EXPLORING THE POTENTIAL OF ORGANIC MOLECULES IN THE TREATMENT OF COVID-19

By

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A thesis submitted to the Department of Mathematics and Natural Sciences in partial fulfillment of the requirements for the degree of
MS in Biotechnology

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Declaration

It is hereby declared that

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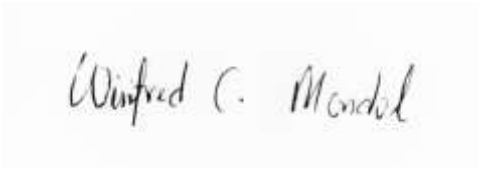

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Approval

The project titled "Exploring the Potential of Organic Molecules in the Treatment of COVID-19" submitted by Winifred Claire Mondol (19176015) of Spring 2019 has been accepted as satisfactory in partial fulfillment of the requirement for the degree of MS in Biotechnology on [Date-of-Defense].

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Ethics Statement

This material is an original work, which has not been previously published elsewhere. It is my own research and analysis in a truthful and complete manner. The paper properly credits all the sources used (correct citation).

Abstract

Abstract

The novel pathogen SARS-CoV2 causing coronavirus disease 2019 (COVID-19) is a global public health concern. COVID-19 has infected over 220 million people worldwide so far. The study and development of novel bioactive chemicals with cost-effective and selective anti-COVID 19 therapeutic power is the primary focus of contemporary medical research. As a result, utilizing the molecular docking technique has become critical in the discovery and development of novel medications. The purpose of this work is to investigate the binding affinity and type of interactions between 30 chemical molecules and M^{pro} using molecular docking. Using UCSFChimera, the PDB data of the target protein and prepared organic molecules (ligands) were docked using AutoDockVina, which provides a set of potential complexes based on the criteria of form complementarity of the natural molecules with their binding affinities. According to the results, hyperoside, aloin, and ginkgetin, were found to have a high affinity with M^{pro}. Hence, these chemicals have the potential to be used as therapeutics against SARS-CoV2.

Keywords: Molecular Docking; M^{pro} ; SARS-CoV-2; COVID-19 treatment

Dedicated to all those who have suffered from COVID-19.

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List of Acronyms

SARS	Severe Acute Respiratory Syndrome
MERS	Middle East Respiratory Syndrome
ICTV	International Committee on Taxonomy of Viruses
CoV	Coronavirus
HCoV	Human Coronavirus
SARS-CoV-2	Severe Acute Respiratory Syndrome Coronaviruses
COVID-19	Coronavirus disease
WHO	World Health Organization
ACE2	Angiotensin Converting Enzyme-II
ARDS	Acute Respiratory Distress Syndrome
M^{pro}	Main protease
ADME	Absorption, Distribution, Metabolism, and Excretion
RNA	Ribonucleic acid
3CLpro	coronavirus main protease
RdRp	RNAdependent RNA polymerase
RBD	Receptor binding domain
TMPRSS2	Transmembrane serine protease II
S	Spike protein
ER	Endoplasmic reticulum

SSEs	Super spreading events
ERGIC	Endoplasmic Reticulum-Golgi Intermediate Compartment
PLP	Papain-like protease
E	Envelop protein
RAAS	Renin-angiotensin-aldosterone system
ARBs	Angiotensin receptor blockers
PDB	Protein Data Bank
ADT	Auto Dock Tools
MGL	Molecular Graphics Laboratory

Chapter 1

Introduction

1.1 Background of Study

On December 31, 2019, a new strain, severe acute respiratory syndrome coronavirus 2 (SARS-Cov-2) was isolated by the International Committee on Taxonomy of Viruses (ICTV) from patients with pneumonia of unknown etiology in Wuhan city, China (Phelan et al., 2020) On March 11, 2020, the World Health Organization (WHO) announced that COVID-19 is a 'public health emergency of international concern' (Li et al., 2020). The primary techniques for controlling an ongoing pandemic were based on regulations and human behavior such as surveillance and isolation, contact tracking, movement restrictions, social distancing, hand washing, and enhanced community awareness (Zhang et al., 2020).

Clinicians are using SARS- CoV, and MERS- CoV antibodies, and a recently recommended combination therapy of hydroxychloroquine and azithromycin were explored and the results of an open-label non-randomized clinical trial were reported. (Huang et al., 2020; Gautret et al., 2020). Meanwhile, the Food and Drug Administration has stated that neither Chloroquine phosphate nor Hydroxychloroquine sulfate is approved for the treatment of COVID-19. Nonetheless, based on some in vitro and clinical findings, chloroquine phosphate and hydroxychloroquine sulfate were recommended as COVID-19 treatments, and enough randomized studies on these compounds were provided, allowing the use of the aforesaid medications for emergency situations (https://www.fda.gov/emergency-use-authorization#covidtherapeutics). Hydroxychloroquine has the potential to suppress viral activities and metabolisms. They may also be implicated in additional methods such as suppression of the ACE2 cellular receptor, acidification of the cell membrane, which prevents

virus entrance, and control of immune response via cytokine release. (COVID-19 Drug Therapy-Elsevier, 09 March 2020). However, new research has indicated that hydroxychloroquine can cause drug poisoning and severe or moderate adverse effects in people who are already receiving diabetes or hypersensitivity medicines, the same patients who are found to be badly affected by COVID-19. Administration of hydroxychloroquine has been found to inhibit pro-inflammatory cytokines which finally leads to Acute Respiratory Distress Syndrome (ARDS) (Guastalegname & Vallone, 2020). It was discovered that after treatment with hydroxychloroquine, an adverse neuropsychiatric condition was observed, which is thought to indicate lysosomal dysfunction leading to mental symptoms, which then led to the patient's return to their normal state (Ali et al., 2018). When hydroxychloroquine was given to patients with acute renal impairment, the adverse impact of retinal toxicity was shown to be fatal. In a trial of diabetic patients treated with high doses of hydroxychloroquine and atorvastatin, the patients who experienced the greatest drop in blood glucose were those who received the highest dosages of hydroxychloroquine and atorvastatin (Wondafrash et al., 2020). When an antimalarial drug, hydroxychloroquine was administered to patients with dermatomycosis, non-life-threatening cutaneous reactions were seen most in dermatomycosis patients than cutaneous lupus erythrematosus (Pelle & Callen, 2002) and many side effects have been reported. It is necessary to find an appropriate natural, non-synthetic pharmaceutical molecule with minimum side effects. Plant chemicals are ideal for identifying medicinal components of interest and are also the most cost-effective and are generated fast. Although several targets for the treatment of COVID-19 have been identified, the main protease (M^{pro}) of SARS-CoV-2 was chosen for this study. The presence of the main protease (M^{pro}) enzyme in SARS-CoV-2 was recently confirmed by Liu and his research group. The activity of M^{pro}, also known as 3CLpro, which regulates polyprotein processing along with papain-like proteases, is required for protein synthesis. M^{pro} activity is thought to be a need

for viral replication, hence blocking M^{pro} activity would prevent viral reproduction (Zhang, 2020).

1.2 Objectives of Study

The objectives of this present study are to employ various bioinformatics tools in order to

- Select natural molecules as ligands
- Perform molecular docking of M^{pro} with selected natural molecules
- Study the interaction of the amino acid of docked molecules
- Evaluate the pharmacokinetic properties of the selected natural molecules

1.3 Literature Review

1.3.1 COVID-19: An Introduction

The CoVs belong to the family Coronaviridae with large RNA genomes and a unique replication method. Four coronavirus genera namely Alpha-, Beta-, Gamma- and Deltacoronavirus have been identified so far, with human coronaviruses (HCoVs) detected in the α coronavirus (HCoV-229E and NL63) and β coronavirus (MERS-CoV, SARS-CoV, HCoV-OC43 and HCoV-HKU1) genera (Perlman, 2009). The new SARS-CoV -2 was identified as a beta-coronavirus (Schoeman and Fielding, 2019). The phylogenetic tree of SARS-like coronaviruses complete genome sequences is clearly portrayed in Fig. 1 A. SARS-CoV-2 is an enveloped, single (+) stranded RNA, with symmetric helical nucleocapsid (Khan et al., 2020). The virus encodes twenty proteins, including four structural proteins (S: spike; E: envelope; M: membrane; N: nucleocapsid), as well as many nonstructural proteins like RNA-dependent RNA polymerase (RdRp), coronavirus main protease (3CLpro), and

papain-like protease (PLpro) (Chen et al., 2020). The angiotensin-converting-enzyme II (ACE2) was found to be a key functional receptor for the SARS-CoV-2 allowing its attachment to human and bat cells and therefore its replication (Walls et al., 2020; Zhou et al., 2020). The receptor-binding motif of the spike protein—receptor-binding domain (RBD) interacts with the ACE2 receptor, allowing SARS-CoV-2 to connect to the host cells. The C-terminal S2 subunit of the spike protein (responsible for virus-cell membrane fusion) undergoes conformational changes due to this interaction. The host cell-type 2II transmembrane serine protease TMPRSS2 subsequently proteolytically processes the complex S protein ACE2, resulting in ACE2 cleavage and so viral entrance into the host cell. (Jiang et al.,2020; Rabi et al., 2020). Following entrance and uncoating, genomic RNA is translated into two polyproteins (pp1a and pp1ab), which are then cleaved by proteases to produce 15–16 nonstructural proteins. The nonstructural proteins cause the cellular membrane to reorganize, resulting in the formation of a double-membrane vesicle. The genomic RNA, on the other hand, is translated into subgenomic RNA, which leads to the production of structural (spike, envelope, membrane, and nucleocapsid) and accessory proteins. Finally, virions are put together in the ERGolgi intermediate complex before being released through the secretory pathway (Fung and Liu, 2020). SARS-CoV-2 proteins have been proven to interfere with host immune responses, and M^{pro}-specific T cells have been seen in SARS-CoV-2 infected individuals (Guo, 2019). Furthermore, earlier research has shown that the papain-like proteases generated by SARS-CoV and MERS-CoV can stymie the immune response and that SARS-CoV can counteract the antiviral response mediated by interferons (Nezhad, 2020).

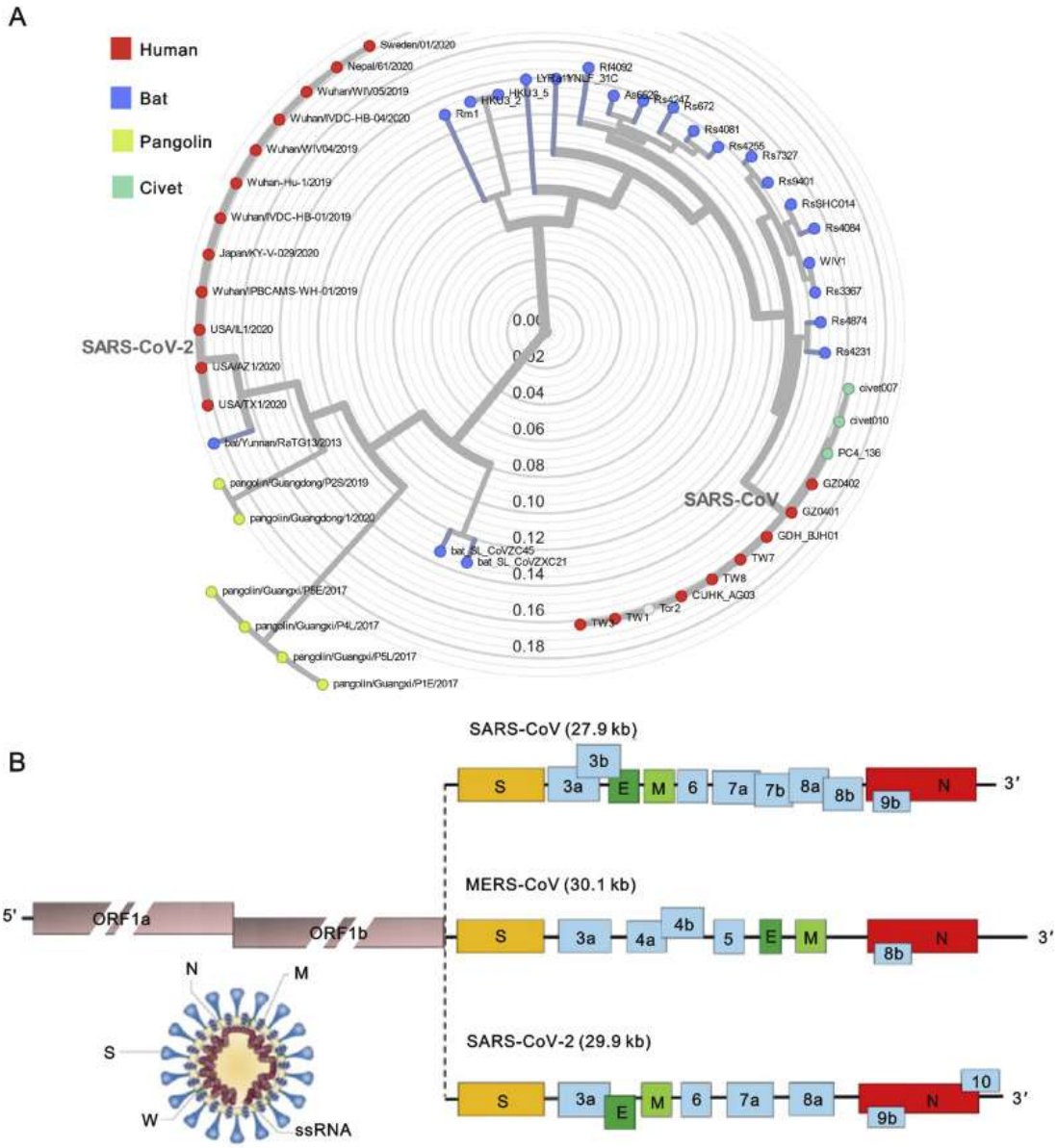


Fig. 1. The phylogenetic tree of SARS-like coronaviruses complete genome sequences and genome of SARS-CoV, MERS-CoV and SARSCoV- 2. (A) This phylogeny shows evolution of SARS-like b-coronaviruses including samples from human (n ¼ 20), bat (n ¼ 22), civet (n ¼ 3) and pangolin (n ¼ 6). The phylogenetic tree of complete genome sequences of coronaviruses was obtained and analyzed with Nextstrain (https://github.com/blab/sars-like-cov). (B) Coronaviruses form enveloped and spherical particles of 100e160 nm in diameter. They contain a positive sense single stranded RNA (ssRNA) genome of 26-32 kb in size. In SARS-CoV, MERS-CoV and SARS-CoV-2, the 50-terminal two-thirds of the genome ORF1a/b encodes polyproteins, which form the viral replicase transcriptase complex. The other ORFs on the one-third of the genome encode four main structural proteins: spike (S), envelope (E), nucleocapsid (N) and membrane (M) proteins, as well as several accessory proteins.

1.3.2 Transmission

COVID-19 appears to have a similar method of transmission to SARS-CoV. SARS-CoV emerged in 2002 as a result of cross-species transmission from animal to human, then spread via human-to-human transmission. COVID-19 is presently following the same pattern as COVID-19, with super spreading events (SSEs) leading to a pandemic (Riou & Althaus 2020). As the outbreak has progressed, it has become clear that droplets of respiratory mucus discharge and direct contact are the most common mechanisms of human-to-human transmission. The virus is released from a person's respiratory secretions when they talk, sneeze, or cough, resulting in droplet transmission. Droplets that come into direct contact with the mucosal membrane of an infected patient are more likely to transmit the virus. Droplets have a six-foot range and do not linger in the air. Contacting a virus-infected surface or object and then touching their lips, nose, or eyes are two more possible ways for a person to become infected (Rothe et al., 2020) In one study, SARS-CoV-2 was discovered in feces and blood swabs, indicating the possibility of additional transmission pathways (Zhang et al.,2020) . In the absence of a viable vaccine, the only way to control and halt this outbreak is to use isolation, frequent hand washing, and social distancing as effective preventive measures. By 14 days after the onset of symptoms, infected people may have developed antibodies to the virus (To et al., 2020). According to preliminary studies, some of these antibodies appear to be protective, albeit this has yet to be proven. It is unknown, however, whether all infected patients will develop a protective immune response or how long this effect would last.

1.3.3 Pathogenesis and Replication

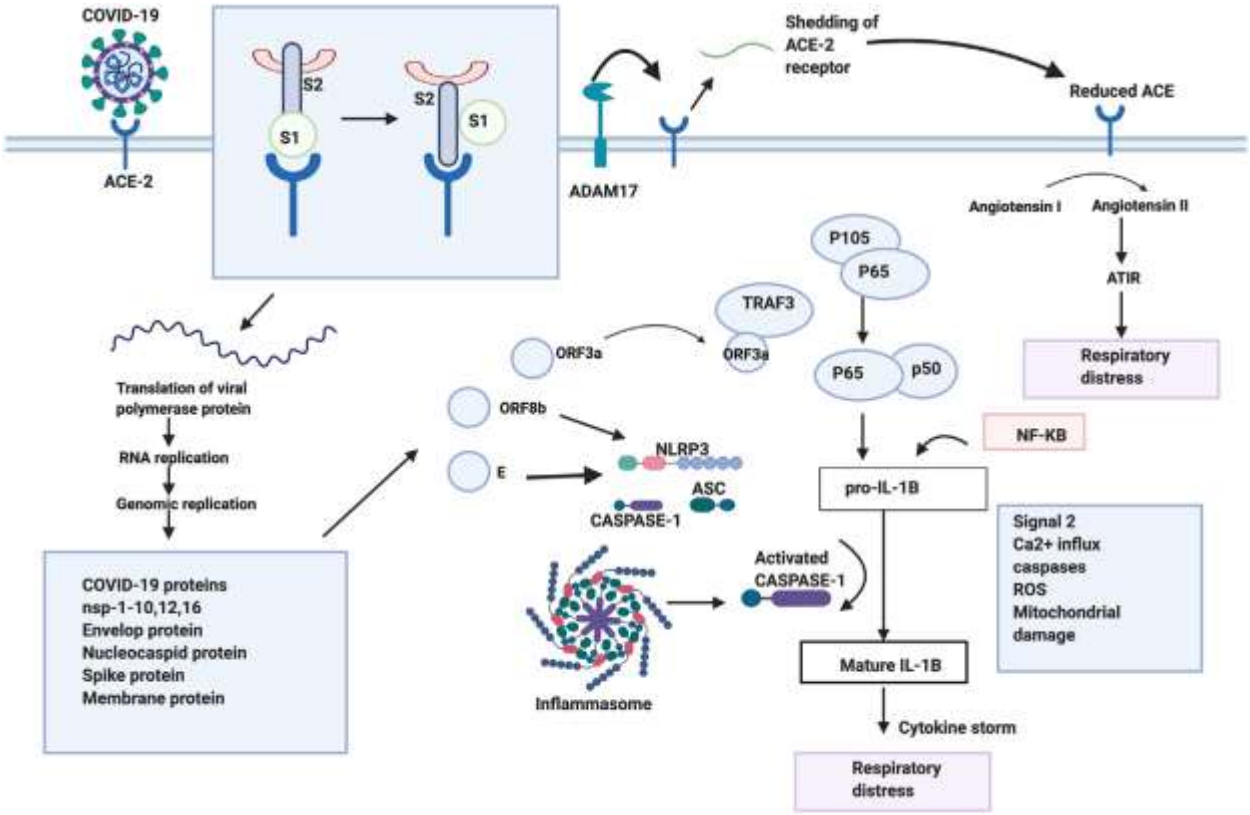


Fig. 2 Possible mechanism of action of SARS-COV-2. Depiction of the binding of SARS-COV-2 to its receptor ACE-2. The S1 and S2 subunits are subsequently cleaved followed by the shedding of ACE-2 by ADAM 17. This resulting in an increased amount of Angiotensin II leading to respiratory distress. Upon binding, the virus fuses with the membrane and enters the cell, followed by translation, and replication of the proteins. ORF3a, ORF8b,E proteins and the NF-KB pathway activates the inflammasome pathway through various means, leading to the activation of cytokine. This results in a cytokine storm, further resulting in respiratory distress.

The life cycle of coronaviruses begins when the virion's spike protein S1 subunit binds to the host cell receptor. (Figure 2, step 1). The virus's host species range and tissue tropism are determined by the S-protein-receptor interaction. Many alpha-coronaviruses, for example, employ aminopeptidase N as a receptor, while SARS-CoV and HCoV-NL63 use angiotensin-converting enzyme 2 (ACE2) as the host receptor. To enter human cells, MHV uses CEACAM1 while MERS-CoV binds dipeptidyl-peptidase 4 (DPP4). The distribution of the receptor within tissues in the human body has a big impact on the disease profile that results. (Hamming et al., 2004; Fehr and Perlman, 2015; Li M-Y et al., 2020). After receptor binding,

the virus gains access to the cytosol by acid-dependent proteolytic cleavage of the S protein into S1 and S2 subunits by a furin, cathepsin, TMPRSS2, or another protease, followed by S2-assisted fusion of the viral and cellular membranes. After release of the viral genome (Figure 2, step 2), the replicase is translated from the genomic RNA (Figure 2, step 3). Viral RNA synthesis then follows (Figure 2, step 4a), with the assembly of viral replication-transcription complexes (Figure 1.2, step 4b). Viral structural proteins (S, E, and M) are translated from the RNA (Figure 2, step 5), inserted into the endoplasmic reticulum (Figure.2, step 6), and move to the endoplasmic reticulum-Golgi intermediate compartment (ERGIC). Multiple copies of the nucleocapsid (N protein) package genomic RNA into helical structures (ribonucleoprotein complexes) in the cytoplasm, where they interact with hydrophobic M proteins (envelope proteins) in the ERGIC to direct virion assembly. (Figure 2, step 7). Virions budded from the ERGIC membranes (Figure 2, step 8) are subsequently transported out of the cell via the constitutive exocytic pathway (Figure 2, step 9) (deHann and Rottier, 2005; Fehr and Perlman, 2015).

1.3.4 Current Therapeutic Drugs

For SARS-CoV-2 infection, there is currently no particular antiviral medication that has been clinically validated. The most critical care strategy continues to be supportive treatment, which includes oxygen therapy, fluid conservation, and the use of broad-spectrum antibiotics to treat subsequent bacterial infections (Huang et al., 2020). There are various possible treatment targets to repurpose existing antiviral medications or build effective interventions against this novel coronavirus, according to research on molecular mechanisms of coronavirus infection (Groneberg et al., 2005) and the genomic organization of SARS-CoV-2 (Roujian et al., 2020) Remdesivir, an adenosine analogue that can target the RNAdependent

RNA polymerase and block viral RNA synthesis in cultured cells (Lo et al., 2017) , mice (Sheahan et al., 2020), and non-human primate models (de Wit, et al., 2020), has shown promise against a wide range of RNA viruses (including SARS/MERS-CoV) infections. The Washington Department of Health first administered remdesivir intravenously and discovered that it could protect against SARS-CoV-2 infection (Ren, 2020). Then, in vitro, remdesivir and chloroquine were shown to effectively inhibit SARS-CoV-2 (Wang et al., 2020). As a result, other nucleoside analogues, such as favipiravir, ribavirin, and galidesivir (Zumla et al., 2016; De Clercq, 2019), could be used to treat SARS-CoV-2. Non-structural proteins such as chymotrypsin-like (3C-like protease, 3CLpro) and papain-like protease (PLP) are required for coronaviral replication and can decrease host innate immune responses (Chen et al., 2020). As a result, 3CLpro inhibitors like flavonoids (Jo et al., 2020), as well as PLP inhibitors such diarylheptanoids (Park et al.,2012), are appealing options for fighting SARS-CoV-2. As a functioning coronavirus receptor, ACE2 mediates SARS-CoV-2 entrance into the cell. As a result, using ACE2 to limit S protein binding is a viable method for preventing SARS-CoV-2 infection (Kuhn, 2004)

1.3.5 Alternative Approach

Traditional medicines are sometimes overlooked in modern drug research and development because their translational potential is often underestimated. Despite their ambiguity, these drugs have a wide range of applications in non-Western medical technologies (Yuan et al., 2016). Many phytochemical elements can be found in a single herb, and they can work alone or in conjunction with other substances to create the desired pharmacological effect. (Parasuraman et al., 2014). Many plant compounds have been researched and modified into medications for various diseases as a result of their use in traditional medicine. The majority of antiviral therapy techniques are non-specific for viruses (Jiang et al., 2015). In medical research, the development of antiviral drugs is a major priority. The antiviral activities of

medicinal plants have been shown to have a significant impact at various phases of viral development (Akram et al., 2018). Medicinal plants provide basic raw materials for significant antiviral medications instead of synthetic antiviral treatments. In several viral infections, synthetic medications have been replaced with medicinal plants as life-saving drugs in the past. Many medicinal plants have antiviral, anti-inflammatory, and antioxidant qualities, thus they might be worth considering for COVID-19 treatment. The majority of the active natural chemicals such as polyphenols and flavonoids, along with some alkaloids, anthraquinones, saponins, terpenes, coumarins, and diarylheptanoids has shown promise against the prior SARS-CoV. Considering the structures of SARS-CoV and SARS-CoV-2 are homologous, they might act as potential candidates to combat COVID-19. However, more standard clinical trials should clearly be conducted to scientifically show the efficacy of such traditional products.

1.3.6 Protein Target

One of the fundamental targets for developing antiviral vaccines or medications has been considered as the primary protease (M-pro) enzyme (Jin et al., 2020). M-pro is found in the polyprotein ORF1ab of the SARS-CoV-2 virus and is required for virus replication. This protease is involved in polyprotein degeneration (Zhang et al., 2020). The M-pro enzyme has a strong relationship with the SARS-CoV virus, except for one residue (Ala285Thr) (Gimeno et al., 2020). SARS-CoV-2 has an almost 89 percent resemblance to SARS-CoV-1, according to a full-length genome phylogenetic analysis (Jiang et al., 2020). This laid the groundwork for the development of SARS-CoV-2, indicating that the SARS-CoV-2 receptor may be similar to the SARS-CoV (ACE2) receptor (Veljkovic et al., 2020). SARS-CoV-2 is reported to use angiotensin-converting enzyme 2 (ACE2) receptors to penetrate the target cells (Wang et al., 2020). As a result, any drug that increases ACE2 synthesis is likely to boost COVID-19

susceptibility by promoting viral cellular invasion. However, Angiotensin II is biochemically transformed to angiotensin (1–7), which protects the lungs by decreasing the ACE2 receptor and vasodilation (Kreutz et al., 2020). There is often conflicting evidence about the continuation or discontinuation of medications inhibiting the renin-angiotensin-aldosterone system (RAAS), including inhibitors of angiotensin-converting enzyme (ACEIs) and angiotensin receptor blockers ARBs, in patients with COVID-19 and co-morbidities such as hypertension, cardiovascular disease, and diabetes (Vaduganathan et al., 2020). 6LU7 has been previously denoted as main protease (M^{pro}) of SARS-CoV-2, M^{pro} is a key enzyme of coronaviruses and has a pivotal role in mediating viral replication and transcription, making it an attractive drug target for SARS-CoV-2. Taking all these considerations into account, M^{pro} has been selected to comply with the binding interactions with the ligands.

Chapter 2

Materials and Methods

2.1 Retrieval of the Protein Sequence

The X-ray crystallographic structure of main protease (M^{pro}, PDB ID 6LU7) of SARS-CoV-2 has been downloaded from the Protein Data Bank (PDB) (http://www.pdb.org.) database. The protein was prepared for docking simulation using the Scripps Research Institute's graphical user interface program "Auto Dock Tools (ADT) 1.5.6" (Molecular Graphics Laboratory tool or MGL tool) (R. Huey, 2008). For the production of receptor protein input file for docking study, Chain A of the protein (6LU7) was chosen. Receptor protein preparation for docking study was initiated by removing water molecules, hetero atoms and co-crystallised ligands from PDB crystal structure of protein 6LU7. Polar hydrogen atoms along with Kollman united atom charges were added subsequently to the receptor protein and finally the receptor protein input file was saved (Meng et al., 2011; Khan et al., 2018; Ferreira et al., 2015). Partial atomic charges were then assigned using the Gasteiger-Marsili approach for accurate ionization and tautomeric states of residues.

2.2 Retrieving Molecular Structures from Databases

The three-dimensional (3D) structures of 30 organic compounds were retrieved in .sdf format from PubChem (https://pubchem.ncbi.nlm.nih.gov/). PubChem is a repository for chemical substances and biological activity that consists of three databases: substance, compound, and bioassay (Salehi et al., 2019) then viewed on UCFS Chimera and saved in .pdb format. Table 1 lists out these 30 substances alongside their sources and prior uses.

Table 1 List of organic molecules and their uses

	Organic compound(ligands)	Uses	References
1	Andrographis Paniculata	analgesic, antipyretic, antiretroviral, antiproliferative, antimalarial, antithrombotic, antihyperglycemic, antiurolethial, antilesihmaniasis,	Poolsup et al., 2004
2	Dehydroandrographolide	hepatoprotective, immune-modulatory, protective against alcohol induced toxicity and cardioproetcive activity and anticancer activity	Poolsup et al., 2004
3	Propylene glycol	Wound, dermetitis	Brinkhaus et al., 2000
4	Asiatic acid	antimicrobial, antioxidant, anti-inflammatory, etc	Lv et al., 2018
5	lupeol	Anticancer and anti-inflammatory activity	Saleem, 2009
6	kaempferol	anti-hypoxic and anti-inflammatory effects	Devi et al., 2015
7	quercetin	anti-hypoxic and anti-inflammatory effects	Heinz et al., 2010
8	6-Gingerol	Inhibits viral attachment and penetration	Chang et al., 2013
9	6-shogaol	Inhibits viral attachment and penetration	Yocum et al., 2020
10	Aegle marmelos Alkaloid C	antioxidant, antidiabetic, antimicrobial, hepatoprotective, cardioprotective and anticancer activity	Manandhar et al., 2018
11	Catechin	Reacts with viral membrane, inhibits viral replication and viral mRNA synthesis	Shin et al., 2010
12	Hyperoside	Reacts with viral membrane, inhibits viral replication and viral mRNA synthesis	Shin et al., 2010
13	Allicin,	Interfere with the glycans on the spike protein during virus entry and virus release, Inhibits viral adsorption or penetration	Sánchez-Gloria et al., 2021
14	Epigallocatechin gallate	antibacterial, antifungal and antiviral effects	Steinmann et al., 2013
15	aloin	Inhibits viral attachment to host cell	Sun et al., 2018
16	Azadirachtin	antimicrobial, larvicidal, antimalarial, antibacterial, antiviral	Gupta et al., 2017
17	Homonojirimycin	Prevents infammatory responses and strengthen host resistance against viral infection by activating secretion of IFN- and IL-10	Zhang et al., 2013
18	Curcumin	Inhibit viral replication	Obata et al., 2013
19	Embelin	Inhibits viral replication	Hossan et al., 2018
20	Ginkgetin	Inhibition of viral sialidase activity	Miki et al., 2007
21	Glycyrrhizin	Inhibits viral adsorption, penetration and replication	Cinatl et al., 2003
22	hydroxycitric acid	Inhibited viral replication and viral antigens and genes expression	Baatartsogt et al., 2016
23	Momordicine	Inhibit various stages of viral life cycle	Sur et al., 2021
24	Myricetin	Inhibits helicase protein	Yu et al., 2012
25	linolenic acid	block the entry of SARS-CoV-2	Goc et al., 2021
26	Thymoquinone, thymol	Inhibit viral replication	Shaterzadeh et al., 2018

27	Eugenol,	Inhibit protease enzyme	Fujisawa & Murakami, 2016
28	Oleuropein	Direct inactivation, interacts with viral envelope	Micol et al., 2005
29	ursolic acid	Inhibits viral replication	Hussain et al., 2017
30	Ellagic acid,	Interfere with viral envelop or mask viral structures which are necessary for adsorption or entry into host cells	BenSaad et al., 2017

2.3 Molecular Docking

Docking studies were attempted to explore the binding mode of the 30 organic compounds onto the 3D model of M^{pro} of COVID-19 using AUTODOCK tools 1.5.6 (Goodsell & Olson 1990) in UCFS Chimera (Pettersen et al., 2004). UCSF Chimera supports AutoDock Vina as a plugin, for performing molecular docking and views the docking results (Douangamath et al., 2020). Before docking, polar-H atoms were added to the COVID-19 model followed by Gasteiger charges calculation using AUTODOCK tools available from Scripps Research Institute (http://www.scripps.edu/mb/olson/doc/Autodock). The macromolecule file was then saved in pdb format, ready for docking. The AutoGrid program was used to build ligand-centered maps with a grid size of X: 30× Y: 30 × Z: 30 points, grid centre set at X: -10.75, Y: 12.33, and Z: 68.84 dimensions. Polar Gasteiger-type H charges were allocated, while non-polar-H atoms were merged with the carbons, along with internal degrees of freedom and torsions. By using AutoDock Vina scoring algorithm Gibbs Free Energy (−ΔG kcal/mol) was measured for (Trott & Olson 2010) between these ligands with 6LU7.

2.4 Interaction with M^{pro}

The two-dimensional and three-dimensional structures of the selected ligands were analyzed using Discovery Studio Visualizer v3.0 software (Inc. 2012; Yang et al., 2012). The protein–compound interactions such as bonded and other non-bonded energies among the ligands with binding energy equal to or greater than the standard, Remdesivir were depicted against

M^{pro} of novel coronavirus. This software visualizes molecular interactions such as hydrogen bonds, hydrophobic interactions, and van der Waals interactions.

2.5 ADME Prediction

To develop orally active drugs, it is important to compute their pharmacokinetic and physicochemical features. This was carried out by using the QikProp (ADMET predictor) of Schrodinger. This will provide data regarding absorption, distribution, metabolism, and excretion of the proposed natural compounds as drugs. Parameters such as Lipinski's rule of five (RO5) were evaluated to predict the drug-likeness of the chemical compounds.

Chapter 3

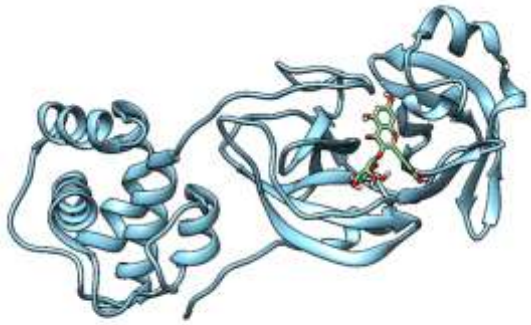
Results and Discussion

3.1 Molecular Docking

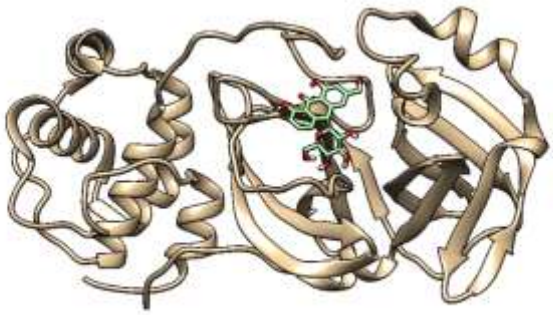
Docking results of all the 30 organic compounds with M^{pro}, 6LU7 are listed below in Table 2 with their respective binding affinity. Remdesivir is considered as a standard and showed a binding affinity of -8.1 kcal/mol with M^{pro} (6LU7). The binding affinities of less than -8.1 kcal/mol are marked red as they are below the standard and hence are discarded for further analysis. The remaining active molecules (based on their binding energy) are considered for the next steps and have a binding affinity score ranging from -8.1 to -9.6kcal/mol. The docking score of M^{pro} (6LU7) with the organic compounds namely hyperoside, aloin, ginkgetin, and glycyrrhizin was found to be -8.5 kcal/mol, -8.1 kcal/mol, -9.6 kcal/mol, and -8.4 kcal/mol respectively. Figure 3 shows the docked structures of the organic compounds with 6LU7 with binding energy greater than -8.1 kcal/mol.

Table 2 The molecular docking analysis results for several organic compounds against 6LU7, Including binding energy/Gibbs Energy

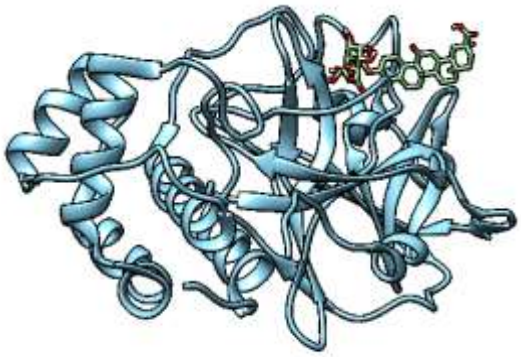
Plant	**Organic compound(ligands)**	**Binding energy (kcal/mol)**	**Plant**	**Organic compound(ligands)**	**Binding energy (kcal/mol)**
Andrographis paniculata	Andrographis paniculata	-6.9	Azadirachta indica	Azadirachtin	-6.8
Andrographis paniculata	Dehydroandrographolide	-7.2	Commelina communis	Homonojirimycin	-5.2
Centella asiatica	Propylene glycol	-3.7	Curcuma longa	Curcumin	-7.3
Centella asiatica	Asiatic acid	-8	Embelia ribes	Embelin	-5.3
Barleria Prionitis	(lupeol)pentacyclic triterpenoid	-7.3	Gingko biloba	Ginkgetin	-9.6
Adhatoda vasica	kaempferol	-7.8	Glycyrrhiza glabra	Glycyrrhizin	-8.4
Adhatoda vasica	quercetin	-7.4	Hibiscus sabdariffa	hydroxycitric acid	-5.3
Zingiber officinale	6-Gingerol	-5.7	Momordica charantia	Momordicine	-7.2
Zingiber officinale	6-shogaol	-6.1	Myrica rubra	Myricetin	-7.3
Aegle marmelos	Aegle marmelos Alkaloid C	-6.5	Moringa oleifera	Linolenic acid	-5.0
Agrimonia pilosa	Catechin	-7.5	Nigella sativa	Thymoquinone	-4.9
Agrimonia pilosa	Hyperoside	-8.5	Ocimum sanctum	Eugenol	-4.9
Allium sativum	Allicin	-3.7	Olea europaea	Oleuropein	-7.7
Camellia sinensis	Epigallocatechin gallate	-7.8	Punica granatum	ursolic acid	-7.6
Aloe vera	aloin	-8.1	Syzygium cumini	Ellagic acid	-7.4



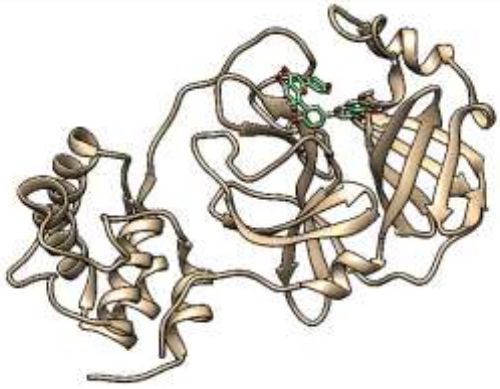
(a)Hyperoside



(b) aloin



(c) Glycyrrhizin



(d) Ginkgetin

Figure 3 The docked structures of (a)hyperoside (b)aloin (c)glycyrrhizin and (d)ginkegetin with 6LU7

3.2 Interaction with M^{pro}

The interacting amino acids of molecular interaction of Remdisivir, Hyperoside, Aloin, Ginkgetin, and Glycyrrhizin in the key residues of M^{pro} are shown in Table 3. The docking simulation of M^{pro} to Hyperoside has shown that hyperoside forms five hydrogen bonds and three hydrophobic bonds which are displayed in Figure 4. Aloin forms six hydrogen bonds and three hydrophobic bonds. Ginkgetin forms six hydrogen bonds and five hydrophobic bonds and glycyrrhizin forms nine hydrogen bonds and two hydrophobic bonds. Table 4 tabulates the interacting amino acids for Remdesivir and the common interacting amino acids

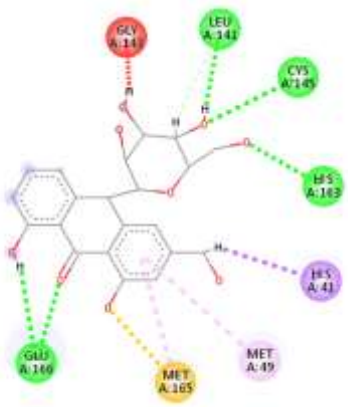
of the other four ligands. Hyperoside has three common interacting amino acids namely, MET 49, HIS 163, and LEU 141 with Remdesivir. Then Aloin gives the best results showing interactions with all the amino acids, MET 49, MET 165, HIS 163, GLU 166, and LEU 141 the same way as Remdesivir with only one mismatch. Besides Ginkgetin also have three common interacting amino acids, MET 165, HIS 163, and GLU 166. Nonetheless, Glycyrrhizin has only one common interacting amino acid, LEU 141 as the standard, Remdesivir, and hence is excluded for further investigation.

Table 3 Common amino acids involved in the binding with 6LU7 (M^{pro}) of the reference and selected candidates

ligands	**Interacting amino acids**
Remdesivir	THR 26, MET49, MET165, HIS163, GLU166, LEU 141
Hyperoside	CYS 145, MET 49, MET 165, SER 144, LEU 141, HIS 163
Aloin	GLY 143,GLU 166, MET 165, MET 49, HIS 41, HIS 163, CYS 145, LEU 141
Ginkgetin	HIS 41, HIS 163, HIS 172, GLY 143, CYS 145, GLU 166, PHE 140, ASN 142, MET 165
Glycyrrhizin	HIS 41, CYS 145, GLN 189, LEU 141, ASN 142

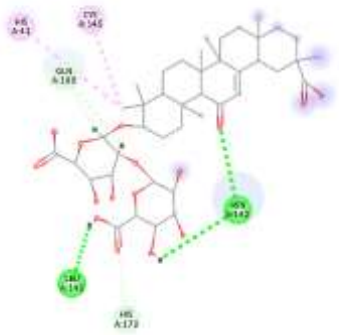
Table 4 Common interacting amino acids as Remdesivir of the organic ligands with 6LU7 (M^{pro})

Ligands	**Amino acids**					
Remdesivir	**THR 26**	**MET 49**	**MET 165**	**HIS 163**	**GLU 166**	**LEU 141**
Hyperoside		√		√		√
Aloin		√	√	√	√	√
Ginkgetin			√	√	√	
Glycyrrhizin						√

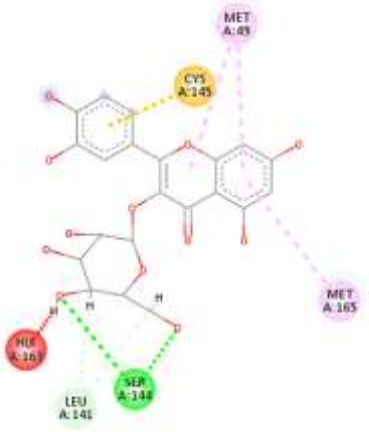


(a) Aloin

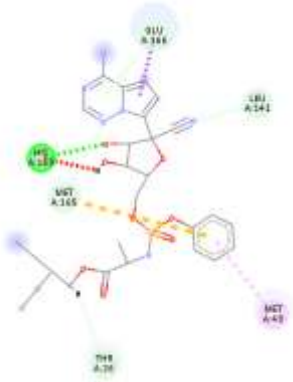
(b) Ginkgetin



(c) Glycyrrhizin



(d) Hyperoside



(e) Remdesivir

Figure 4 2D Diagram generated on Biovia Discovery Studio of (a)aloin (b)ginketin (c) glycyrrhizin and (d) hyperoside (e) Remdesivir with M^{pro}

3.3 ADME Prediction

The physical properties and drug-related characteristics of these compounds were calculated using the QikProp tool. All of the properties were identified based on Lipinski's rule of 5 (Lipinski et al., 2001) and other criteria (Kier, 2012; Krämer, 1999). The ADME properties of the lead organic compounds are shown in Table 5. The properties include percentage HOA which determines the human oral absorption ranging from 0 to 100% : >80% (high), 25–80% (medium) e < 25% (low). QPPCaco2 is another property what predicts apparent Caco-2 cell permeability in intestinal cells in nm/sec, >500 nm/s (good) e < 25 nm/s (low); QPPMDCK predicts MDCK cell permeability in kidney cells in nm/sec >500 nm/s (good) e < 25 nm/s (low); QPlogKhsa predicts binding to human serum albumin:−1.5 (low) a 1.5 (high); CNS Predicted central nervous system activity on a –2 (inactive) to + 2 active scale: −2 (low permeability) e > −2 (high permeability); QPlogBB Predicted brain/blood partition coefficient : <−1 (low) e > −1 (easy permeation); PSA Van der Waals surface area of polar nitrogen and oxygen atoms; area):>60 (does not cross the blood/brain barrier) ; < 60 (to cross the blood/brain barrier). All probable candidates show moderate human oral absorption levels including Remdesivir. Also considering the other parameters all the organic compounds show unsatisfactory results which are not within the range to be a good orally consumed drug. The binding energies of aloin, hyperoside, and ginkgetin were above the control drug and also had satisfactory interaction. Therefore it is necessary to look for other likely methods of drug delivery. The use of engineered nanocarriers to deliver these therapeutic candidates safely and effectively can be explored. Nanoparticulate drug delivery techniques include nanospheres, micelles, solid lipid nanoparticles, nanoliposomes, dendrimers, magnetic nanoparticles, and nanocapsules, among other dosage forms (Witika et al., 2020).

Table 5 Predicted pharmacokinetic properties of the reference and selected candidates								
SI no	**Molecules**	**Absorption**	**Distribution**			**CNS permeability**		
		%HOA	**QPPCaco2**	**QPPMDCK**	**QPlogKhsa**	**CNS**	**QPlogBB**	**PSA**
	Remdesivir (Reference inhibitor)	34.504	28.486	10.568	-0.59	-2	-3.278	198.913
	Aloin	32.443	14.983	5.277	-0.665	-2	-2.67	170.548
	Ginkgetin	63.865	23.199	8.465	1.031	-2	-2.88	165.943
	Hyperoside	0	2.537	0.774	-0.895	-2	-3.823	217.584

Conclusion

The current medical treatment for COVID-19 infection is mostly supportive, with no specific therapy available. Several drugs, including antimalarials such as chloroquine and hydroxychloroquine the anti-retroviral combination lopinavir/ritonavir, an investigational nucleotide analog with broad-spectrum antiviral activity initially intended to treat hepatitis C and Ebola, viz., remdesivir , and the macrolide antibiotic azithromycin, have been tested in clinical trials as a potential treatment for the virus. However, none of these treatments provide a permanent cure or are appropriate for prevention. The disadvantages of these treatments include exposing patients to medications with well-documented systemic side effects or innovative therapies with unknown consequences without confirmation of clinical benefit. The molecular docking method was used to attain the intended goal. This technique comprises looking into the binding affinity and type of interactions between 30 chemicals derived from medicinal plants (ligands) and M^{pro}, the target protein involved in SARS-CoV-2 infection. To assign each compound an ID and retrieve its chemical structure, the PubChem database was used. The ligands and protein were prepared for docking using UCSF Chimera. Default parameters were used to prepare the ligands and proteins for docking. Then, utilizing the PDB files of the target proteins and generated compounds, AutoDock Vina was used to predict the structure of the protein-ligand complexes and analyze the binding energy. The results of molecular docking were analysed using Discovery Studio 2020 to view the interactions. Hyperoside, aloin and ginkgetin were selected as they showed the best results in the investigation with greater binding affinities than the standard, Remdesivir, and the interactions proved that the protein-ligand complex formed would be stable. Unfortunately, none of these organic compounds possess the quality to be administered orally, and hence it is crucial to find other delivery pathways. Nonetheless, these three organic compounds: hyperoside, aloin and ginkgetin can be of great importance in the treatment of COVID-19

with minimal side effects. Thus, additional laboratory experiments are required to put the prospective organic molecules to use.

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