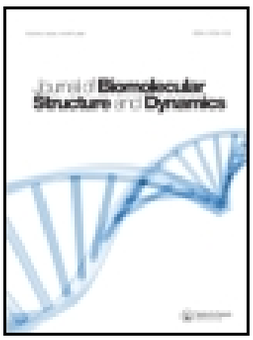

Journal of Biomolecular Structure and Dynamics

ISSN: 0739-1102 (Print) 1538-0254 (Online) Journal homepage: https://www.tandfonline.com/loi/tbsd20

Computational investigation on *Andrographis paniculata* phytochemicals to evaluate their potency against SARS-CoV-2 in comparison to known antiviral compounds in drug trials

Natarajan Arul Murugan, Chitra Jeyaraj Pandian & Jeyaraman Jeyakanthan

To cite this article: Natarajan Arul Murugan, Chitra Jeyaraj Pandian & Jeyaraman Jeyakanthan (2020): 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, DOI: 10.1080/07391102.2020.1777901

To link to this article: https://doi.org/10.1080/07391102.2020.1777901

Published online: 16 Jun 2020.

JOURNAL OF BIOMOLECULAR STRUCTURE AND DYNAMICS
https://doi.org/10.1080/07391102.2020.1777901

Taylor & Francis
Taylor & Francis Group

Computational investigation on *Andrographis paniculata* phytochemicals to evaluate their potency against SARS-CoV-2 in comparison to known antiviral compounds in drug trials

Natarajan Arul Murugan[a], Chitra Jeyaraj Pandian[b] and Jeyaraman Jeyakanthan[c]

[a]Department of Theoretical Chemistry and Biology, School of Engineering Sciences in Chemistry, Biotechnology and Health, KTH Royal Institute of Technology, Stockholm, Sweden; [b]Department of Biotechnology, Dr. Umayal Ramanathan College for Women, Karaikudi, India; [c]Department of Bioinformatics, Alagappa University, Karaikudi, Tamilnadu, India

Communicated by Ramaswamy H. Sarma

ABSTRACT

The outbreak due to SARS-CoV-2 (or Covid-19) is spreading alarmingly and number of deaths due to infection is aggressively increasing every day. Due to the rapid human to human transmission of Covid-19, we are in need to find a potent drug at the earliest by ruling-out the traditional time-consuming approach of drug development. This is only possible if we use reliable computational approaches for screening compounds from chemical space or by drug repurposing or by finding the phytochemicals and nutraceuticals from plants as they can be immediately used without the need for carrying out drug-trials to test safety and efficacy. A number of plant products were routinely suggested as drugs in traditional Indian and Chinese medicine. Here using molecular docking approach, and combined molecular dynamics and MM-GBSA based free energy calculations approach, we study the potency of the four selected phytochemicals namely andrographolide (AGP1), 14-deoxy 11,12-didehydro andrographolide (AGP2), neoandrographolide (AGP3) and 14-deoxy andrographolide (AGP4) from *A. paniculata* plant against the four key targets including three non-structural proteins (3 L main protease (3CLpro), Papain-like proteinase (PLpro) and RNA-directed RNA polymerase (RdRp)) and a structural protein (spike protein (S)) of the virus which are responsible for replication, transcription and host cell recognition. The therapeutic potential of the selected phytochemicals against Covid-19 were also evaluated in comparison with a few commercially available drugs. The binding free energy data suggest that AGP3 could be used as a cost-effective drug-analog for treating covid-19 infection in developing countries.

ARTICLE HISTORY
Received 20 April 2020
Accepted 27 May 2020

KEYWORDS
SARS-CoV-2; *Andrographis paniculata*; andrographolide; neoandrographolide; RNA-directed RNA polymerase; spike protein; Covid-19

1. Introduction

The current outbreak of highly transmissible fatal pneumonia in human referred as Coronavirus Disease-2019 (Covid-19) has spread globally with over 5.5 M confirmed cases and over 340000 deaths worldwide as of 15th April 2020 (Wu, Liu, et al., 2020). Covid-19 is caused by a novel zoonotic pathogenic virus termed as Severe Acute Respiratory Syndrome Coronavirus-2(SARS-CoV-2) which has been first reported to break out during December 2019 in Wuhan, Hubei Province, China with a fatality rate of 4%. The most common symptoms of SARS-CoV-2 include sore throat, dry cough, fever, and shortness of breath (Cheng et al., 2007). In most severe cases, the clinical presentation of SARS-CoV-2 include pneumonia that progresses to multi-organ failure and death (Zaki et al., 2012). SARS-CoV-2 also belongs to β genus of Coronaviridae family of order Nidovirales similar to SARS-CoV and MERS-CoV that had emerged worldwide in 2002 and 2012 with fatality rate of 10% and 36%, respectively (Reusken et al., 2013). However the increasing pandemic potential of SARS-CoV-2 is due to its high human to human transmissible efficiency which makes it difficult to contain (Wu, Zhao, et al., 2020). SARS-CoV-2 has enveloped positive sense single stranded RNA genome and sequence analysis of its 30,000 base-pair genome has evidenced about 14 open reading frames (ORF) (Fehr & Perlman, 2015). The 5′ end ORF1a/b codes for polyprotein that are consequently processed by proteolytic cleavage into 16 non-structural and in 3′ end nine subgenomic RNAs of the viral genome express 13 ORF which includes four structural proteins (Envelope protein, spike protein, nucleo-capsid protein and viral cell membrane protein) along with 9 putative accessory factors (Boopathi et al., 2020; Chan et al., 2020). The 16 non-structural proteins (Nsp) mainly includes RNA dependent RNA polymerase (RdRp), helicase, Papain-like protease (PLP) and 3-Chymotrypsin-like protease (3CLpro)) (Boopathi et al., 2020; Simmons et al., 2005). SARS-CoV-2 spike protein facilitate the entry of viral particles into the host cell after binding with the host Angiotensin-converting enzyme 2 (ACE-2) and the

CONTACT Natarajan Arul Murugan murugan@kth.se Department of Theoretical Chemistry and Biology, School of Engineering Sciences in Chemistry, Biotechnology and Health, KTH Royal Institute of Technology, S-106 91, Stockholm, Sweden; Jeyaraman Jeyakanthan jjkanthan@gmail.com Department of Bioinformatics, Alagappa University, Karaikudi, Tamilnadu, India

spike protein also determines the host range (Simmons et al., 2005). Entry of virus into host cells is mediated by the cleavage of viral spike protein by host proteases namely Cysteine proteases (Cathepsin B/L) or Serine proteases (TMPRSS2) (Boopathi et al., 2020; Yan et al., 2020). According to an earlier research (Glowacka et al., 2011) on single cell RNA sequencing of Human tissues, the Lung pneumocytes (Type II), nasal secretory (goblet) cells and Ileum enterocytes (absorptive) were reported to co-express ACE-2 and TMPRSS2 (Matsuyama et al., 2010). Moreover both the 3CLpro and PLpro proteases are involved in the replication and transcription of the SARS-CoV-2 and 3CLpro plays a key role in the replication of the virus (Prussia et al., 2011). The SARS-CoV-2 proteases also cleave the pp1a and pp1b which is encoded by open reading frame (ORF1a/b) of virus (Sheahan et al., 2020). This protease is a potent therapeutic target essential for polyprotein processing which is translated from viral RNA (Sheahan et al., 2020). The sequence of 3CLpro in SARS-CoV-2 has shown 96% similarity with the 3CLpro of SARS-CoV that caused 2003 outbreak (Zhu et al., 2020). Though SARS-CoV-2 shows similarity to SARS-CoV, it differs in having complement of 3′ORF namely ORF 3b and ORF 10 along with intact ORF 8 while SARS-CoV encodes for ORF 8a/b (Omrani et al., 2014). About 332 interacting proteins were identified between human proteins and viral proteins that includes wide range of function including lipid modification, DNA replication, regulation of gene expression, trafficking of vesicles, RNA processing, ligases, extracellular matrix, mitochondria, signaling, cytoskeleton and transport machinery in nucleus by spike proteins such as Nsp 1, 4, 5, 6, 7, 8, 9, 10 and Nsp 13 as well as ORF 10, 9b, 6 and 9c. SARS-CoV-2 proteins Nsp 13 were also found to interact with host innate immune pathways namely TAN-binding Kinase 1 and its binding protein (TBKBP1) along with its adaptor protein (SINTBAD) (Ge et al., 2013). Viral ORF 10 also hijacks ubiquitin ligase pathway for its pathogenesis (Chan et al., 2013). Viral transmembrane protein E mimics the host histone structure and interacts with bromodomain binding protein which changes the expression of host protein and makes it favorable for viral replication (de Wilde et al., 2014). It is evident from a recent study that similar to other viruses, SARS-CoV-2 genome also exhibits random mutation over time with the rate of one to two mutations per month and the phylogenetic trees could predict the association between various cases of SARS-CoV-2. Therapy for coronavirus be categorized into two strategies which is acting either on (i) coronavirus or (ii) on human cells or immune system (Omrani et al., 2014). The first strategy includes three approaches in analyzing therapeutic drugs for coronavirus namely (a) testing with antiviral activity of broad spectrum of anti-viral drugs, ribavirin, Interferon and inhibitors like cyclophilin that are used to treat pneumonia caused by the virus (Ge et al., 2013; Muralidharan et al., 2020), (b) High-throughput screening of therapeutic molecules in the available molecular databases (Chan et al., 2013) and (c) to develop potent drug based on the pathological and genomic characteristics of several other coronaviruses (de Wilde et al., 2014). The second strategy in-terms of immune system involves the innate immune response and Interferon that play a key role in preventing viral replication as well as blocking the human signal pathways (ACE2) for viral binding and replication (Dyall et al., 2014). Subunit vaccines or live attenuated vaccines or DNA vaccines developed for SARS-CoV-2 may lose their efficiency if the virus mutates constantly and changes its antigenicity. Therefore plant-based drugs for viral infection could combat viral infection by targeting viral receptors (Chang & Woo, 2003; Keyaerts et al., 2007), viral integration (Kim et al., 2010), reverse transcription (Zhang et al., 2014), viral replication and viral protein translation (Mansouri et al., 2009). Moreover the developing countries like India needs efficient and economical anti-viral drugs to combat prevailing viral infectious diseases. Plants have always been used as good source of antiviral drug in folk medicine to treat viral infections. One such medicinal plant with numerous medicinal properties is *A. paniculata* also known as 'King of bitters' belonging to Acanthaceae family (Pholphana et al., 2013). This plant is widely used herb in Asia as traditional medicine to treat fever, diarrhoea, common cold etc. and it has varied range of pharmacologically active substances such as andrographolides and its derivatives that have been recorded high antimalarial, anti-cancer, antioxidant, hepato-protective and anti-HIV activities (Niranjan et al., 2010). More than 10 flavonoids and 20 diterpenoids in *A. paniculata* have been confirmed to have medicinal properties (Thisoda et al., 2006). These phytochemicals are found in higher concentration in the leaves of *A. paniculata* when compared to other parts of the plant (Parasher et al., 2011). Andrographolide and its derivatives in *A. paniculata* have been reported to have potent antiviral activity against diverse group of viruses belonging to different families including influenza A virus (H1N1), Hepatitis B virus (HBV), Hepatitis C virus (HCV), Herpes simplex virus 1 (HSV-1), Chikungunaya virus (CHV), Human immunodeficiency virus (HIV), Human papillomavirus (HPV) and Epstein-Barr virus (EBV) that belongs to various viral family such as Orthomyxoviridae, Hepadnaviridae, Flaviviridae, Herpesviridae, Togaviridae, Retroviridae, Papillomaviridae and Herpesviridae, respectively (Gupta et al., 2017). Andrographolide in *A. paniculata* induced cell mortality in H1N1 virus and andrographolide analogues showed highest potency against H3N2 influenza A virus (Yuan et al., 2016) by inhibiting receptor signaling pathway (RLR pathway) in bronchial endothelial cells of human (Yu et al., 2014). Andrographolide and dehydroandrographolide also exhibited anti-HBV activity by inhibiting the viral replication and viral envelop antigen of HBV (Chen et al., 2014). Combination treatment of andrographolide with IFN-a or telaprevir or PSI-7977 showed synergistic activity in suppressing HCV replication by activating p38 MAPK phosphorylation pathway and increasing the expression of heme oxygenase-1 and Nrf2 (Lee et al., 2014). Chandramohan et al. (2015) predicted through molecular docking analysis that andrographolide could inhibit NS3/4A protease in andrographolide. 14-deoxy-11,12-didehydroandrographolide and neoandrographolide extracted from *A. paniculata* inhibited HSV entry into host cells and its replication through decreased expression of gp C/D (Seubsasana et al., 2011). Earlier studies have also reported that andrographolide could induce the expression of PKR and RIG-1 in CHV infection and thereby decrease the copy number

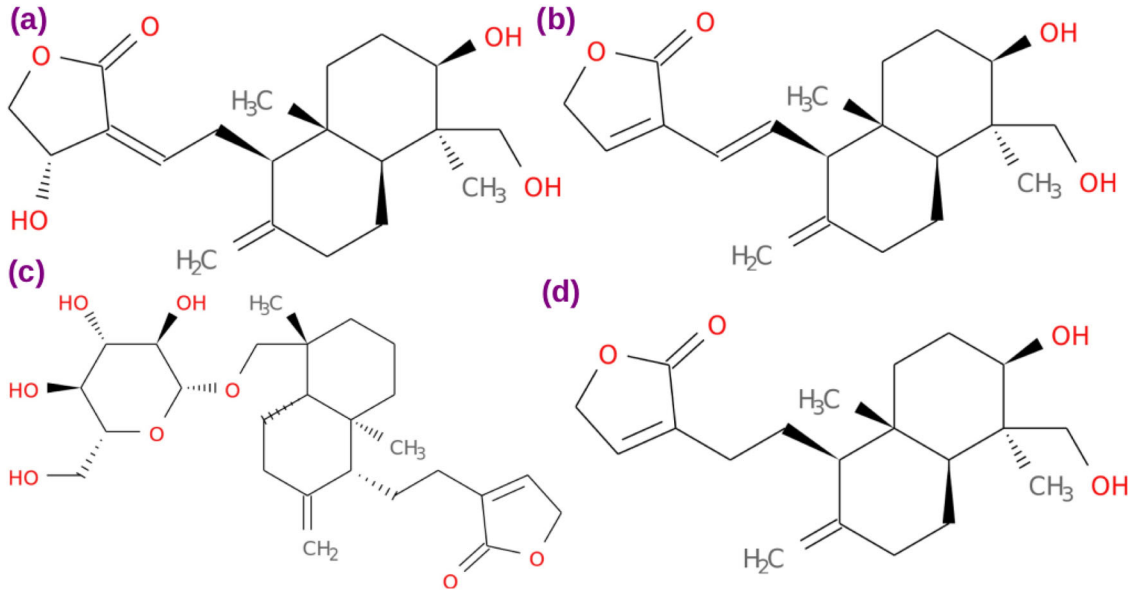

Figure 1. The chemical structure of studied phytochemicals (a) andrographolide, (b) 14-deoxy 11,12-didehydro andrographolide, (c) neoandrographolide and (d) 14-deoxy andrographolide from *A. paniculata*.

of CHV RNA and protein expression (Wintachai et al., 2015). Andrographolide and monoester of dehydroandrographolide succinic acid were found to exhibit effective anti-HIV activity by interfering in the binding of HIV to host cells, decreasing the copy number of HIV RNA, dysregulating the signaling pathways in cells and increasing the T-lymphocytes in HIV-infected individuals (Wintachai et al., 2015). Ekalasananan et al. (Calabrese et al., 2000) have also recorded anti-HPV activity of andrographolide and its derivatives in HPV16 pseudovirus by inhibiting the E6 oncogene expression, restoration of tumour suppressor protein p53 and induction of apoptosis in cervical cell. 25 $\mu g/mL$ of andrographolide in *A. paniculata* ethanolic extracts were reported to inhibit the expression of lytic proteins (Zta, EA-D and Rta) of Epstein-Barr virus (EBV) (Ekalaksananan et al., 2015). Moreover, andrographolides in *A. paniculata* were found to have immunomodulatory activity in human peripheral blood mononuclear cells that strengthens host innate immune response (Tsuey-Pin et al., 2008). Therefore andrographolides as well as its derivatives have promising immunomodulatory and anti-viral activities that can be used either alone or in combination as an effective drug against SARS-CoV-2 (Peng et al., 2002).

With the above considerations, the objective of the study is designed to computationally predict the potency of the compounds against key targets in SARS-CoV-2 and to find out the most active compound in *A. paniculata*. Our study includes therapeutics compounds in *A. paniculata* such as AGP1, AGP2, AGP3 and AGP4 (the chemical structures of all four compounds are shown in Figure 1) and its mode of action against the non-structural protein targets in SARS-CoV-2 namely 3 L main protease (3CLpro), Papain-like proteinase (PLpro, and RNA-directed RNA polymerase (RdRp) and spike (S) protein (Liu et al., 2020). We have employed the autodockvina (Trott & Olson, 2010) to estimate the high affinity binding mode/pose of these compounds in the viral targets along with their binding affinities. Further, for the most stable binding mode, we have carried out molecular dynamics calculations and binding free energy calculations using molecular mechanics generalized Born surface area (MM-GBSA) approach (Rastelli et al., 2010). We have also performed the same set of calculations (i.e. molecular docking, molecular dynamics and free energy calculations) for the drugs namely as Remdesivir, Lopinavir, Ritonavir, Oseltamivir, Hydroxychloroquine and Azithromycin which are being tested for their potency against Covid-19. This is to assess the relative performance of the phytochemicals from *A. paniculata* in comparison to the aforementioned commercially available medications. Remdesivir is an adenosine analog used in Ebola treatment as it acts by inserting itself into the RNA of virus and interferes in the action of RdRp which causes premature termination of viral RNA production and currently it is used as promising Covid-19 drug for post infection treatment. HIV drug Lopinavir is an inhibitor of HIV-1 dimeric aspartic acid protease which cleaves the 'gag' polyprotein of the virus essential for viral life cycle and it is often administered in combination with protease booster ritonavir. Oseltamivir is also an antiviral drug used in treatment of Influenza A and B that inhibits viral neuraminidase enzymes and prevents the release of new viral particles from the infected cell. Hydroxychloroquine is a malarial drug also used for rheumatoid arthritis and lupus erythematosus treatments act by inhibiting Toll-like receptor-9 signaling thereby decreasing the inflammation process and this drug is used in combination with macrolide antibiotic azithromycin. All the selected drugs are used either alone or in combination as drug cocktails in drug trails for Covid-19 treatment.

2. Materials and methods

2.1. Retrieval of 3D structures of phytochemicals

The three dimensional structure for AGP1 has been obtained from drug databank (DB05767) (Wishart et al., 2008). The chemical structure has been modified using Molden software

(Schaftenaar et al., 2017) to generate the coordinates for the remaining three compounds namely AGP2, AGP3, AGP4. Using openbabel (O'Boyle et al., 2011), the mol2 files were converted to pdbqt and then molecular docking was performed using Autodockvina software (Trott & Olson, 2010). For those remaining compounds (will be referred as trial compounds) in this study where ever the 3D structure is not available, we manually build the structures using Molden software and then optimized the geometry by carrying out electronic structure theory calculations at B3LYP/6-31G* level of theory as implemented in Gaussian09. 3D structures for the compounds such as Hydroxychloroquine (DB01611), Remdesivir (DB14761) and Oseltamivir (DB00198) are available in the drugbank database.

2.2. Molecular docking

Four different targets from virus were considered for the docking studies namely 3CLpro, PLpro, RdRp and spike protein-ACE2 complex. Recently, the crystal structures for spike protein and 3CLpro have been available in the open access databases. In particular, for 3CLpro, the structures of both apo form and complex form with certain inhibitor N3 are reported in pdb databank (Berman et al., 2007). For the spike protein, the structure has been solved using cryogenic-electron microscopy experiments for both closed and open forms and also in complex with ACE-2 receptor (Lan et al., 2020; Walls et al., 2020; Wrapp et al., 2020; Yan et al., 2020). For the remaining two targets, the structures are not available. So, we have proposed the three dimensional structures for these targets by homology modelling using the protein structures of SARS-CoV-1 which has a larger sequence similarity. The structure for PLpro is based on the pdb id 5Y3E of SARS-CoV-1 which had 83% sequence identity to that of SARS-CoV-2 protein. Similarly, the three dimensional structure for RdRp has been obtained from homology modelling by using the template structure from the same protein of SARS-CoV-1 (PDB ID 6NUR) which had sequence identity of 96.4%. In all cases, the binding sites were selected carefully and then the center and size of gridbox were chosen accordingly. For example, in the case of spike protein, there are two domains namely S1 and S2 and we have selected the receptor binding domain S1 which is bound to mammalian ACE-2 receptor (Lan et al., 2020; Yan et al., 2020). In the case of 3CLpro, the binding site was selected based on the location of inhibitor, N3 which has been co-crystallized with the target protein as in 6LU7. The active site in this target is made of the residues THR24, THR26, PHE140, ASN142, GLY143, CYS145, HIS163, HIS164, GLU166 and HIS172 (Khaerunnisa et al., 2020). We have used the apo form of the 3CLpro for carrying out molecular docking. In the case of PLpro, the binding site location has been chosen based on the PLpro-ligand complex of SARS-CoV-1 (PDB id is 4OW0). The binding site in RdRp has been chosen based on the information available in the literature. A recent paper evidenced that the active site of RdRp is highly conserved in many RNA viruses (Hepatitis C and Zika) and human coronaviruses such as 229E, NL63, OC43, HKU1, SARS and MERS (Elfiky, 2020a; Narayanan & Nair, 2020). In particular, the binding site of RdRp is characterized by two residues D645 and D646 (Elfiky, 2020b). In the molecular docking using autodockvina, the protein is kept rigid while the ligand is fully flexible. Upto 20 least energy binding modes and binding poses were stored in all molecular docking studies. The relative binding affinities of all four ligands along with the trial compounds with the four viral targets were analyzed to find out the most stable complex formed.

2.3. Molecular dynamics simulation

The ligand binding mode with the least binding free energy (and so the one with larger binding affinity) has been used for preparing the initial configuration for the protein-ligand complexes for carrying out subsequent molecular dynamics (MD) simulations. The ligands included here are those phytochemicals from *A. paniculata* and trial compounds. The MD simulations were carried out to study the finite temperature effect and stability of the complexes formed in the ambient condition. However, this requires the charges and force-fields available for all the subsystems in the complex to be studied. So, the electrostatic potential fitted charges for the ligands were computed by employing CHELPG approach (Breneman & Wiberg, 1990) and B3LYP/6-31G* level of theory as implemented in Gaussian09 software.(Frisch et al.) Further, the GAFF force-field (Wang et al., 2004) has been used to describe the ligand interaction with other subsystems like protein and solvents. Similarly the FF99SB force-field has been adopted for proteins and for water solvent, TIP3P force-field has been employed. In all the cases, sufficient number of counter-ions were added to neutralize the system. In case of any hot spots present in the protein or in the complex, firstly minimization run was performed. Followed by this, molecular dynamics simulation was carried out at a constant volume ensemble. Further simulations in the isothermal isobaric ensemble have been carried out to study the system stability in ambient condition. The Langevin thermostat and Barendsen barostat were adopted to maintain the temperature and pressure in the simulation. The time step for solving Newton's equation of motion was set to 1 fs. The simulations were carried out for a total time scale of 20 ns. All simulations were carried out using Amber16 software (Case et al., 2016). The time evolution of various properties such as density and energies were analysed to ensure that the simulations were equlibrated appropriately . The configurations from trajectory corresponding to final 5 ns timescale were used for calculating binding free energies using molecular mechanics- Generalized Born surface area approach (Rastelli et al., 2010). In this method, the binding free energy has been estimated as the difference between the complex and individual subsystems' free energies. The free energy for each system is computed as the sum of van der Waals, electrostatic, polar and non-polar solvation energies and contributions due to entropy. Since the calculations of entropies are computationally demanding, usually they are not estimated. Further it is usually approximated that in comparing the relative stabilities of different complexes, the entropic contributions are not significant and

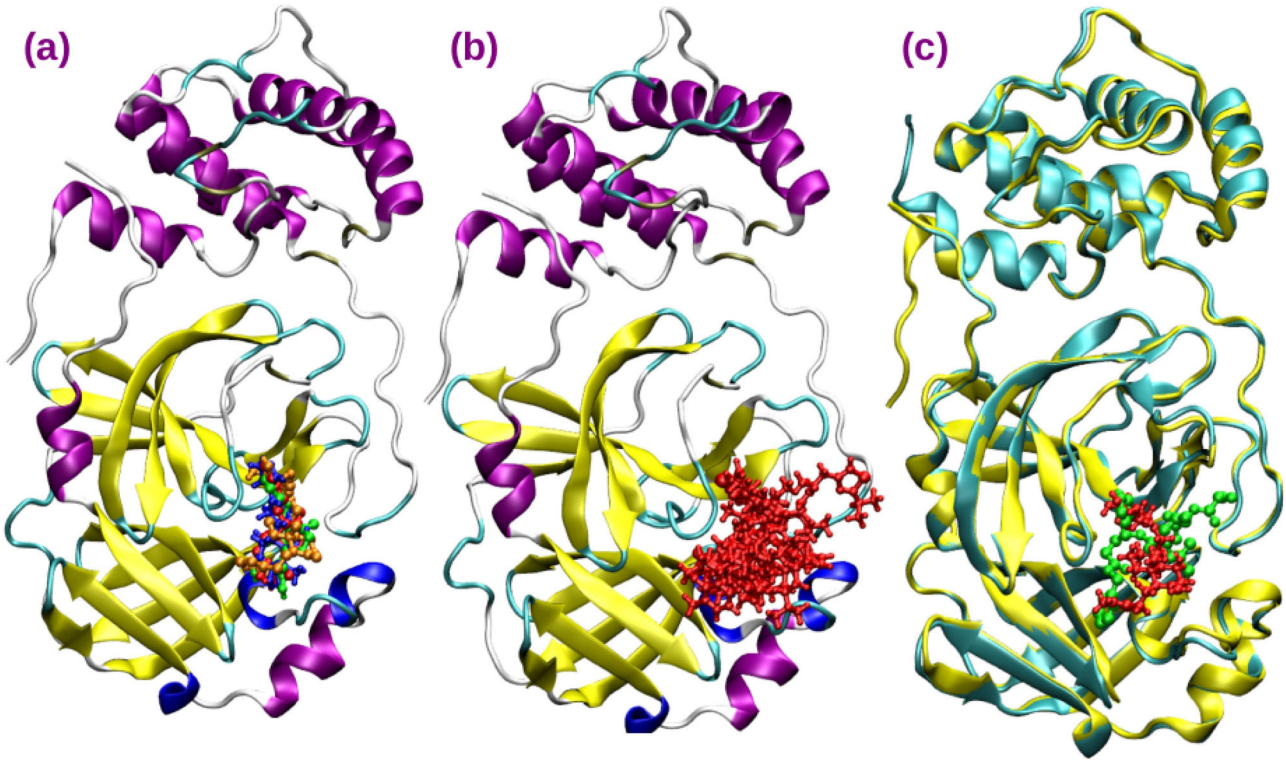

Figure 2. (a) Binding sites for AGP compounds within 3CLpro (b) Binding sites for trial compounds within 3CLpro (c) Comparison of the binding site of AGP3 with that of N3 inhibitor (based on 6LU7).

they were not included in this study. Since the computed binding free energies are not absolute, the relative free energy differences are computed to classify a compound as an inhibitor or non-inhibitor. In order to estimate the relative potencies of compounds from *A. paniculata*, we have also included many trial compounds which are being considered in drug trials against covid-19.

3. Results and discussion

3.1. Analysis of binding modes for AGP1-4 with viral targets

First the study focuses on the binding modes of AGP compounds and trial compounds with the two proteases, 3CLpro and PLpro. In the case of 3CLpro, all ligands bind to the substrate recognition catalytic site involved in proteolytic processing of RNA replicase which is a key process for viral replication (Figure 2(a and b)). Also the catalytic site is highly conserved among all the coronaviruses such as SARS-CoV-1, MERS-CoV and other human coronaviruses such as HCoV-229E, HCoV-OC43, HCoV-NL63, and HCoV-HKU1. There are a few crystallographic reports for 3CLpro of different coronaviruses where certain irreversible inhibitors are bound to the cys144. This reaction occurs through the formation of a reversible association complex in the first step. The investigated ligands (phytochemicals and trial compounds) in the current work are reversible inhibitors (i.e. they do not form covalent bonding with the target) that target the same substrate binding site in 3CLpro and will have therapeutic values. The binding modes of AGP compounds and trial compounds are shown in Figure 2(a and b), respectively. It is evident from the Figure 2(a, b) that the AGP compounds also bind to the same binding site as the trial compounds. Just to establish that the AGP compounds are binding to the binding site as the inhibitor, we have superimposed the crystal structure of 3CLpro: N3 inhibitor complex (as in PDB id 6LU7) with that of the 3CLpro: AGP3 complex structure obtained from molecular docking study (refer to Figure 2(a)). The spatial location of AGP3 is the same as that of N3 which suggests that all these compounds studied also bind to the substrate binding site in 3CLpro and have therapeutic activity by inhibiting the functionality of this enzyme in replication.

In the case of PLpro, the drug molecules bind to S3/S4 domains of the target and so we searched for binding modes/poses for the ligands in this region. The binding modes of AGP compounds and trial compounds are shown in Figure 3(a and b), respectively. As shown in the Figure 3(a), AGP compounds also bind to the same binding site as the trial compounds (refer to Figure 3(b)) except azithromycin which binds to a unique binding site. In addition, it is evidenced that this binding site is the same as the inhibitors of PLpro. We have superimposed the PLpro from SARS-CoV-1 bound to an inhibitor GRM (pdb id is 3MJ5) with the AGP1 in complex using the homology model of PLpro of Covid-19 as shown in Figure 3(c). The overlap of both the ligands GRM and AGP3 suggests that the both the selected phytochemicals and trial compounds target the same inhibitor site as GRM. In the case of RdRp the active sites are characterized by the aspartic residues D645 and D646 (Elfiky, 2020b). The binding mode for all the phytochemicals and trial compounds are shown in Figure 4(a and b), respectively and the

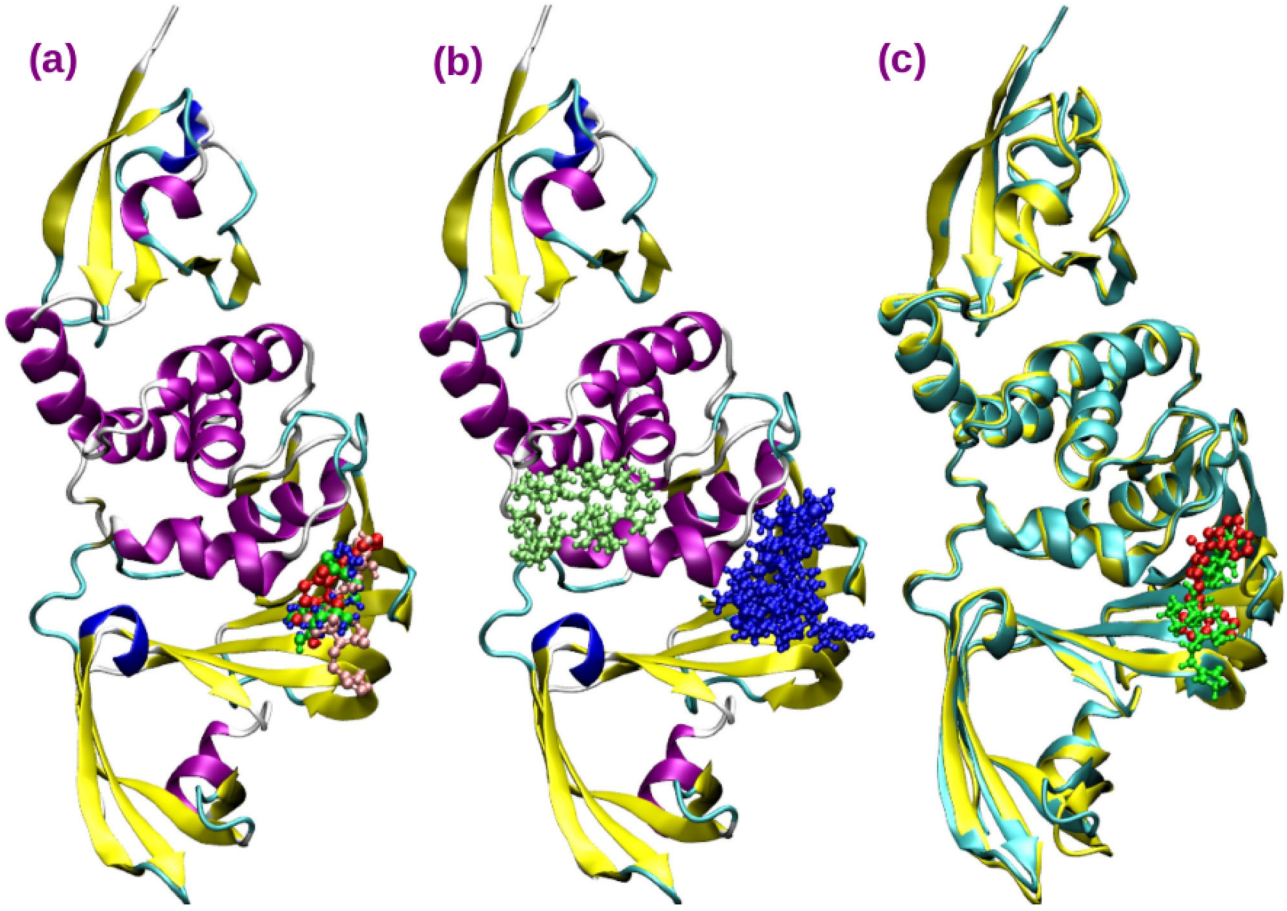

Figure 3. (a) Binding sites for AGP compounds within PLpro (b) Binding sites for trial compounds within PLpro (c) Comparison of the binding site of AGP3 with that of inhibitor, GRM as in PLpro of SARS coronavirus (based on 3mj5).

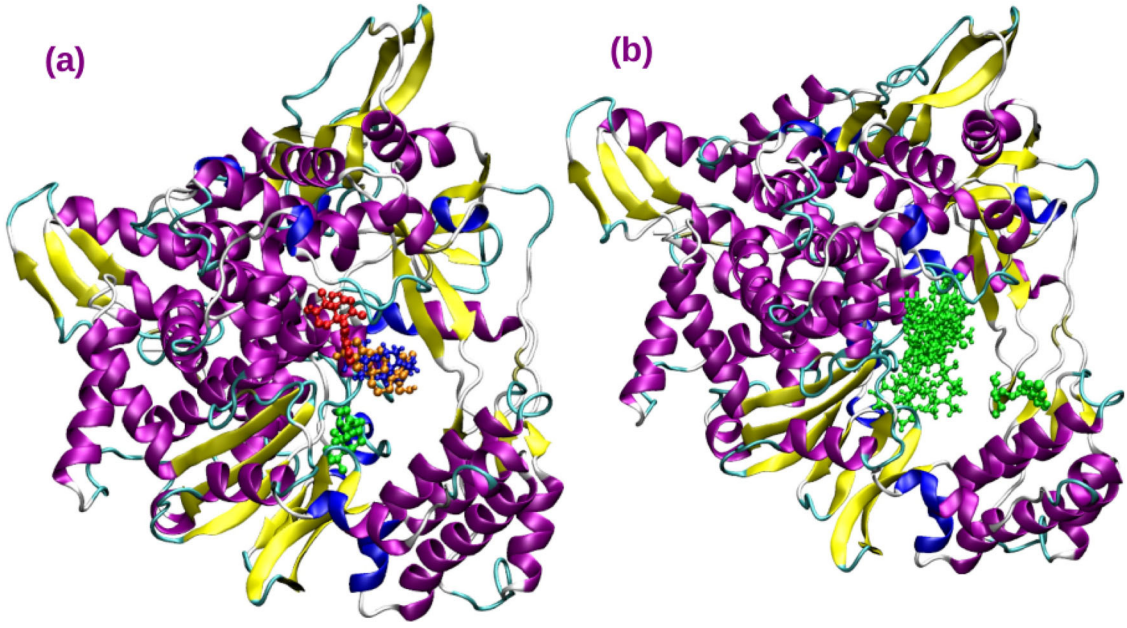

Figure 4. (a) Binding sites for AGP compounds within RdRp target (b) Binding sites for trial compounds within RdRp.

ligands such as AGP-3, AGP-4 and most of the trial compounds are bound to the entry site of nucleotide along the channel. Due to this reason, the ligands AGP-1 and AGP-2 will have less significant effect on the entry of the incoming nucleotides while the remaining ligands will have substantial therapeutic effect.

Finally, the study demonstrates the effect of AGP and trial compounds to S protein-ACE2 complex. The binding modes

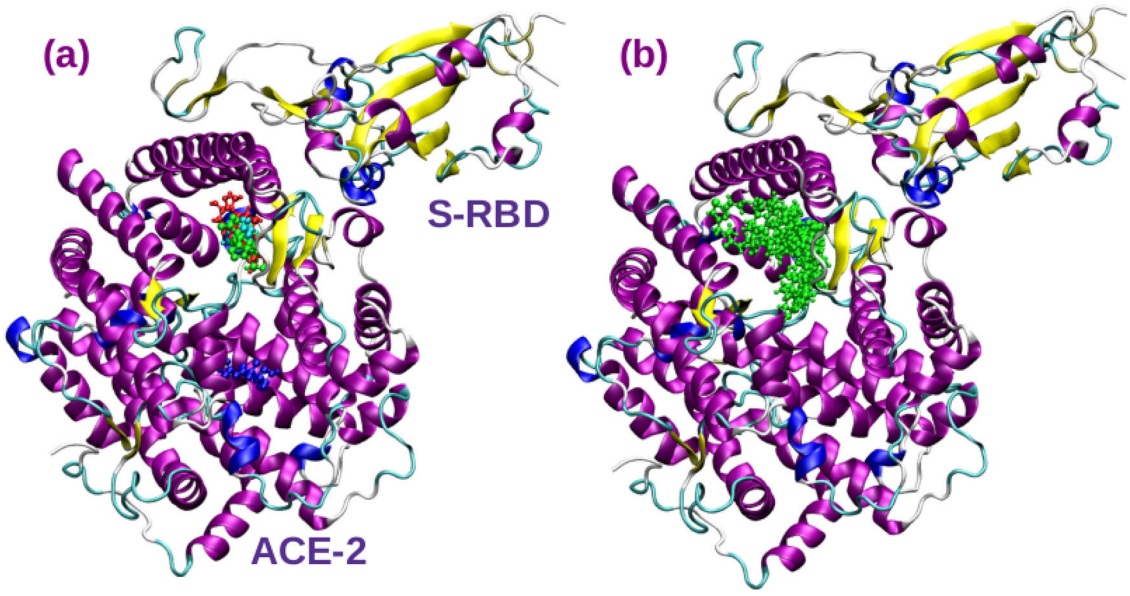

Figure 5. (a) The binding site for AGP1-4 in spike protein-ACE2 complex. (b) The binding site for trial compounds in the ACE2 receptor of the complex. The AGP1, AGP3, AGP4 and trial compounds occupy the same binding site which is localized near the helices involved in the binding to RBD domain of spike protein. AGP-2 binds to a unique site located near ASN260.

Table 1. The binding affinity for the phytochemicals and trial compounds with the four vital targets in Covid-19.

Compound	3CLpro	PLpro	RdRp	Spike-ACE2
Phytochemicals from *A. paniculata*				
AGP1	−7.2	−6.8	−6.7	−6.9
AGP2	−7.0	−6.5	−6.7	−6.8
AGP3	−7.1	−7.3	−7.4	−7.8
AGP4	−6.9	−6.7	−6.3	−6.7
Trial compounds				
Remdesivir	−7.3	−7.5	−7.5	−8.3
H.Chloroquine	−5.6	−5.8	−6.3	−6.3
Oseltamivir	−5.6	−5.5	−5.9	−5.9
Ritonavir	−6.5	−6.7	−7.6	−7.2
Lopinavir	−7.1	−7.3	−7.5	−7.5
Azithromycin	−5.7	−6.3	−6.5	−6.7

for the four compounds, AGP1-4 and trial compounds with this complex are shown in Figure 5(a and b), respectively. Interestingly the ligand AGP1, 3 and 4 and remdesivir target the same binding site (which is located just below the interfacial region in the ACE-2 mammalian receptor) while the AGP-2 bind to another site located near the residue ASN260. In order for a compound to serve as a drug, it should bind to the interfacial area of spike protein-ACE2 complex and weaken the interaction. However here the four compounds are binding to a core site located underneath the interfacial area and may only indirectly modulate the protein-protein interaction. The small organic molecules have usually less significant surface area and so are less effective in modulating the protein-protein interaction and this is the reason for preferring peptides for targeting spike protein (even though for other targets, small organic molecules are suitable). However, we cannot exclude the possibility that the ligand interaction to helices will modulate their interaction with the receptor binding domain of spike protein. The interaction brings in the rigidity of the ACE-2 residues which will affect their conformational flexibility and consequently affect the host cell recognition. Since the AGP-2 ligand binds to a site which is located far away from the interfacial region, it may have less therapeutic value when compared to other ligands studied. It is worth mentioning that the remdesivir has been considered to be a potential drug compound against covid-19 infection and has been subjected to the clinical trial phase recently. It was originally developed for treating viral infection caused by Ebola and Marburg viruses but was also found to be active against SARS and MERS viruses. Even though it is suggested as a RdRp target, its potency to other targets is not studied in detail. Therefore here we report that it could have a potent inhibitory activity in the host cell recognition and binding as it binds to the spike protein with significant binding affinity (−8.5 kcal/mol).

3.2. Analysis of binding affinities for AGP1-4 with viral targets

In order for a ligand to serve as a therapeutic molecule, it has to bind to a specific catalytic site with significant binding affinity. In certain cases, the ligands bind to allosteric sites (as in non-nucleoside reverse transcriptase inhibitors in HIV (Poongavanam et al., 2018)) which leads to specific structural/conformational changes in the binding site causing the modulation or inhibition of substrate binding. We speculate that in the case of spike protein-ACE2 complex, even though the ligands are not binding to interfacial regions, they may indirectly affect the binding of spike protein to ACE-2 receptors. The stronger the ligand binding, the stronger the modulating effect and therefore the ligand with larger binding affinity to spike protein-ACE2 complex can have a better therapeutic effect. Table 1 shows that the phytochemical AGP3 has the stronger binding affinity towards spike protein-ACE2 complex (−8.9 kcal/mol) when compared to 3CLpro (−7.1 kcal/mol), PLpro (−7.3 kcal/mol) and RdRp (−7.4 kcal/mol).

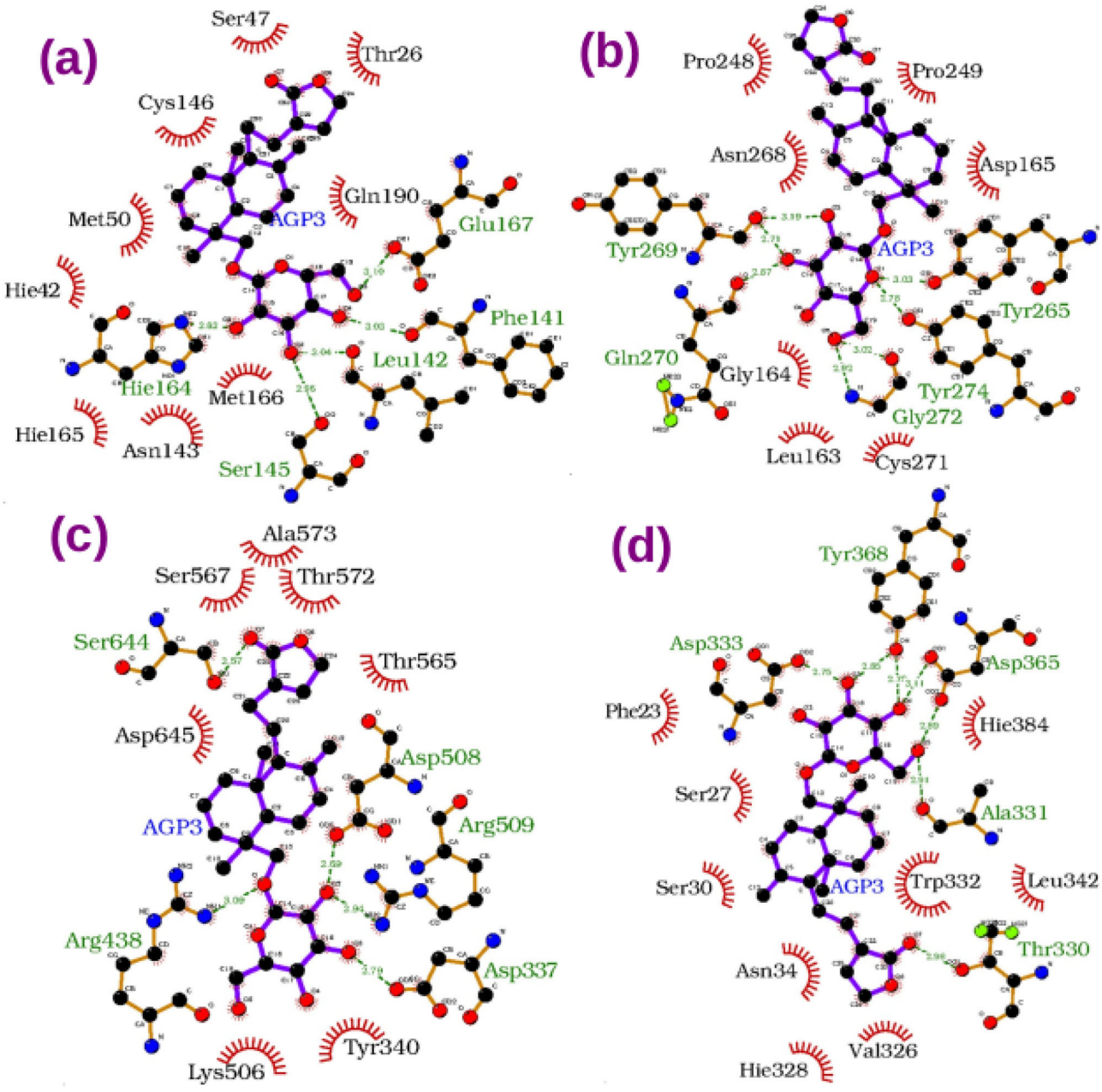

Figure 6. Protein-ligand interaction diagram for AGP3 with viral targets (a) 3CLpro, (b) PLpro, (c) RdRp and (d) with ACE2 of spike-ACE2 complex.

Interestingly the remdesivir which is known to target RdRp also binds to spike protein-ACE2 complex with considerable affinity (−8.5 kcal/mol). There are no experimental studies available to confirm this or to contradict this. Interestingly, Lopinavir also binds to RdRp with a larger binding affinity (−9.3 kcal/mol). In the case of 3CLpro, we have established all four phytochemicals bind to the substrate binding site. In this case, all the ligands have comparable binding affinity (−6.9 to −7.2 kcal/mol). Again, remdesivir shows comparable binding affinity towards this target (7.1 kcal/mol). Another trial compound that showed significant binding affinity to both proteases is lopinavir. Now, for the PLpro, except the AGP-3 (−7.3), other ligands show lower binding affinity and a similar trend is observed in the case of RdRp target. Overall it can be inferred that AGP-3 is the best therapeutic compound among the four tested phytochemicals as it has the larger binding affinity (or the least binding free energy) towards all four selected targets. This result could be attributed to the larger molecular surface and presence of many hydroxyl functional groups as they respectively increase the magnitude of both van der Waals and electrostatic interactions. The protein-ligand interaction diagrams computed using Ligplus + Wallace et al. (1995) displayed that many intermolecular hydrogen bonds are formed between the aminoacids and polar groups in AGP3 (refer to Figure 6).

Table 2 shows more accurate and reliable results obtained for binding free energies computed as averages over various configurations for all the ligands as well as phytochemicals with four different targets. Similar to autodockvina results, AGP3 is found to be the most potential compound for the four targets namely 3CLpro, PLpro and RdRp. Only in the

Table 2. The binding free energies (in kcal/mol) for AGP and trial compounds with four vital targets of covid-19 virus. The free energies are computed as an average over 2500 configurations extracted from molecular dynamics trajectories (of time scale 5 ns). The free energies were computed using molecular Mechanics-Generalized Born approach.

Compound	3CLPro	PLpro	RdRp	Spike-ACE2
Phytochemicals from *A. paniculata*				
AGP1	−21.7	−12.7	−3.0	−12.7
AGP2	−18.9	−22.9	−9.8	−13.3
AGP3	−31.4	−28.5	−17.1	−23.9
AGP4	−26.1	−26.6	−7.8	−24.1
Trial compounds				
Remdesivir	−44.4	−27.3	−36.5	−33.3
H.Chloroquine	−12.1	−14.7	−25.8	−24.5
Oseltamivir	−15.8	0.00	−19.8	−10.5
Ritonavir	−30.9	−30.4	−33.5	−43.2
Lopinavir	−43.3	−3.5	−21.6	−33.5
Azithromycin	−15.7	−20.0	0.00	−11.9

Table 3. Contributions from van der Waals (ΔE_{vdw}), electrostatic (ΔE_{elec}), polar (ΔG_{GB}) and non-polar solvation (ΔG_{SA}) free energies to the total binding free energies (unit in kcal/mol). The data are only shown for AGP-3 which has potency to target all viral targets of covid-19.

Site	ΔG_{vdw}	ΔG_{elec}	ΔG_{GB}	ΔG_{SA}	$\Delta G_{binding}$
3CLpro	−37.0	−20.5	36.0	−4.6	−26.1
PLpro	−38.7	−27.3	44.2	−4.8	−26.6
RdRp	−46.3	−73.2	108.6	−6.2	−17.1
Spike-ACE2	−30.9	−40.1	51.2	−4.3	−24.1

case of spike protein-ACE2 complex, AGP-4 is found to be the slightly better compound (only by 0.2 kcal/mol) when compared to AGP-3 in terms of potency. Table 3 lists various contributions to total binding free energy namely van der Waals, electrostatic, polar and non-polar solvation free energies. As discussed above both van der Waals and electrostatic interactions for AGP-3 with different viral targets are larger in magnitude. In the case of spike protein and RdRp, the contributions from electrostatic interaction between the protein-ligand are larger than the van der Waals contributions. However, in all cases the polar solvation free energy contributions nullifies the favourable electrostatic contributions. Overall, it is the van der Waals interactions between the protein-ligand that drives the formation of stable complexes between the phytochemicals and viral targets. It is quite striking to observe that APG-3 has comparable binding free energies for both proteases 3CLpro (−31.4 kcal/mol) and PLpro (−28.5 kcal/mol) which catalyzes the cleavage in viral polyprotein and are responsible for viral replication. Inhibition of these two proteases have favourable therapeutic effects and so the current study supports that AGP-3 has potency against the viral infection (the binding free energies are in the range −28.1 to −31.4 kcal/mol).

Furthermore AGP-3 has shown significant binding affinity (free energy of binding is −24.1 kcal/mol) towards RdRp which catalyzes the RNA synthesis (with the consumption of nucleoside triphosphate). In addition to the potency against the proteases and RdRp, we also report significant binding to spike protein which is responsible for host cell recognition. Even though we could not confirm if this modulates protein-protein interaction between spike protein and ACE-2, we cannot exclude the allosteric modulation of such interaction. This observation suggests that the AGP3 can be effective during both initial and matured state of viral infection and has a multi-targeting character similar to the drug-cocktails used for the treatment of infections like HIV. It is worth recalling that in the case of HIV treatment, a cocktail of drugs is usually recommended which can be a combination of drugs that are nucleoside reverse transcriptase inhibitor, non-nucleoside reverse transcriptase inhibitor, protease inhibitor and integrase inhibitor. This gives an advantage that the subscribed drug cocktails can act during any stage of infection and cure the patient. However, if a single drug can inhibit all viral targets simultaneously it will be much more desirable. Here, we see that the ligand AGP-3 has better binding affinity for all the key viral targets and can serve as an efficient drug compound having potency against multiple viral targets necessary for viral lifecycle.

Though the AGP-3 has recorded significant potency towards different viral targets, we need to confirm whether they bind with the optimal binding affinity since in the case of RdRp the binding free energies are larger compared to the RdRp inhibitor remdesivir. Therefore there is a need to further analyze the possibilities to enhance the potency of AGP-3 towards the selected viral targets. Lower the free energy of binding, larger will be the binding affinity and this will minimize the drug dosage. To evidence this, we have carried out the residue wise decomposition analysis of binding free energies and the results are shown in the Figure 7. This has been performed only for the ligand AGP3 which had shown good binding affinity to all the targets. As expected, the number of residues contributing favorably are more in number. In the case of 3CLpro, the aminoacid residues ASN143 and CYS146 contribute binding affinity lower than −2. kcal/mol while for PLpro and TYR268 residue contributes higher binding affinity of −3.2 kcal/mol. For the RdRp, the residues ASP336 (−3.1 kcal/mol), THR440 (−2.1 kcal/mol) and ASP507 (−4.9) are dominantly contributing to the stability of the complex. In addition to these stabilizing residues, in all cases, there are certain residues which contribute to the binding unfavourably (i.e. with positive value for $\Delta E_{Residue}$). This indicates that the binding affinity can be further enhanced if the repulsive interaction between these residues and AGP3 ligand can be changed to become attractive through suitable chemical tuning of the ligand. The number of destabilizing residues are more in the case of 3CLpro when compared to PLpro. In the case of RdRp, certain residues contribute to a larger extent for destabilization (for example the residues such as LYS429 (+2.3 kcal/mol), LYS560 (+1.5 kcal/mol)). Due to these destabilizing residues the binding free energy of AGP3 is higher than that of remdesivir. Further the binding affinity of AGP3 towards the selected viral targets can be further enhanced to develop a potential target for Covid-19.

4. Conclusions

Amidst the wildly spreading Covid-19 infection which has harvested more than 1 lakh human lives worldwide, the researchers are searching for drugs which can be of

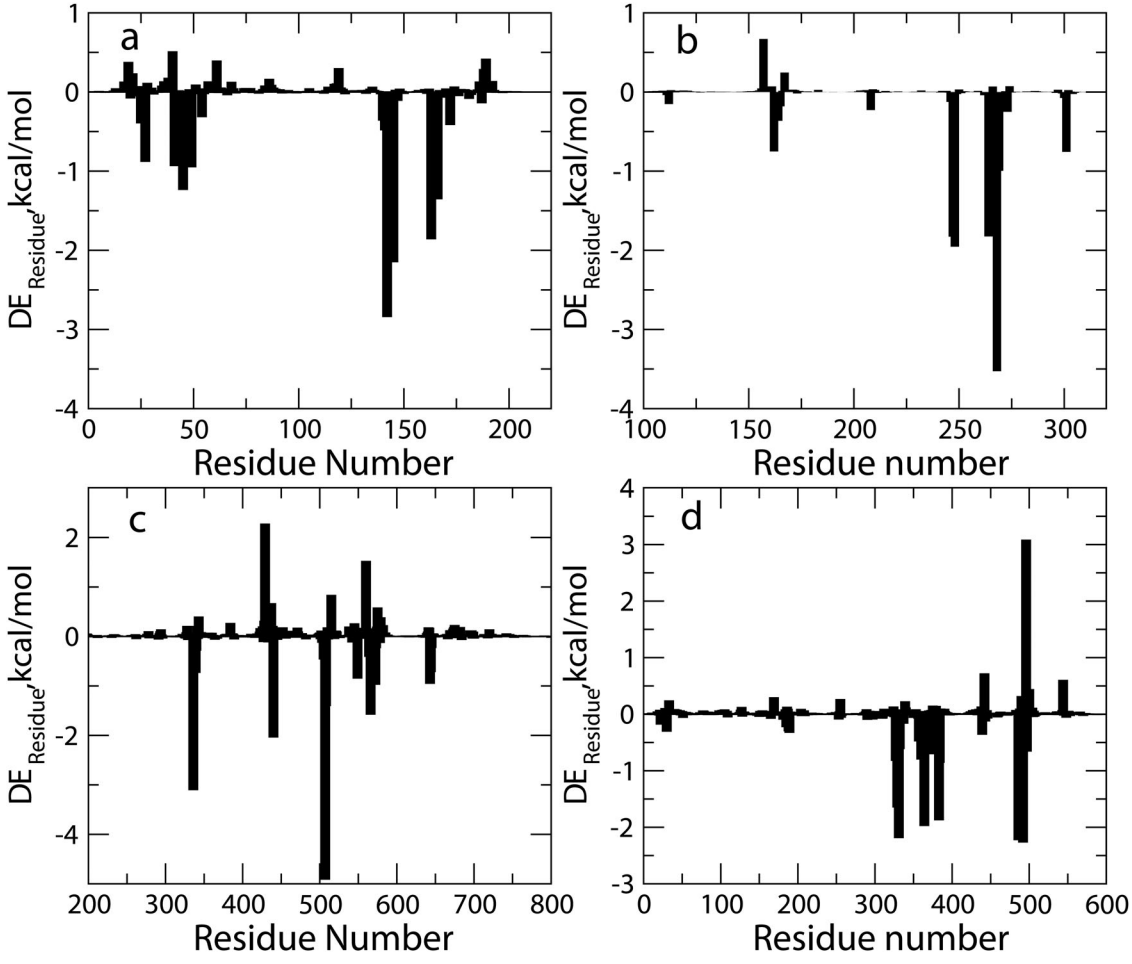

Figure 7. Residue wise decomposition analysis of binding free energies in the case of various AGP3: viral target complex systems. (a) 3CLpro (b) PLpro (c) RdRp (d) ACE-2 receptor of spike protein-ACE2 complex.

immediate use. Therefore, drug repurposing and use of compounds from phytochemicals are best strategies for the given situation. For the developing and underdeveloped countries the former option is still not suitable as the population and poor economy may disable the capacity to buy such drugs. The latter approach where the use of phytochemicals from plants and herbs appears highly relevant as they can be made available for a larger population. Moreover, such compounds have least side effects and have favorable pharmacokinetic and pharmacodynamics properties which again allow them to be excluded for tedious, expensive and time-consuming clinical trials. In this way, the phytochemicals from *A. paniculata* were shown to have potency against the Covid-19 and we have evidenced its microscopic mechanism through rational computational modeling. Among the four phytochemicals, AGP3 has shown promising binding affinity towards all the four targets namely, 3CLpro, PLpro, RdRp and spike protein with precise binding to the catalytic site required for inhibiting the targets in a therapeutic way. The residue-wise contributions of binding free energies has shown that in the case of 3CLpro and RdRp there is still scope for the improvement through the chemical modification of the ligand. Other option in using the ligand structure as a template is that it facilitates to screen the drug databank, nutraceuticals and Chinese medicine databases for identifying more active compounds against the viral targets.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This work was supported by the grant from the Swedish Infrastructure Committee (SNIC) for the projects "In-silico Diagnostic Probes Design" (snic2020-5-2). JJ gratefully acknowledges the MHRD-RUSA 2.0 [F. 24/51/2014-U, Policy (TNMulti-Gen), Dept. of Edn. Govt. of India] for the infrastructure facilities provided to the Department of Bioinformatics, Alagappa University.

References

Berman, H., Henrick, K., Nakamura, H., & Markley, J. L. (2007). The worldwide protein data bank (wwpdb): ensuring a single, uniform archive of pdb data. *Nucleic Acids Research*, *35*(Database issue), D301–D303. https://doi.org/10.1093/nar/gkl971

Boopathi, S., Poma, A. B., & Kolandaivel, P. (2020). Novel 2019 coronavirus structure, mechanism of action, antiviral drug promises and rule out against its treatment. *Journal* of Biomolecular Structure and Dynamics, (0), 1–14. https://doi.org/10.1080/07391102.2020.1758788

Breneman, C., & Wiberg, K. (1990). Determining atom-centered monopoles from molecular electrostatic potentials. the need for high sampling density in formamide conformational analysis. *Journal of Computational Chemistry*, *11*(3), 361–373. https://doi.org/10.1002/jcc.540110311

Calabrese, C., Berman, S. H., Babish, J. G., Ma, X., Shinto, L., Dorr, M., Wells, K., Wenner, C. A., & Standish, L. J. (2000). A phase i trial of andrographolide in HIV positive patients and normal volunteers. *Phytotherapy Research*, *14*(5), 333–338. https://doi.org/10.1002/1099-1573(200008)14:5<333::AID-PTR584>3.0.CO;2-D

Case, D., Betz, R., Cerutti, D., Cheatham, T., III, Darden, T., Duke, T., Giese, T., Gohlke, H., Goetz, A., Homeyer, N., Izadi, S., Janowski, P., Kaus, J., Kovalenko, A., Lee, T., LeGrand, S., Li, P., Lin, C., Luchko, T., … Kollman, P. (2016). Amber 16, university of California, San Francisco.

Chan, J. F., Chan, K.-H., Kao, R. Y., To, K. K., Zheng, B.-J., Li, C. P., Li, P. T., Dai, J., Mok, F. K., Chen, H., Hayden, F., & Yuen, K. (2013). Broad-spectrum antivirals for the emerging Middle East respiratory syndrome coronavirus. *The Journal of Infection*, *67*(6), 606–616. https://doi.org/10.1016/j.jinf.2013.09.029

Chan, J. F.-W., Kok, K.-H., Zhu, Z., Chu, H., To, K. K.-W., Yuan, S., & Yuen, K.-Y. (2020). Genomic characterization of the 2019 novel human-pathogenic coronavirus isolated from a patient with atypical pneumonia after visiting Wuhan. *Emerging Microbes & Infections*, *9*(1), 221–236. https://doi.org/10.1080/22221751.2020.1719902

Chandramohan, V., Kaphle, A., Chekuri, M., Gangarudraiah, S., & Bychapur Siddaiah, G. (2015). Evaluating andrographolide as a potent inhibitor of ns3-4a protease and its drug-resistant mutants using in silico approaches. *Advances in Virology*, *2015*, 972067– 972069. https://doi.org/10.1155/2015/972067

Chang, Y.-S., & Woo, E.-R. (2003). Korean medicinal plants inhibiting to human immunodeficiency virus type 1 (hiv-1) fusion. *Phytotherapy Research: PTR*, *17*(4), 426–429. https://doi.org/10.1002/ptr.1155

Chen, H., Ma, Y.-B., Huang, X.-Y., Geng, C.-A., Zhao, Y., Wang, L.-J., Guo, R.-H., Liang, W.-J., Zhang, X.-M., & Chen, J.-J. (2014). Synthesis, structure-activity relationships and biological evaluation of dehydroandrographolide and andrographolide derivatives as novel anti-hepatitis B virus agents . *Bioorganic & Medicinal Chemistry Letters*, *24*(10), 2353–2359. https://doi.org/10.1016/j.bmcl.2014.03.060

Cheng, V. C., Lau, S. K., Woo, P. C., & Yuen, K. Y. (2007). Severe acute respiratory syndrome coronavirus as an agent of emerging and reemerging infection. *Clinical Microbiology Reviews*, *20*(4), 660–694. https://doi.org/10.1128/CMR.00023-07

de Wilde, A. H., Jochmans, D., Posthuma, C. C., Zevenhoven-Dobbe, J. C., van Nieuwkoop, S., Bestebroer, T. M., van den Hoogen, B. G., Neyts, J., & Snijder, E. J. (2014). Screening of an FDA-approved compound library identifies four small-molecule inhibitors of Middle East respiratory syndrome coronavirus replication in cell culture. *Antimicrobial Agents and Chemotherapy*, *58*(8), 4875–4884. https://doi.org/10.1128/AAC.03011-14

Dyall, J., Coleman, C. M., Hart, B. J., Venkataraman, T., Holbrook, M. R., Kindrachuk, J., Johnson, R. F., Olinger, G. G., Jahrling, P. B., Laidlaw, M., Johansen, L., Lear-Rooney, C., Glass, P., Hensley, L., & Frieman, M. (2014). Repurposing of clinically developed drugs for treatment of middle east respiratory syndrome coronavirus infection. *Antimicrobial Agents and Chemotherapy*, *58*(8), 4885–4893. https://doi.org/10.1128/AAC.03036-14

Ekalaksananan, T., Sookmai, W., Fangkham, S., Pientong, C., Aromdee, C., Seubsasana, S., & Kongyingyoes, B. (2015). Activity of andrographolide and its derivatives on HPV16 pseudovirus infection and viral oncogene expression in cervical carcinoma cells. *Nutrition and Cancer*, *67*(4), 687–696. https://doi.org/10.1080/01635581.2015.1019630

Elfiky, A. A. (2020a). Anti-hcv, nucleotide inhibitors, repurposing against covid-19. *Life Sciences*, *248*(1), 117477. https://doi.org/10.1016/j.lfs.2020.117477

Elfiky, A. A. (2020b). Sars-cov-2 RNA dependent RNA polymerase (RDRP) targeting: An in silico perspective. *Journal* of Biomolecular Structure and Dynamics, *0*(0), 1–9. PMID: 32338164.

Fehr, A. R., & Perlman, S. (2015). Coronaviruses: An overview of their replication and pathogenesis. In *Coronaviruses* (pp. 1–23). Springer.

Frisch, M. J., Trucks, G. W., Schlegel, H. B., Scuseria, G. E., Robb, M. A., Cheeseman, J. R., Scalmani, G., Barone, V., Mennucci, B., Petersson, G. A., Nakatsuji, H., Caricato, M., Li, X., Hratchian, H. P., Izmaylov, A. F., Bloino, J., Zheng, G., Sonnenberg, J. L., Hada, M., … Fox, D. J. (2009). Gaussian, 09, Revision A.02. Gaussian, Inc.

Ge, X.-Y., Li, J.-L., Yang, X.-L., Chmura, A. A., Zhu, G., Epstein, J. H., Mazet, J. K., Hu, B., Zhang, W., Peng, C., Zhang, Y.-J., Luo, C.-M., Tan, B., Wang, N., Zhu, Y., Crameri, G., Zhang, S.-Y., Wang, L.-F., Daszak, P., & Shi, Z.-L. (2013). Isolation and characterization of a bat SARS-like coronavirus that uses the ace2 receptor. *Nature*, *503*(7477), 535–538. https://doi.org/10.1038/nature12711

Glowacka, I., Bertram, S., Müller, M. A., Allen, P., Soilleux, E., Pfefferle, S., Steffen, I., Tsegaye, T. S., He, Y., Gnirss, K., Niemeyer, D., Schneider, H., Drosten, C., & Pöhlmann, S. (2011). Evidence that tmprss2 activates the severe acute respiratory syndrome coronavirus spike protein for membrane fusion and reduces viral control by the humoral immune response. *Journal of Virology*, *85*(9), 4122–4134. https://doi.org/10.1128/JVI.02232-10

Gupta, S., Mishra, K., & Ganju, L. (2017). Broad-spectrum antiviral properties of andrographolide. *Archives of Virology*, *162*(3), 611–623. https://doi.org/10.1007/s00705-016-3166-3

Keyaerts, E., Vijgen, L., Pannecouque, C., Van Damme, E., Peumans, W., Egberink, H., Balzarini, J., & Van Ranst, M. (2007). Plant lectins are potent inhibitors of coronaviruses by interfering with two targets in the viral replication cycle. *Antiviral Research*, *75*(3), 179–187. https://doi.org/10.1016/j.antiviral.2007.03.003

Khaerunnisa, S., Kurniawan, H., Awaluddin, R., Suhartati, S., & Soetjipto, S. (2020). Potential inhibitor of covid-19 main protease (mpro) from several medicinal plant compounds by molecular docking study. Prepr. doi10. 20944/preprints202003. 0226. v1, 1–14.

Kim, Y., Narayanan, S., & Chang, K.-O. (2010). Inhibition of influenza virus replication by plant-derived isoquercetin. *Antiviral Research*, *88*(2), 227–235. https://doi.org/10.1016/j.antiviral.2010.08.016

Lan, J., Ge, J., Yu, J., Shan, S., Zhou, H., Fan, S., Zhang, Q., Shi, X., Wang, Q., Zhang, L., & Wang, X. (2020). Structure of the sars-cov-2 spike receptor-binding domain bound to the ace2 receptor. *Nature*, *581*(7807), 215–220. https://doi.org/10.1038/s41586-020-2180-5

Lee, J.-C., Tseng, C.-K., Young, K.-C., Sun, H.-Y., Wang, S.-W., Chen, W.-C., Lin, C.-K., & Wu, Y.-H. (2014). Andrographolide exerts anti-hepatitis c virus activity by up-regulating haeme oxygenase-1 via the p38 mapk/n rf2 pathway in human hepatoma cells. *British Journal of Pharmacology*, *171*(1), 237–252. https://doi.org/10.1111/bph.12440

Liu, C., Zhou, Q., Li, Y., Garner, L. V., Watkins, S. P., Carter, L. J., Smoot, J., Gregg, A. C., Daniels, A. D., Jervey, S., & Albaiu, D. (2020). Research and development on therapeutic agents and vaccines for covid-19 and related human coronavirus diseases. *ACS Central Science*, *6*(3), 315–331. https://doi.org/10.1021/acscentsci.0c00272

Mansouri, S., Choudhary, G., Sarzala, P. M., Ratner, L., & Hudak, K. A. (2009). Suppression of human t-cell leukemia virus i gene expression by pokeweed antiviral protein. *The Journal of Biological Chemistry*, *284*(45), 31453–31462. https://doi.org/10.1074/jbc.M109.046235

Matsuyama, S., Nagata, N., Shirato, K., Kawase, M., Takeda, M., & Taguchi, F. (2010). Efficient activation of the severe acute respiratory syndrome coronavirus spike protein by the transmembrane protease tmprss2. *Journal of Virology*, *84*(24), 12658–12664. https://doi.org/10.1128/JVI.01542-10

Muralidharan, N., Sakthivel, R., Velmurugan, D., & Gromiha, M. M. (2020). Computational studies of drug repurposing and synergism of lopinavir, oseltamivir and ritonavir binding with sars-cov-2 protease against covid-19. *Journal of Biomolecular Structure and Dynamics*, 1–6. https://doi.org/10.1080/07391102.2020.1752802

Narayanan, N., & Nair, D. T. (2020). Vitamin b12 May inhibit RNA-dependent-RNA polymerase activity of NSP12 from the Covid-19 virus. preprints.org https://doi.org/ https://doi.org/10.20944/preprints202003.0347.v1

Niranjan, A., Tewari, S. K., & Lehri, A. (2010). Biological activities of kalmegh (andrographis paniculata nees) and biological activities of kalmegh (*Andrographis paniculata* nees). *Indian Journal of Natural Products and Resources*, *1*(2), 125–135.

O'Boyle, N. M., Banck, M., James, C. A., Morley, C., Vandermeersch, T., & Hutchison, G. R. (2011). Open babel: An open chemical toolbox. *Journal of Cheminformatics*, *3*(1), 33.

Omrani, A. S., Saad, M. M., Baig, K., Bahloul, A., Abdul-Matin, M., Alaidaroos, A. Y., Almakhlafi, G. A., Albarrak, M. M., Memish, Z. A., & Albarrak, A. M. (2014). Ribavirin and interferon alfa-2a for severe Middle East respiratory syndrome coronavirus infection: A retrospective cohort study. *The Lancet. Infectious Diseases*, *14*(11), 1090–1095. https://doi.org/10.1016/S1473-3099(14)70920-X

Parasher, R., Upadhyay, A., Khan, N. A., & Dwivedi, S. K. (2011). Biochemical estimation and quantitative determination of medicinally important andrographolide in andrographis peniculata at different growth stages. *Electronic Journal of Environmental, Agricultural and Food Chemistry*, *10*(7), 2479–2486.

Peng, G.-Y., Zhou, F., Ding, R-l., Li, H.-D., & Yao, K. (2002). Modulation of lianbizi injection (andrographolide) on some immune functions. *Zhongguo Zhong Yao za Zhi = Zhongguo Zhongyao Zazhi = China Journal of Chinese Materia Medica*, *27*(2), 147–150.

Pholphana, N., Rangkadilok, N., Saehun, J., Ritruechai, S., & Satayavivad, J. (2013). Changes in the contents of four active diterpenoids at different growth stages in *Andrographis paniculata* (burm. f.) nees (chuanxinlian). *Chinese Medicine*, *8*(1), 2. https://doi.org/10.1186/1749-8546-8-2

Poongavanam, V., Namasivayam, V., Vanangamudi, M., Al Shamaileh, H., Veedu, R. N., Kihlberg, J., & Murugan, N. A. (2018). Integrative approaches in hiv-1 non-nucleoside reverse transcriptase inhibitor design. *Wiley Interdisciplinary Reviews: Computational Molecular Science.*, *8*(1), e1328.

Prussia, A., Thepchatri, P., Snyder, J. P., & Plemper, R. K. (2011). Systematic approaches towards the development of host-directed antiviral therapeutics. *International Journal of Molecular Sciences*, *12*(6), 4027–4052. https://doi.org/10.3390/ijms12064027

Rastelli, G., Rio, A. D., Degliesposti, G., & Sgobba, M. (2010). Fast and accurate predictions of binding free energies using mm-pbsa and mm-gbsa. *Journal of Computational Chemistry*, *31*(4), 797–810. https://doi.org/10.1002/jcc.21372

Reusken, C. B., Haagmans, B. L., Müller, M. A., Gutierrez, C., Godeke, G.-J., Meyer, B., Muth, D., Raj, V. S., Smits-De Vries, L., Corman, V. M., Drexler, Jan-Felix Smits, S. L., Tahir, Y. E. E., De Sousa, R., Beek, J. v., Nowotny, N., Maanen, K. v., Hidalgo-Hermoso, E., Bosch, B.-J., … Koopmans, M. (2013). Middle east respiratory syndrome coronavirus neutralising serum antibodies in dromedary camels: A comparative serological study. *The Lancet. Infectious Diseases*, *13*(10), 859–866. https://doi.org/10.1016/S1473-3099(13)70164-6

Schaftenaar, G., Vlieg, E., & Vriend, G. (2017). Molden 2.0: Quantum chemistry meets proteins. *Journal of Computer-Aided Molecular Design*, *31*(9), 789–800. https://doi.org/10.1007/s10822-017-0042-5

Seubsasana, S., Pientong, C., Ekalaksananan, T., Thongchai, S., & Aromdee, C. (2011). A potential andrographolide analogue against the replication of herpes simplex virus type 1 in vero cells. *Medicinal Chemistry (Shariqah (United Arab Emirates))*, *7*(3), 237–244. https://doi.org/10.2174/157340611795564268

Sheahan, T. P., Sims, A. C., Leist, S. R., Schäfer, A., Won, J., Brown, A. J., Montgomery, S. A., Hogg, A., Babusis, D., Clarke, M. O., Spahn, J. E., Bauer, L., Sellers, S., Porter, D., Feng, J. Y., Cihlar, T., Jordan, R., Denison, M. R., & Baric, R. S. (2020). Comparative therapeutic efficacy of remdesivir and combination lopinavir, ritonavir, and interferon beta against MERS-COV. *Nature* Communications, *11*(1), 1–14.

Simmons, G., Gosalia, D. N., Rennekamp, A. J., Reeves, J. D., Diamond, S. L., & Bates, P. (2005). Inhibitors of cathepsin l prevent severe acute respiratory syndrome coronavirus entry. *Proceedings of the National Academy of Sciences of the United States of America*, *102*(33), 11876–11881. https://doi.org/10.1073/pnas.0505577102

Thisoda, P., Rangkadilok, N., Pholphana, N., Worasuttayangkurn, L., Ruchirawat, S., & Satayavivad, J. (2006). Inhibitory effect of *Andrographis paniculata* extract and its active diterpenoids on platelet aggregation. *European Journal of Pharmacology*, *553*(1-3), 39–45. https://doi.org/10.1016/j.ejphar.2006.09.052

Trott, O., & Olson, A. J. (2010). Autodock vina: Improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. *Journal of Computational Chemistry*, *31*(2), 455–461. https://doi.org/10.1002/jcc.21334

Tsuey-Pin, L., Shih-Ying, C., Pin-Der, D., Chang, L.-K., & Yen-Ni, L. (2008). Inhibition of the epstein-barr virus lytic cycle by andrographolide. *Biological & Pharmaceutical Bulletin*, *31*(11), 2018–2023. https://doi.org/10.1248/bpb.31.2018

Wallace, A. C., Laskowski, R. A., & Thornton, J. M. (1995). Ligplot: A program to generate schematic diagrams of protein-ligand interactions. *Protein Engineering*, *8*(2), 127–134. https://doi.org/10.1093/protein/8.2.127

Walls, A. C., Park, Y.-J., Tortorici, M. A., Wall, A., McGuire, A. T., & Veesler, D. (2020). Structure, function, and antigenicity of the sars-cov-2 spike glycoprotein. *Cell*, *181*(2), 281–292.e6. https://doi.org/10.1016/j.cell.2020.02.058

Wang, J., Wolf, R., Caldwell, J., Kollman, P., & Case, D. (2004). Development and testing of a general amber force field. *Journal of Computational Chemistry*, *25*(9), 1157–1174. https://doi.org/10.1002/jcc.20035

Wintachai, P., Kaur, P., Lee, R. C. H., Ramphan, S., Kuadkitkan, A., Wikan, N., Ubol, S., Roytrakul, S., Chu, J. J. H., & Smith, D. R. (2015). Activity of andrographolide against Chikungunya virus infection. *Scientific Reports*, *5*, 14179. https://doi.org/10.1038/srep14179

Wishart, D. S., Knox, C., Guo, A. C., Cheng, D., Shrivastava, S., Tzur, D., Gautam, B., & Hassanali, M. (2008). Drugbank: A knowledgebase for drugs, drug actions and drug targets. *Nucleic Acids Research*, *36*(Database issue), D901–D906. https://doi.org/10.1093/nar/gkm958

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/ https://doi.org/10.1016/j.apsb.2020.02.008

Wu, F., Zhao, S., Yu, B., Chen, Y.-M., Wang, W., Song, Z.-G., Hu, Y., Tao, Z.-W., Tian, J.-H., Pei, Y.-Y., Yuan, M.-L., Zhang, Y.-L., Dai, F.-H., Liu, Y., Wang, Q.-M., Zheng, J.-J., Xu, L., Holmes, E. C., & Zhang, Y.-Z. (2020). A new coronavirus associated with human respiratory disease in china. *Nature*, *579*(7798), 265–269. https://doi.org/10.1038/s41586-020-2008-3

Yan, R., Zhang, Y., Li, Y., Xia, L., Guo, Y., & Zhou, Q. (2020). Structural basis for the recognition of sars-cov-2 by full-length human ace2. *Science (New York, N.Y.).)*, *367*(6485), 1444–1448. https://doi.org/10.1126/science.abb2762

Yu, B., Dai, C.-Q., Jiang, Z.-Y., Li, E.-Q., Chen, C., Wu, X.-L., Chen, J., Liu, Q., Zhao, C.-L., He, J.-X., Ju, D-h., & Chen, X-y. (2014). Andrographolide as an anti-h1n1 drug and the mechanism related to retinoic acid-inducible gene-i-like receptors signaling pathway. *Chinese Journal of Integrative Medicine*, *20*(7), 540–545. https://doi.org/10.1007/s11655-014-1860-0

Yuan, L., Zhang, C., Sun, H., Liu, Q., Huang, J., Sheng, L., Lin, B., Wang, J., & Chen, L. (2016). The semi-synthesis of novel andrographolide analogues and anti-influenza virus activity evaluation of their derivatives. *Bioorganic & Medicinal Chemistry Letters*, *26*(3), 769–773. https://doi.org/10.1016/j.bmcl.2015.12.100

Zaki, A. M., Van Boheemen, S., Bestebroer, T. M., Osterhaus, A. D., & Fouchier, R. A. (2012). Isolation of a novel coronavirus from a man with pneumonia in Saudi Arabia. *The New England Journal of Medicine*, *367*(19), 1814–1820. https://doi.org/10.1056/NEJMoa1211721

Zhang, X., Huang, S.-Z., Gu, W.-G., Yang, L.-M., Chen, H., Zheng, C.-B., Zhao, Y.-X., Wan, D., & Zheng, Y.-T. (2014). Wikstroelide m potently inhibits HIV replication by targeting reverse transcriptase and integrase nuclear translocation. *Chinese Journal of Natural Medicines*, *12*(3), 186–193. https://doi.org/10.1016/S1875-5364(14)60031-5

Zhu, N., Zhang, D., Wang, W., Li, X., Yang, B., Song, J., Zhao, X., Huang, B., Shi, W., Lu, R., Niu, P., Zhan, F., Ma, X., Wang, D., Xu, W., Wu, G., Gao, G. F., & Tan, W. (2020). China novel coronavirus investigating and research team. A novel coronavirus from patients with pneumonia in china, 2019. *New England Journal of Medicine*, *382*(8), 727–733. https://doi.org/10.1056/NEJMoa2001017