In silico studies reveal potential antiviral activity of phytochemicals from medicinal plants for the treatment of COVID-19 infection

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Abstract

The spread of COVID-19 across continents has led to a global health emergency. COVID-19 disease caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has affected nearly all the continents with around 1.52 million confirmed cases worldwide. Currently only a few regimes have been suggested to fight the infection and no specific antiviral agent or vaccine is available. Repurposing of the existing drugs or use of natural products are the fastest options available for the treatment. The present study is aimed at employing computational approaches to screen phytochemicals from the medicinal plants targeting the proteins of SARS-CoV2 for identification of antiviral therapeutics. The study focuses on three target proteins important in the life cycle of SARS-CoV-2 namely Spike (S) glycoprotein, main protease (Mpro) and RNA-dependent RNA-polymerase (RdRp). Molecular docking was performed to screen phytochemicals in medicinal plants to determine their feasibility as potential inhibitors of these target viral proteins. Of the 30 plant phytochemicals screened, Silybin, an active constituent found in *Silybum marianum* exhibited higher binding affinity with targets in SARS-CoV-2 in comparison to currently used repurposed drugs against SARS-CoV-2. Withaferin A from *Withania somnifera* also showed significant binding to the targets proteins. In addition, phytochemicals from *Tinospora cordifolia* and *Aloe barbadensis* displayed good binding energetics with the target proteins in SARS-CoV-2. These results provide a basis for the use of traditional medicinal plants as alternative lines of treatment for COVID-19 infection.

Introduction

COVID–19 disease caused by the novel coronavirus SARS-CoV–2 has been declared as a global pandemic by WHO. Severe Acute Respiratory Syndrome Corona Virus 2 (SARS-CoV–2), previously named 2019 n-CoV, first emerged in late 2019 in China [1]. Since December 2019, COVID–19 infection has spread to nearly all the continents. According to the latest situation report released by WHO, globally 1.61 million confirmed cases and around 99690 deaths have been reported due to COVID–19 as on 11th April, 2020 [2]. India records 6634 confirmed cases and 242 deaths due to COVID–19 infection (as on 11th April, 2020) [3]. The virus has high rate of transmissibility and spreads via droplets, physical contact with infected individuals, contaminated surfaces and possibly through oral-fecal route [4]. Common symptoms of a person infected with coronavirus include fever, cough, shortness of breath, and dyspnea. In more severe cases, the infection can cause pneumonia, severe acute respiratory syndrome, kidney failure, and even death due to multiple organ failure [5].

SARS-CoV–2 is an enveloped RNA virus belonging to the *Coronaviridae* family and genus beta-coronavirus and is distant from SARS-CoV with 79% identity. Phylogenetic analysis of the SARS-CoV–2 shows 50% identity with Middle East respiratory syndrome coronavirus MERS-CoV, 88% identity to two bat-derived (SARS)-like coronaviruses bat-SL-CoVZC45 and bat-SL-CoVZXC21 [6] and 96.2% identity to bat CoV RaTG13 [7]. The complete genome of Wuhan-Hu–1 coronavirus (WHCV), a strain of SARS-CoV-2
with a size of 29.9 kb was first isolated from a pneumonia patient in Wuhan [8]. The genome has variable number of Open reading frames (around 6–11) [9]. Viral RNA located in the ORF1 translates two polyproteins, pp1a and pp1ab, and encodes 16 non-structural proteins (NSP), while remaining ORF codes for structural proteins. Corona virus has four major structural proteins, namely the Spike (S) protein, envelope (E) protein, membrane (M) protein, and nucleocapsid (N) protein [10]. Among these, S glycoprotein of SARS-CoV–2 binds to host cell receptors, angiotensin-converting enzyme 2 (ACE2) that is a critical step for virus entry [11]. Both the structural proteins and NSPs have played important roles from drug design perspectives. The therapies for SARS-CoV–2 can target different pathways—structural proteins that block the entry of virus into the human host cell, critical enzymes involved in viral replication and virus RNA synthesis, proteins that cause virulence or aid virus assembly process and many more. Availability of crystal structures of SARS-CoV–2 proteins in PDB (Table 1) has guided structure-based drug design endeavors for development of antiviral agents.

To date, no specific therapeutic drug or vaccine has been approved for the treatment of coronavirus. There is an urgent need to discover novel antivirals for the ongoing pandemic situation caused by Severe Respiratory Corona Virus 2 (SARS-Cov–2). Drug discovery for the very infectious COVID–19 is a challenging job owing to frequent mutations [12]. In addition, researchers across the globe are racing to develop potential vaccines [13]. The development of both novel antiviral compounds as well as vaccines presents several challenges and requires significant amounts of effort and time for validation. Therefore, exploring the repurposing of already-approved pharmaceuticals or the use of natural compounds can provide alternatives to the development of novel antiviral drugs. Chloroquine, a repurposed drug known widely for the treatment of malaria is used to treat COVID–19 infection [14]. In vitro studies have shown hydroxychloroquine, a less hepatotoxic derivative of chloroquine, to be foremost effective drug in inhibiting SARS-CoV–2 infection [15]. Few clinical trials have also shown chloroquine phosphate to be effective in COVID–19 associated pneumonia [16]. Other antivirals in combination or alone are also being used to determine their effectiveness against SARS-CoV–2 [17–18]. However, at present the treatment is only symptomatic and more effective antivirals need to be developed to fight the deadly disease.

Medicinal plants are valuable sources of drugs used globally as alternative medicines. India is a rich source of biodiversity with more than 7000 plants species used as medicinal plants [19].

India also has a rich ancient tradition of alternative medicines – Ayurveda, Yoga, Unani and Siddha and Homeopathy system (AYUSH) that is still in use today. It has also been estimated that 70- 80 % of people in developing countries are totally dependent on herbal drugs for their primary healthcare [20]. Traditional medicinal plants with strong antiviral activity have long been used to treat viral infection [21–22]. The beneficial medicinal effects of plant products typically result from the secondary metabolites present in the plants. A variety of phytochemicals derived from the plant like alkaloids, steroids, diterpenoid lactones, aliphatics, and glycosides have proved to induce antiviral effects in humans [23]. The world has started exploring traditional medicines for the treatment of viral diseases, which are comparatively more economical, easily available and bear fewer chances of side effects and toxicity [24].
Bioinformatics and systems biology approaches can aid to study the therapeutic potential of traditional medicinal plants, making drug development faster, cheaper, and safer [25]. Receptors and enzymes involved in various stages in the life cycle of the SARS-CoV–2 are being used as drug targets. Viral proteases have long been shown to be effective targets of antiviral therapies as seen in case of human immunodeficiency virus (HIV) and hepatitis C virus (HCV) infections. Proteases represent potential targets for the inhibition of viral replication, One of the best characterized drug targets among coronaviruses is the main protease (M\text{pro}, also called 3CL\text{pro}) [26]. The protein sequences of the SARS-CoV–2 Mpro and the SARS-CoV Mpro show 96% identity [27]. The functional importance of Mpro in the viral life cycle, together with the absence of closely related homologues in humans, identifies Mpro as an attractive target for antiviral drug design. Many of the protease inhibitors are being used in the treatment of COVID–19, e.g., lopinavir–ritonavir combinations [28–29]. Recent studies have also reported the importance of viral Spike (S) protein in receptor binding and shown that human angiotensin-converting enzyme 2 (ACE2) promotes entry of SARS-CoV–2 into the cells [30]. Spike proteins offer as excellent drug targets at the early infection stage. Angiotensin-converting enzyme 2 (ACE2) binds to the receptor-binding motif (RBM) in the receptor-binding domain (RBD) of S protein and functions as a receptor for SARS-CoV [31–32]. Many inhibitors have been proposed that block the binding of spike proteins protein to ACE2 receptor in the human host. Repurposing of drug Nafamostat mesylate (Fusan), used to treat acute pancreatitis, has showed inhibition of MERS-SARS entry in human cells [33]. Targeting RNA-dependent RNA-polymerase (RdRp), required for viral RNA replication is another good choice for the development of antiviral therapeutics. It has been targeted in the development of therapeutics for several viral infections, including the hepatitis C virus (HCV), the Zika virus (ZIKV), and coronaviruses (CoVs) [34–42].

India is a country rich with diverse repository of medicinal plants used for treatment of several human disorders. *Tinospora cordifolia* also known as *amrita, guduchi, shindilkodi, giloy,* is widely used in indigenous systems of Indian medicine [43]. The chemical constituents of *T. cordifolia* belong to different classes, namely terpenoids, alkanoids, glycosides, liganans, steroids among others [43–45]. The natural phytochemicals found in *Tinospora cordifolia* are known to show antiviral activity for many viral infections [46–48]. Curcumin, a component of turmeric (*Curcuma longa*) has been described to have several functions in preventing or treating diseases, including cancers and viral infections [49]. It has also been demonstrated that curcumin is an antiviral compound, with activity against diverse viruses such as dengue virus (serotype 2) [50], herpes simplex virus [51], human immunodeficiency virus [52], Zika and Chikungunya [53] among others. Curcumin and its analogues have proved to be useful as HIV–1 integrase inhibitor [51, 54]. *Aloe Barbadensis* (Aloe Vera) has been used medicinally for several thousands of years in many cultures; from ancient Egypt, Greece, and Rome to China and India. For centuries, the gel of Aloe vera has been used for healing and therapeutic purposes [55]. The biologically active components include anthraquinones, anthrones, chromanes, alkaloids, flavonoids, terpenes, minerals, carbohydrates and pyrans. An extract of Aloe Vera has been found to be effective against a broad range of viruses, especially causing the infections of the upper respiratory tract [56]. Anthraquinones isolated from Aloe vera has shown to inhibit herpes simplex virus–2 [57], hepatitis virus [58], Influenza virus [59] and Human Immunodeficiency virus HIV [60]. It has also been reported that consumption of A. vera might be helpful
to human immunovirus-infected individuals since it enhances the CD4 count and thereby improves the functioning of the immune system. [60]. Lignans are a class of natural products that possess diverse pharmacological properties and are known to be effective as antitumor, antioxidant, antibacterial and antiviral agents [61]. Milk thistle (Silybum marianum) is another medicinal plant that has been used for thousands of years as a remedy for a variety of ailments [62]. The major component of S. marianum fruit extract (silymarin) is a flavonoid lignan called silybin that has been used in Indian medicines for liver and gallbladder problems [63–64]. Many studies have also reported that silymarin is an effective antiviral treatment for hepatitis C virus (HCV) [65–66]. Withania somnifera (Ashwagandha) is one of the most important herbs of Ayurvedic system of Indian medicine [67]. Withaferin A (WA) is an active constituent of Withania somnifera that has been shown to have a broad range of medicinal properties including antiviral activity [68–69]. Withonalides are steroidal lactones present in ashwagandha possessing diverse pharmacological activities [70].

Based on the above observations, this work focused on the study of phytochemicals derived from the medicinal plants for their antiviral activity towards SARS-CoV–2. The targets were carefully identified that played a key role in the viral attachment to host cell, viral replication and viral synthesis. A library of 30 natural metabolites in medicinal plants included curcumin and its derivatives, chemical constituents of giloy (Tinospora cordifolia), Aloe Barbadensis, S. marianum, Withania somnifera and plant lignans were studied by docking to three targets Main protease, Spike S protein and RNA dependent RNA polymerase (RdRp) in SARS-CoV–2. In addition to the library of phytochemicals, we also included the currently used hydroxychloroquine along with other repurposed drugs as ligands for molecular docking.

**Methodology**

*Retrieval of Structures of Target Proteins*

The three-dimensional structures of protein targets from SARS-CoV–2 were retrieved from Protein Data Bank [71]. Targets chosen for this study included SARS-CoV–2 main protease (PDB ID: 6W63) [72], Spike receptor binding domain (PDB ID: 6M0J) [73] and RNA-dependent RNA-polymerase (RdRp) (PDB ID: 6M71) [74] crucial for the viral entry and replication.

*Ligand Preparation*

A library of phytochemicals in medicinal plants was compiled as ligands from the review of literature and their 2D structures were retrieved from PubChem database [75]. For the ligands whose structures were not available, the 2D structures were drawn using MarvinSketch [76]. The protein and ligand structures were prepared using the preparation wizard in Flare (module from CRESSET software) [77–78].

*Prediction of Binding site*

Binding site of the main protease was analyzed by using the information about the amino acid residues interacting with the known, co-crystallized ligand. The interface residues of the spike receptor binding
domain with ACE2 (Angiotensin converting enzyme 2) were selected for grid generation for spike protein. The binding site for RNA-dependent RNA-polymerase (RdRp) was predicted using CASTp web server [79].

**Protein-Ligand Molecular Docking**

Molecular docking of the ligand library composed of natural compounds and repurposed drugs currently in use for COVID–19 treatment was carried out with target proteins main protease, spike protein (S) and RNA-dependent RNA-polymerase (RdRp) using Flare module provided by CRESSET software. For the docking process, the targets were prepared and minimized, the grid box was defined according to the binding site information and the docking calculations were carried out in Cresset Flare software in normal mode and default settings.

**Results And Discussion**

Molecular docking of target proteins namely main protease, spike protein and RNA-dependent RNA-polymerase from SARS-CoV–2 was performed with ligands using Flare docking protocol that use the polarizable extended electron distribution (XED) force field [78]. A library of ligands comprising of 30 phytochemicals from medicinal plants *Tinospora cordifolia*, *Aloe Barbadensis*, *Withania somnifera*, *Silybum marianum* and plant lignans along with seven repurposed drugs including hydroxychloroquine currently in use for COVID–19 treatment (*Table 3*) were prepared for docking. The amino acid residues of target proteins considered for binding during molecular docking are enlisted in *Table 4*. It was observed that several phytochemicals screened showed significant binding to the target proteins of SARS-CoV–2 upon docking in comparison to hydroxycholoroquine and other antiviral drugs binding. Analysis of molecular docking revealed Silybin (flavonolignan), the major active component of silymarin (from *Silybum marianum*) to be the most promising inhibitor of the target proteins in SARS-CoV–2. Significant binding energy values of −11.928 kcal/mol with Silybin-main protease complex, −10.572 kcal/mol with Silybin-S spike glycoprotein complex and −11.499 kcal/mol with Silybin-RNA-dependent RNA-polymerase were estimated (*Figure 1*). To obtain deeper insight into interaction pattern of silybin with target proteins, protein-ligand interaction maps were plotted and key amino acid residues involved in interactions were identified. The interaction plot of main protease complexed with silybin highlighted Thr 25, Met 49, His 163 and Glu 166 as important residues involved in hydrogen bonding (*Figure 2a*). In the spike protein/ACE2-silybin docked complex, strong hydrogen bonds were formed by residues Lys 353, Arg 403, Tyr 453, Ser 494 and amino acid residue like His 34 was observed to be involved in aromatic-aromatic interaction with the ligand (*Figure 2b*). Stable hydrogen bonds were also seen in RdRp-silybin dock complex formed by residues Val 315, Thr 394, Arg 457, Asn 459 and Asn 628 (*Figure 2c*).

Withaferin A, a major pharmacological constituent from Ashwagandha (*Withania somnifera*) also displayed very strong binding with main protease having binding energy value of −11.242 kcal/mol. Analysis of interaction interface in the active site showed hydrogen bond formation of the compound with residues Cys 44 and Glu 166 in the main protease. Amino acid residues Glu 35 and Gln 42 in spike protein/ACE2 participated in hydrogen bond formation with Withaferin A which contributed to a binding
energy value of $-9.631$ kcal/mol. On the other hand, in RdRp-Withaferin A docked complex, amino acid residues Lys 621, Asp 623, Glu 811 and Ser 814 mediated hydrogen bond interactions with a total binding energy value of $-9.27$ kcal/mol. Glu 166 in the main protease was identified as a key residue facilitating strong interactions with ligands analyzed by docking. Withanolide A found in *Withania somnifera* showed significant interaction with main protease ($-10.292$ kcal/mol) and RdRp ($-9.668$ kcal/mol). The results also implicated that compounds like catechin (phenolic constituent from aloe vera plant) and phillygenin (lignan) showed significant interaction with protease (*Table 5*). In addition, cordioside, a phytochemical constituent in *Tinospora cordifolia*, interacted strongly with the main protease having binding energy of $-10.577$ kcal/mol. Furthermore, quercetin, a constituent of Aloe vera, displayed good binding with RNA-dependent RNA-polymerase with an energy value of $-9.131$ kcal/mol. The above results implicate the antiviral activity of medicinal plants that can aid in treatment of COVID–19 infection.

**Conclusion**

Highly pathogenic viruses such as SARS-Corona pose a significant threat to human health, yet in most cases, therapies to prevent or treat these diseases are lacking. The COVID–19 outbreak is an unprecedented global public health challenge and needs immediate intervention. With the current threat looming over the world, there is urgency to develop both effective diagnostics and newer therapeutics at an affordable cost with minimum or no side effects. Structural bioinformatics has emerged during the last thirty years as a powerful tool for rational drug discovery. In this context, three-dimensional structures of target proteins have played essential roles for the design and discovery of newer or alternative drugs. Traditional Indian medicinal plants have long been used for treatment of several diseases including antiviral therapeutics against several viruses. This study observed that phytochemicals in medicinal plants displayed better binding affinities than the synthetic repurposed drugs currently in use for treatment for COVID–19 infection. Among the phytochemicals screened, natural compounds derived from *Silybum marianum* (Silybin), *Withania somnifera* (Withaferin A), *Tinospora cordifolia* (Cordioside) and *Aloe Barbadensis* Catechin and Quercetin) exhibited higher binding energetics than the widely used hydroxychloroquine and other repurposed drugs used for treatment of COVID–19 infection. The proposed *in silico* studies hence suggest the medicinal plants extracts or as an herbal cocktail could serve as effective alternative therapeutics for treatment of COVID–19 affected patients. However this would require validation from clinical cases tested upon. In addition, many more such bioactive components from medicinal plants existing in the rich Indian biodiversity need to be further explored. This study could provide basis for alternative therapeutics against the invading biological pathogens including the currently threatening coronavirus SARS-CoV–2.

**Declarations**

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Conflict of interest

Authors declare no conflict of interest

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**Tables**

**Table 1**: List of crystal structures of SARS-CoV-2 proteins in PDB.
| PDB ID | Description                                                                                            |
|--------|--------------------------------------------------------------------------------------------------------|
| 6VWW   | Crystal Structure of NSP15 Endoribonuclease from SARS CoV-2.                                          |
| 6VXS   | Crystal Structure of ADP ribose phosphatase of NSP3 from SARS CoV-2                                    |
| 6VXX   | Structure of the SARS-CoV-2 spike glycoprotein (closed state)                                         |
| 6VYB   | SARS-CoV-2 spike ectodomain structure (open state)                                                     |
| 6W4B   | The crystal structure of Nsp9 RNA binding protein of SARS CoV-2                                        |
| 6W9C   | The crystal structure of papain-like protease of SARS CoV-2                                           |
| 5R8T   | PanDDA analysis group deposition of ground-state model of SARS-CoV-2 main protease screened against DSI poised (Enamine), Fraglites and Peplites (Newcastle university), Mini Frags (Astex), York 3D (York university), electrophile cysteine covalent (Weizman institute) fragment libraries |
| 6W41   | Crystal structure of SARS-CoV-2 receptor binding domain in complex with human antibody CR3022          |
| 5RE4   | Crystal Structure of SARS-CoV-2 main protease in complex with Z1129283193                              |
| 5RFJ   | Crystal Structure of SARS-CoV-2 main protease in complex with PCM-0103067                              |
| 5RFI   | Crystal Structure of SARS-CoV-2 main protease in complex with PCM-0102353                              |
| 5RFH   | Crystal Structure of SARS-CoV-2 main protease in complex with PCM-0102277                              |
| 5RFG   | Crystal Structure of SARS-CoV-2 main protease in complex with PCM-0102372                              |
| 5RFF   | Crystal Structure of SARS-CoV-2 main protease in complex with PCM-0102704                              |
| 5RFE   | Crystal Structure of SARS-CoV-2 main protease in complex with Z509756472                               |
| 5RFD   | Crystal Structure of SARS-CoV-2 main protease in complex with Z126932614                               |
| 5RFC   | Crystal Structure of SARS-CoV-2 main protease in complex with Z979145504                               |
| 5RFB   | Crystal Structure of SARS-CoV-2 main protease in complex with Z1271660837                              |
| 5RFA   | Crystal Structure of SARS-CoV-2 main protease in complex with Z2643472210                              |
| 5RF9   | Crystal Structure of SARS-CoV-2 main protease in complex with Z217038356                               |
| 5RF8   | Crystal Structure of SARS-CoV-2 main protease in complex with Z271004858                               |
| 5RF7   | Crystal Structure of SARS-CoV-2 main protease in complex with Z316425948_minor                          |
| 5RF6   | Crystal Structure of SARS-CoV-2 main protease in complex with Z1348371854                              |
| 5RF5   | Crystal Structure of SARS-CoV-2 main protease in complex with Z3241250482                              |
| 5RF4   | Crystal Structure of SARS-CoV-2 main protease in complex with Z1741982125                              |
| 5RF3   | Crystal Structure of SARS-CoV-2 main protease in complex with Z1741970824                              |
| 5RF2   | Crystal Structure of SARS-CoV-2 main protease in complex with Z1741969146                              |
| 5RF1   | Crystal Structure of SARS-CoV-2 main protease in complex with NCL-00023830                             |
| 5RF0   | Crystal Structure of SARS-CoV-2 main protease in complex with POB0073                                 |
| 5REZ   | Crystal Structure of SARS-CoV-2 main protease in complex with POB0129                                 |
| 5REY   | Crystal Structure of SARS-CoV-2 main protease in complex with PCM-0102911                              |
| 5REX   | Crystal Structure of SARS-CoV-2 main protease in complex with PCM-0102287                              |
| Code | Description |
|------|-------------|
| 5REW | Crystal Structure of SARS-CoV-2 main protease in complex with PCM-0102275 |
| 5REV | Crystal Structure of SARS-CoV-2 main protease in complex with PCM-0103072 |
| 5REU | Crystal Structure of SARS-CoV-2 main protease in complex with PCM-0102395 |
| 5RET | Crystal Structure of SARS-CoV-2 main protease in complex with PCM-0102269 |
| 5RES | Crystal Structure of SARS-CoV-2 main protease in complex with PCM-0102281 |
| 5RER | Crystal Structure of SARS-CoV-2 main protease in complex with PCM-0102615 |
| 5REP | Crystal Structure of SARS-CoV-2 main protease in complex with PCM-0102201 |
| 5REO | Crystal Structure of SARS-CoV-2 main protease in complex with PCM-0102578 |
| 5REN | Crystal Structure of SARS-CoV-2 main protease in complex with PCM-0102425 |
| 5REM | Crystal Structure of SARS-CoV-2 main protease in complex with PCM-0103016 |
| 5REL | Crystal Structure of SARS-CoV-2 main protease in complex with PCM-0102340 |
| 5REK | Crystal Structure of SARS-CoV-2 main protease in complex with PCM-0102327 |
| 5REJ | Crystal Structure of SARS-CoV-2 main protease in complex with PCM-0102241 |
| 5REI | Crystal Structure of SARS-CoV-2 main protease in complex with PCM-0102432 |
| 5REH | Crystal Structure of SARS-CoV-2 main protease in complex with PCM-0102301 |
| 5REG | Crystal Structure of SARS-CoV-2 main protease in complex with PCM-0102340 |
| 5REF | Crystal Structure of SARS-CoV-2 main protease in complex with PCM-0102237 |
| 5REE | Crystal Structure of SARS-CoV-2 main protease in complex with PCM-0102219 |
| 5RED | Crystal Structure of SARS-CoV-2 main protease in complex with PCM-0102179 |
| 5REC | Crystal Structure of SARS-CoV-2 main protease in complex with PCM-0102179 |
| 5REB | Crystal Structure of SARS-CoV-2 main protease in complex with PCM-0102241 |
| 5REA | Crystal Structure of SARS-CoV-2 main protease in complex with PCM-0102432 |
| 5RE9 | Crystal Structure of SARS-CoV-2 main protease in complex with PCM-0102301 |
| 5RE8 | Crystal Structure of SARS-CoV-2 main protease in complex with PCM-0102237 |
| 5RE7 | Crystal Structure of SARS-CoV-2 main protease in complex with PCM-0102219 |
| 5RE6 | Crystal Structure of SARS-CoV-2 main protease in complex with PCM-0102179 |
| 5RE5 | Crystal Structure of SARS-CoV-2 main protease in complex with PCM-0102179 |
| 5RFK | Crystal Structure of SARS-CoV-2 main protease in complex with PCM-0102575 |
| 5RFL | Crystal Structure of SARS-CoV-2 main protease in complex with PCM-0102389 |
| 5RFM | Crystal Structure of SARS-CoV-2 main protease in complex with PCM-0102539 |
| 5RFN | Crystal Structure of SARS-CoV-2 main protease in complex with PCM-0102868 |
| 5RFO | Crystal Structure of SARS-CoV-2 main protease in complex with PCM-0102972 |
| 5RFP | Crystal Structure of SARS-CoV-2 main protease in complex with PCM-0102190 |
| 5RFQ | Crystal Structure of SARS-CoV-2 main protease in complex with PCM-0102179 |
| 5RFR | Crystal Structure of SARS-CoV-2 main protease in complex with PCM-0102169 |
| 5RFS | Crystal Structure of SARS-CoV-2 main protease in complex with PCM-0102739 |
| 5RFT | Crystal Structure of SARS-CoV-2 main protease in complex with PCM-0102432 |
| 5RFU | Crystal Structure of SARS-CoV-2 main protease in complex with PCM-0102121 |
| ID | Description |
|----|-------------|
| 5RFV | Crystal Structure of SARS-CoV-2 main protease in complex with PCM-0102306 |
| 5RFU | Crystal Structure of SARS-CoV-2 main protease in complex with PCM-0102121 |
| 5RFT | Crystal Structure of SARS-CoV-2 main protease in complex with PCM-0102432 |
| 5RFS | Crystal Structure of SARS-CoV-2 main protease in complex with PCM-0102739 |
| 5RFR | Crystal Structure of SARS-CoV-2 main protease in complex with PCM-0102169 |
| 5RFQ | Crystal Structure of SARS-CoV-2 main protease in complex with PCM-0102179 |
| 5RFP | Crystal Structure of SARS-CoV-2 main protease in complex with PCM-0102190 |
| 5RFO | Crystal Structure of SARS-CoV-2 main protease in complex with PCM-0102972 |
| 5RFN | Crystal Structure of SARS-CoV-2 main protease in complex with PCM-0102868 |
| 5RFM | Crystal Structure of SARS-CoV-2 main protease in complex with PCM-0102539 |
| 5RFL | Crystal Structure of SARS-CoV-2 main protease in complex with PCM-0102389 |
| 5RFK | Crystal Structure of SARS-CoV-2 main protease in complex with PCM-0102575 |
| 6Y2F | Crystal structure (monoclinic form) of the complex resulting from the reaction between SARS-CoV-2 (2019-nCoV) main protease and tert-butyl (1-((S)-1-(((S)-4-(benzylamino)-3,4-dioxo-1-((S)-2-oxopyrrolidin-3-yl)butan-2-yl)amino)-3-cyclopropyl-1-oxopropan-2-yl)-2-oxo-1,2-dihydropyridin-3-yl)carbamate (alpha-ketoamide 13b) |
| 6Y2G | Crystal structure (orthorhombic form) of the complex resulting from the reaction between SARS-CoV-2 (2019-nCoV) main protease and tert-butyl (1-((S)-1-(((S)-4-(benzylamino)-3,4-dioxo-1-((S)-2-oxopyrrolidin-3-yl)butan-2-yl)amino)-3-cyclopropyl-1-oxopropan-2-yl)-2-oxo-1,2-dihydropyridin-3-yl)carbamate (alpha-ketoamide 13b) |
| 6VSB | Prefusion 2019-nCoV spike glycoprotein with a single receptor-binding domain |
| 6LVN | Structure of the 2019-nCoV HR2 Domain |
| 6M03 | The crystal structure of COVID-19 main protease in apo form |
| 6W63 | Structure of COVID-19 main protease bound to potent broad-spectrum non-covalent inhibitor X77 |
| 6M71 | 2019-nCoV RNA-dependent RNA polymerase in complex with cofactors |
| 5R80 | Crystal Structure of COVID-19 main protease in complex with Z18197050 |
| 6LU7 | The crystal structure of COVID-19 main protease in complex with an inhibitor N3 |
| 5R7Y | Crystal Structure of COVID-19 main protease in complex with Z45617795 |
| 5R7Z | Crystal Structure of COVID-19 main protease in complex with Z1220452176 |
| 5R81 | Crystal Structure of COVID-19 main protease in complex with Z1367324110 |
| 5R82 | Crystal Structure of COVID-19 main protease in complex with Z219104216 |
| 5R83 | Crystal Structure of COVID-19 main protease in complex with Z44592329 |
| 5R84 | Crystal Structure of COVID-19 main protease in complex with Z31792168 |
| 6VW1 | Structure of 2019-nCoV chimeric receptor-binding domain complexed with its
| PDBID | Description |
|-------|-------------|
| 6M0J  | Crystal structure of 2019-nCoV spike receptor-binding domain bound with ACE2 |
| 6M17  | The 2019-nCoV RBD/ACE2-B0AT1 complex |
| 6Y84  | SARS-CoV-2 main protease with unliganded active site (2019-nCoV, coronavirus disease 2019, COVID-19) |
| 6YB7  | SARS-CoV-2 main protease with unliganded active site (2019-nCoV, coronavirus disease 2019, COVID-19) |
| 6Y2E  | Crystal structure of the free enzyme of the SARS-CoV-2 (2019-nCoV) main protease |
| 6W75  | 1.95 Angstrom Resolution Crystal Structure of NSP10 - NSP16 Complex from SARS-CoV-2 |
| 6W61  | Crystal Structure of the methyltransferase-stimulatory factor complex of NSP16 and NSP10 from SARS CoV-2. |
| 6W4H  | 1.80 Angstrom Resolution Crystal Structure of NSP16 - NSP10 Complex from SARS-CoV-2 |
| 6M3M  | Crystal structure of SARS-CoV-2 nucleocapsid protein N-terminal RNA binding domain |
| 6VYO  | Crystal structure of RNA binding domain of nucleocapsid phosphoprotein from SARS coronavirus 2 |
| 6W9Q  | Peptide-bound SARS-CoV-2 Nsp9 RNA-replicase |
| 7BTF  | SARS-CoV-2 RNA-dependent RNA polymerase in complex with cofactors in reduced condition |
| 6Y13  | The N-terminal RNA-binding domain of the SARS-CoV-2 nucleocapsid phosphoprotein |

The list provides the PDBID with description of crystal structures of proteins in SARS-CoV-2 deposited in PDB (as on April 11, 2020)

**Table 2: Details of proteome of SARS-Cov-2**
| Protein Name           | Length | Mature Protein                                                                 | PDB Structure |
|------------------------|--------|-------------------------------------------------------------------------------|---------------|
| orf1ab polyprotein     | 7096   | NSP1 (Leader Protein)                                                         | NO            |
|                        |        |                                                                               |               |
|                        |        | NSP2                                                                          | NO            |
|                        |        |                                                                               |               |
|                        |        | NSP3 (papain-like proteinase, ADP ribose phosphatase)                         | YES           |
|                        |        |                                                                               |               |
|                        |        | NSP4                                                                          | NO            |
|                        |        |                                                                               |               |
|                        |        | NSP5A and NSP5B (3C-like proteinase)                                          | YES           |
|                        |        |                                                                               |               |
|                        |        | NSP6                                                                          | NO            |
|                        |        |                                                                               |               |
|                        |        | NSP7                                                                          | YES           |
|                        |        |                                                                               |               |
|                        |        | NSP8                                                                          | YES           |
|                        |        |                                                                               |               |
|                        |        | NSP9 (ssRNA-binding protein)                                                  | YES           |
|                        |        |                                                                               |               |
|                        |        | NSP10                                                                         | IN COMPLEX WITH NSP16 |
|                        |        |                                                                               |               |
|                        |        | NSP12 (RNA-dependent RNA polymerase)                                          | YES           |
|                        |        |                                                                               |               |
|                        |        | NSP13 (helicase)                                                              | NO            |
|                        |        |                                                                               |               |
|                        |        | NSP14 (exonuclease)                                                           | NO            |
|                        |        |                                                                               |               |
|                        |        | NSP15 (endoRNAse)                                                             | NO            |
|                        |        |                                                                               |               |
|                        |        | NSP16 (2'-O-ribose methyltransferase)                                         | IN COMPLEX WITH NSP10 |
|                        |        |                                                                               |               |
| orf1a polyprotein      | 4405   | NSP1 (Leader Protein)                                                         | NO            |
|                        |        |                                                                               |               |
|                        |        | NSP2                                                                          | NO            |
|                        |        |                                                                               |               |
|                        |        | NSP3 (papain-like proteinase, ADP ribose phosphatase)                         | YES           |
|                        |        |                                                                               |               |
|                        |        | NSP4                                                                          | NO            |
|                        |        |                                                                               |               |
|                        |        | NSP5A and NSP5B (3C-like proteinase)                                          | YES           |
|                        |        |                                                                               |               |
|                        |        | NSP6                                                                          | NO            |
|                        |        |                                                                               |               |
|                        |        | NSP7                                                                          | YES           |
|                        |        |                                                                               |               |
|                        |        | NSP8                                                                          | YES           |
|                        |        |                                                                               |               |
|                        |        | NSP9 (ssRNA-binding protein)                                                  | YES           |
|                        |        |                                                                               |               |
|                        |        | NSP10                                                                         | IN COMPLEX WITH NSP16 |
|                        |        |                                                                               |               |
|                        |        | NSP11                                                                         | NO            |
|                        |        |                                                                               |               |
| Surface glycoprotein   | 1273   | Surface (spike) glycoprotein                                                  | YES           |
| ORF3a protein          | 275    | ORF3a protein                                                                 | NO            |
| Envelope protein       | 75     | Envelope protein                                                              | NO            |
| Membrane glycoprotein  | 222    | Membrane glycoprotein                                                         | NO            |
| ORF6 protein           | 61     | ORF6 protein                                                                  | NO            |
| ORF7a protein          | 121    | ORF7a protein                                                                 | NO            |
| ORF7b                  | 43     | ORF7b                                                                         | NO            |
| ORF8 protein           | 121    | ORF8 protein                                                                  | NO            |
The list provides proteins details of SARS-CoV-2 as obtained from GenBank: NC_045512.2

| Nucleocapsid phosphoprotein | 419 | Nucleocapsid phosphoprotein | YES |
|-----------------------------|-----|-----------------------------|-----|
| ORF10 protein               | 38  | ORF10 protein               | NO  |

**Table 3: List of ligands used for docking studies**
The list provides the names of ligands with 2D structures screened by molecular docking with target proteins in SARS-CoV-2. The ligands 1-30 include phytochemicals compiled from medicinal plants and the ligands 31-37 are the repurposed drugs (with 2D structures) currently in use for treatment of COVID-19 infection.
| S.No. | Ligands         | 2D structure |
|-------|----------------|--------------|
| 1.    | Curcumin       | ![Curcumin](image1) |
| 2.    | Bisdemethoxycurcumin | ![Bisdemethoxycurcumin](image2) |
| 3.    | Demethoxycurcumin | ![Demethoxycurcumin](image3) |
| 4.    | Tetrahydrocurcumin | ![Tetrahydrocurcumin](image4) |
| 5.    | Corydine       | ![Corydine](image5) |
|   | 6. Cordioside | 7. Cordiofolioside A | 8. Tinosporin | 9. Aloe-emodin | 10. Aloin |
|---|---------------|---------------------|--------------|---------------|---------|
|   | ![Cordioside](image1) | ![Cordiofolioside A](image2) | ![Tinosporin](image3) | ![Aloe-emodin](image4) | ![Aloin](image5) |
|   | Chemical Name   | Molecular Structure |
|---|----------------|---------------------|
|11.| Chrysophanol    | ![Chrysophanol](image) |
|12.| Catechin        | ![Catechin](image)  |
|13.| Aloin A         | ![Aloin A](image)   |
|14.| Isoaloresin     | ![Isoaloresin](image) |
|15.| Quercetin       | ![Quercetin](image) |
16. 6-deoxyglucose-diphyllin

17. Arctigenin

18. Bicyclol

19. Diphyllin

20. Hinokinin
| No. | Compound                      |
|-----|-------------------------------|
| 21. | Niranthin                     |
| 22. | Nordihydroguaiaretic acid     |
| 23. | Phillygenin                   |
| 24. | Rubrifloralignin A            |
|   |   |
|---|---|
| 25. | Schisandrin |
| 26. | Silybin |
| 27. | Terameprocol |
| 28. | Yatein |
|   |   |
|---|---|
| 29. | Withaferin A |
| 30. | Withanolide A |
|   | Repurposed Drugs |
| 31. | Arbidol |
| 32. | Chloroquine |
| 33. | Hydroxychloroquine |
|   | Name       | Structure                           |
|---|------------|-------------------------------------|
| 34. | Lopinavir  | ![Lopinavir Structure](image)       |
| 35. | Remdesivir | ![Remdesivir Structure](image)      |
| 36. | Ribavirin  | ![Ribavirin Structure](image)       |
| 37. | Ritonavir  | ![Ritonavir Structure](image)       |
### Table 4: Amino acid residues in the binding site of Spike protein and RNA polymerase

The table lists the amino acid residues identified in the binding pocket of Spike protein and predicted by CASTp server for RNA polymerase.

| Target protein | Binding site residues                                                                 |
|----------------|----------------------------------------------------------------------------------------|
| Interface residues of spike receptor binding domain with ACE2 | LYS 417, VAL 445, GLY 446, GLY 447, TYR 449, TYR 453, LEU 455, PHE 456, TYR 473, ALA 475, GLY 476, GLU 484, PHE 486, ASN 487, TYR 489, GLN 493, GLY 496, GLN 498, THR 500, ASN 501, GLY 502, TYR 505 |
| RNA-dependent RNA-polymerase | ASP 164, VAL 166, GLU 167, LYS 438, HIS 439, PHE 441, ASP 452, TYR 455, TYR 456, ILE 494, ASN 496, ASN 497, ASP 499, LYS 500, SER 501, GLY 503, ASN 507, LYS 511, THR 540, GLN 541, MET 542, ASN 543, LEU 544, LYS 545, TYR 546, ALA 547, ILE 548, SER 549, ALA 550, LYS 551, ARG 553, ALA 554, ARG 555, THR 556, VAL 557, ALA 558, GLY 559, THR 565, ASN 568, ARG 569, HIS 572, GLN 573, LEU 576, LYS 577, ALA 580, VAL 588, ILE 589, GLY 590, THR 591, SER 592, LYS 593, PHE 594, TRP 598, MET 601, LEU 602, GLY 616, TRP 617, ASP 618, TYR 619, PRO 620, LYS 621, CYS 622, ASP 623, ARG 624, GLU 665, VAL 667, LYS 676, THR 680, SER 681, SER 682, GLY 683, ASP 684, ALA 685, THR 686, THR 687, ALA 688, TYR 689, ASN 691, MET 756, LEU 758, SER 759, ASP 760, ASP 761, ALA 762, VAL 763, VAL 792, PHE 793, SER 795, ALA 797, LYS 798, CYS 799, TRP 800, HIS 810, GLU 811, PHE 812, CYR 813, SER 814, GLN 815, HIS 816, ASP 833, SER 835, ARG 836, ILE 837, ALA 840, GLY 841, PHE 843, VAL 844, ASP 845, LEU 854, MET 855, GLU 857, ARG 858, PHE 859, SER 861, LEU 862, ILE 864, ASP 865 |

### Table 5: Molecular docking of ligands (phytochemicals and repurposed drugs) with target proteins of SARS-CoV-2 using FLARE.

The table shows the binding energy values (in kcal/mol) of ligands (phytochemicals) docked to all three target proteins, namely main protease, Spike glycoprotein and RNA polymerase in SARS-CoV-2. The repurposed drugs were docked to their respective drug targets as shown.
highlighted values in the table denote the higher binding energy values of phytochemical-docked complexes in comparison to repurposed drug-target complexes.

| Ligands                          | Protease (PDB ID: 6W63) | Spike glycoprotein - ACE2 (PDB ID: 6M0J) | RNA-dependent RNA-polymerase (PDB ID: 6M71) |
|----------------------------------|--------------------------|------------------------------------------|---------------------------------------------|
| Curcumin and its derivatives     |                          |                                          |                                             |
| Curcumin                         | -8.104                   | -6.278                                   | -7.556                                      |
| Bisdemethoxy-curcumin            | -8.424                   | -8.436                                   | -7.698                                      |
| Demethoxycurcumin               | -8.611                   | -8.642                                   | -7.565                                      |
| Tetrahydrocurcumin              | -8.793                   | -8.009                                   | -7.518                                      |
| Constituents of *Tinospora cordifolia* |                  |                                          |                                             |
| Cordyline                        | -7.91                    | -6.041                                   | -6.942                                      |
| Cordioside                       | -10.577                  | -8.77                                    | -8.142                                      |
| Cordiofolioside A               | -8.02                    | -6.964                                   | -7.657                                      |
| Tinosporin                       | -7.924                   | -7.288                                   | -7.553                                      |
| Constituents of *Aloe Vera*      |                          |                                          |                                             |
| Aloe-emodin                      | -7.784                   | -5.888                                   | -7.283                                      |
| Aloin                            | -9.185                   | -8.383                                   | -8.889                                      |
| Chrysophanol                     | -7.322                   | -6.153                                   | -6.895                                      |
| Catechin                         | -9.582                   | -9.243                                   | -7.264                                      |
| Aloin A                          | -9.533                   | -8.432                                   | -8.761                                      |
| Isoaloresin                      | -9.759                   | -7.835                                   | -8.492                                      |
| Quercetin                        | -7.78                    | -8.664                                   | -9.131                                      |
| Constituents of *Silymarin*      |                          |                                          |                                             |
| Silybin                          | -11.928                  | -10.572                                  | -11.499                                     |
| Ashwagandha (*Withania somnifera*) |                        |                                          |                                             |
| Withaferin A                     | -11.242                  | -9.631                                   | -9.27                                       |
| Withanolide A                    | -10.292                  | -8.708                                   | -9.668                                      |
| Lignans                          |                          |                                          |                                             |
| 6-deoxyglucose-diphyllin         | -8.495                   | -7.241                                   | -7.487                                      |
| Arctigenin                       | -8.456                   | -8.055                                   | -7.766                                      |
| Bicyclol                         | -7.906                   | -7.042                                   | -7.464                                      |
| Diphyllin                        | -8.508                   | -7.917                                   | -7.339                                      |
| Hinokinin                        | -7.665                   | -7.106                                   | -7.025                                      |
| Niranthin                        | -9.333                   | -8.463                                   | -7.287                                      |
| Nordihydroguaiaretic acid        | -9.018                   | -7.696                                   | -8.39                                       |
| Phillygenin                      | -9.503                   | -9.102                                   | -7.807                                      |
| Rubrifloralignin A              | -9.236                   | -7.868                                   | -8.221                                      |
| Schisandrin                      | -9.056                   | -7.189                                   | -8.386                                      |
| Terameprocol                     | -7.778                   | -7.203                                   | -7.363                                      |
| Yatein                           | -8.236                   | -7.373                                   | -6.939                                      |
| Repurposed Drugs currently used in treating COVID-19 | | | |
| Arbidol                          | NA                       | -8.913                                   | NA                                          |
| Chloroquine                      | NA                       | -8.019                                   | NA                                          |
| Hydroxychloroquine               | NA                       | -7.862                                   | NA                                          |
| Lopinavir                        | -9.71                    | NA                                       | NA                                          |
| Remdesivir                       | NA                       | NA                                       | -6.02                                       |
| Ribavirin                        | NA                       | NA                                       | -5.229                                      |
| Ritonavir                        | -9.135                   | NA                                       | NA                                          |
Figure 1

The figure shows Silybin (green) and Withaferin A (yellow CPK model) docked to a) main protease (PDB ID: 6W63), b) Spike glycoprotein-ACE2 (PDB ID: 6M0J) and c) RNA-dependent RNA-polymerase (PDB ID: 6M71)
Figure 2

The figure depicts interactions of silybin (ball and stick model) with a) SARS-CoV-2 main protease (PDB ID: 6W63), b) Spike glycoprotein-ACE2 (PDB ID: 6M0J) in SARS-CoV-2 and c) RNA-dependent RNA-polymerase (PDB ID: 6M71) (Green- hydrogen bonds; purple- aromatic-aromatic interactions) in SARS-CoV-2