results using PASS Online showed 5 compounds highly potential to be candidates for SARS-CoV-2 antivirals, namely: *12S-Hydroxyandrographolide, 14-Deoxy-11,12-didehydroandrographolide, Andrographolide, Andropanolide,* and *Isoandrographolide.* In conclusion, *A. paniculata* may be potential herbal treatments as SARS-CoV-2 antivirals by inhibiting the NSP5 protein, but these studies must be validated in vitro and in vivo.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare that there are - conflicts of interest.

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