Shortlisting Phytochemicals Exhibiting Inhibitory Activity against Major Proteins of SARS-CoV-2 through Virtual Screening

Saranya Nallusamy
Tamil Nadu Agricultural University
Jayakanthan Mannu
Tamil Nadu Agricultural University
Caroline Ravikumar
Tamil Nadu Agricultural University
Kandavelmani Angamuthu
Tamil Nadu Agricultural University
Bharathi Nathan
Tamil Nadu Agricultural University
Kumaravadivel Nachimuthu
Tamil Nadu Agricultural University
Gnanam Ramasamy
Tamil Nadu Agricultural University
Raveendran Muthurajan
Tamil Nadu Agricultural University
Mohankumar Subbarayalu (smktnau@gmail.com)
Tamil Nadu Agricultural University
Kumar Neelakandan
Tamil Nadu Agricultural University

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Abstract

Severe Acute Respiratory Syndrome Corona Virus 2 (SARS-CoV-2) declared as a pandemic by WHO that has affected more than 40 lakh peoples and caused death of more than 2 lakh individuals across the globe. Limited availability of genomic information of SARS-CoV-2 and non-availability of vaccines and effective drugs are major problems responsible for the ineffective control and management of this pandemic. Several attempts have been made to explore repurposing existing drugs known for their anti-viral activities, and test the traditional herbal medicines known for their health benefiting and immune boosting activity against SARS-CoV-2.In this study, efforts were made to examine the potential of 721 phytochemicals of 37 plant species in inhibiting major protein targets namely, spike glycoprotein, main protease (M^{Pro}), NSP3, NSP9, NSP15, NSP10-NSP16 and RNA dependent RNA polymerase of SARS-CoV-2 through virtual screening approach. Results of our experiments revealed that SARS-CoV-2 M^{Pro} shared significant dissimilarities against SARS-CoVM^{Pro} and MERS-CoVM^{Pro} indicating the need for discovering novel drugs. This study has identified the phytochemical cyanin (*Zingiber officinale*) exhibiting broad spectrum inhibitory activity against main proteases of all the three Coronaviruses. Amentoflavone, agathisflavone, catechin-7-o-gallate and chlorogeninwere shown to exhibit multi target inhibitory activity. This study has identified *Mangifera indica, Anacardium occidentale, Vitex negundo, Solanum nigrum, Pedalium murex, Terminalia chebula, Azadirachta indica, Cissus quadrangularis, Clerodendrum serratum and Ocimum basilicum* as potential sources of phytochemicals combating nCOVID-19. More interestingly, this study has generated evidences for the anti-viral properties of the traditional herbal formulation “Kabasura kudineer” recommended by AYUSH, a unit of Government of India. Testing of short listed phytochemicals through clinical trials will help in developing effective formulation for management of this pandemic disease. Genomic analysis of identified herbal plants will help in unravelling molecular complexity of therapeutic and anti-viral properties and will pave way for designing synthetic drugs.

Introduction

Novel Coronavirus disease (nCOVID-19) caused by SARS-CoV-2 virus has become a global threat and WHO has declared it as a pandemic [1]. nCOVID-19 is the third life threatening virus in the SARS family of viruses after SARS-CoV occurred during 2002-03 and MERS-CoV which occurred during 2012[2-5]. As of April 29, 2020, a total of 216,563 deaths have been reported globally due to nCOVID-19. It is named as a novel Coronavirus as it shares significant dissimilarity against other members of the SARS family of viruses viz., SARS-CoV (30%) and MERS-CoV (60%) [6]. Its unique genetic makeup has made it not responsive to available vaccines and drugs. Ineffectiveness of existing drugs and vaccines against nCOVID’19 is attributed to its unique genetic makeup which necessitated search for novel targets for vaccine development and drugs for effective prevention and treatment of nCOVID-19.

Exploding increase in the nCOVID’19 affected cases has brought this globe to a halt. Scientific community is trying to unravel genome complexity of nCOVID’19 for identifying novel targets for

development of vaccines, screen available anti-viral drugs for effective management and shortlisting effective botanicals for therapeutic interventions. This has resulted in the accumulation enormous genomic information of nCOVID'19 in the public domain (https://www.ncbi.nlm.nih.gov/genbank/sars-cov-2-seqs/). Genomic analysis of nCOVID'19 revealed that it is approximately 30 kb in size (NCBI Accession # NC_045512) and further investigations identified three key genes viz., 1) coronavirus main protease ($3CL^{pro}$)/papain-like protease (PL^{pro}); 2) RNA-dependent RNA polymerase (RdRp) and 3) spike glycoprotein (S protein) as potential targets for drug designing[7-9].

Screening of existing antiviral drugs including interferon α (IFN-α), lopinavir/ritonavir, chloroquine phosphate, ribavirin, chloroquine, hydroxychloroquine and arbidol is in progress and many of these experiments require pre-clinical and clinical validation [7]. Non availability of vaccines and ineffectiveness of existing anti-viral drugs have made the doctors to resort using traditional medicines in nCOVID'19 treatments[8, 10]. Several attempts have been made to exploit the potential of several herbal products having potential to inhibit the main protease (Mpro)/chymotrypsin-like protease (3CLpro) using molecular modelling and docking studies [11-13]. SR et al. [7] made an attempt to screen 27 different ligands present in commonly used herbals of indian cuisines against SARS-CoV-2 Main protease and identified 15 different ligands effective in binding the viralprotease [10]. made a systematic review of herbal drugs used in the effective treatment of SARS-CoV and MERS-CoV and emphasized the urgent need for evolving procedures involving complementary and alternative treatments in managing nCOVID'19. Studies conducted so far have made attempts by using limited number ligands which may hinder discovery of effective viral inhibitor in the herbal gene pool. In this context, shortlisting potential herbal drugs effective against nCOVID'19 through *in silico* docking of globally available ligands and validating them through laboratory and clinical trials is one of the viable approaches in managing this pandemic. India is one of the richest biodiversity centers in the world and known for its vast repository of medicinal plants. Considering India's richest biodiversity of herbal medicinal plants and regular use of such medicinal plants in Indian health care system, the present study was undertaken to screen about 721 ligands including small molecules and phytochemicals from 37 different indian medicinal plants against 7 different protein targets of nCOVID'19 through molecular docking. Protein-Ligand interactions were analyzed carefully to shortlist potential small molecules and phytochemicals for drug development.

Materials And Methods

Phylogenetic analysis of main protease of nCOVID'19

Protein sequence of SARS-CoV-2 encoding for main protease was used for PSI BLAST (NCBI) [14] search to identify its homologs for understanding the evolutionary relationship with main proteases of other viruses. Multiple sequence alignment and phylogenetic analysis of SARS-CoV-2 main protease with other viral proteins was done using MAFFT server [15].

Virtual screening of herbal ligands against potential targets of nCOVID'19

Protein targets

Corona virus genome was reported to encode for 29 proteins, out of which main protease is considered to be an important drug target. ORF1ab of the coronavirus genome contains 15 polypeptide chains encoding for non-structural proteins (NS proteins). Other part of the genome encodes for envelope and coat proteins. Availability of X-ray crystal structures for most of the proteins in the coronavirus 2 genome facilitates virtual screening to search for potential inhibitors. We have performed molecular docking of 721 ligands against seven different target proteins of nCOVID'19 genome (Table 1). Inaddition, virtual screening was also performed against M^{Pro} of SARS CoV and MERS CoV with a view to identify inhibitors exhibiting inhibitory activity against main protease of all three viruses and inhibitors specific to nCOVID'19.

Ligand Library Preparation

Chemical structures of all the small molecules were retrieved from Dukes database [16], PubChem[17] and DrugBank [18] From the DrugBank database, Chemical structures of drugs approved for the treatment of respiratory diseases and compounds exhibiting antiviral activity were collected from DrugBank Database. Structures of phytochemicals belonging to 37 different herbals and spices used in South Indian Traditional Medicine were also used for virtual screening (Table 2). Known active ingredients of 8 herbal plants included in the Tamil traditional medicine "Kabasura Kudineer" (meaning water capable of boosting immunity) were also included in the screening. Overall, a total of 721 small molecules/ligands (Table S1) were used for virtual screening against 7 different protein targets.

Virtual Screening

Virtual screening was performed using Python Prescription Virtual Screening tool (PyRx 0.8) containing AutoDock Vina module [19]. Protein structure was prepared by using SWISS PDB Viewer by adding hydrogen atoms and energy minimization. Prepared proteinstructure was fed into the PyRx tool along with the structure of 721 ligands. Both the ligands and protein molecules were converted to pdbqt file using the AutoDock module of PyRx tool. Binding sites were predicted using CASTP server[20] and the same were used for setting grid (XYZ dimensions: 25*25*25) in the AutoDock Vina for virtual screening experiment with the exhaustiveness value of 8. Furthermore, phylogenetic analysis of SARS-CoV-2 $M^{Pro}$was carried out using PSI-BLAST (NCBI)[14] and MAFFT server [15]. Top 10 ligand hits against each of the 7 protein targets were taken for further analysis. 2D and 3D interactions between the protein-ligand were analysed using Schrodinger Maestro visualizer[21]. Properties of top 10 ligands against individual protein targets are given in Table S2.

Results And Discussion

Phylogenetic analysis on corona virus main proteases

Main protease (M^{pro}, also called $3CL^{pro}$) is considered as one of the important molecular targets for designing novel drugs against corona viruses[22]. With a view to design drugs/inhibitors specifically targeting main protease of nCoVID'19, *in-silico* analysis was performed using main protease sequences of SARS-CoV-2, SARS-CoV and MERS-CoV. Multiple sequence alignment identified 12 significant differences between main proteases of SARS CoV and SARS-CoV-2 (Fig. 1). Out of the 12 differences, S45 to A45 was found to reside within in the binding site of SARS-CoV-2 main protease. This may play a crucial role in determining differential binding affinity of the two proteases.

Phylogenetic analysis was performed using main protease sequences sharing >50 percentage similarity against SARS-CoV-2homolog revealed its significant genetic relatedness with main proteases of SARS CoV (96.08% similarity) and bat coronavirus (76.84% similarity) (Fig. 2). Next to this, it shared significant similarity with ORF1ab of Rousettus bat coronavirus. Main protease of nCoVID'19 shared only 50.65% similarity against main proteases of MERS-CoV. Above results clearly indicated the need for a highly specific novel drug specifically inhibiting main proteases of SARS-CoV-2.

Virtual screening of potential herbal ligands against major protein targets of SARS-CoV-2

Virtual screening of 721 ligands belonging to small molecules and active compounds from 37 medicinal herbs against 7 major protein targets of nCOVID'19 identified potential inhibitors. Information regarding the binding site residues predicted using CASTp server is provided in Table 3. Top 10 hits reported with higher binding affinity for each target protein is considered for downstream analysis (Table 4). Seven molecular targets of SARS-CoV-2 include, Main protease, RNA-dependent RNA polymerase (RdRp), NSP3, NSP9, NSP10-NSP16, NSP15 and Spike protein.

Small molecules/herbal compounds exhibiting significant inhibitory activity against Main Protease

Despite of significant structural (RMSD: 0.71 Å) and binding site volume similarity between SARS-CoV and SARS-CoV-2, they showed differential binding affinity against different inhibitors (Table 1).Virtual screening of small molecules against M^{Pro} identified agathisflavone as the best inhibitor exhibiting the binding affinity value of -8.2 kcal/mol.

Out of 721 ligands screened against SARS-CoV main protease, a ligand namely rutin abundantly found in *Terminalia chebula*, *Azadirachta indica* and *Ocimum basilicum* exhibited highest binding affinity value of -9.0 kcal/mol. In case of MERS-CoV main protease, amentoflavone predominantly found in *Mangifera indica* and *Garcinia species* showed the maximum binding affinity value of -8.6kcal/mol. Interestingly, a cytotoxic biflavonoid agathisflavone found in cashew nut (Anacardium occidentale) was shown to exhibit significant binding affinity with -8.0 kcal/mol against the main protease of SARS-CoV-2. Agathisflavones have been reported for their cytotoxicity against malignant cell lines [23]. Agathisflavone is a biflavanoid derived from plant source and has been found to possess several biological activities[24]. Various studies have found that agathisflavone possesses antioxidant, anti-inflammatory, antiviral, antiparasitic, cytotoxic, neuroprotective, and hepatoprotective activities. It has also been suggested that agathisflavone could be used in the treatment of oxidative stress, inflammatory diseases, microbial

infection, hepatic and neurological diseases and cancer[25]. This compound was found to involve in the formation of 3 hydrogen bonds at ASP 187, PRO 52 and ARG 40. This was followed by Rubusic acid (*Pedalium murex*), solanocapsine (*Solanum nigrum*), chlorogenin (*Solanum torvum*), Lupeol (*Carica papaya* and *Azadirachta indica*), Cyanin (*Zingiber officinale*), 3-O-trans-cafeeoyltormentic acid, Luteolin-7-O-(6”-malonylglucoside) (*Vitex negundo*), Agnuside (*Vitex negundo*) and Luteolin 7-O-beta-D-glucoside (*Vitex negundo*) exhibiting significant binding affinity in the decreasing order against M^{Pro}.

The phytochemical cyanin found in *Zingiber officinale* was found to bind effectively against main proteases of all three coronaviruses (Fig. 3a, c and d) and placed in the top 10 hits list. It showed a binding affinity value of -8.3 kcal/mol, -8.2 kcal/moland -7.7 kcal/mol against SARS-CoV-2, SARS COV and MERS CoV main proteases respectively.

RNA-dependent RNA polymerase (RdRp):

RdRp is responsible for replication of COVID19 genome inside the host. Among the ligands tested, ivermectin (Fig. 3b) showed the higher binding affinity value of -9.4 kcal/mol through the hydrogen bonding at ASN 497 residue. Amentoflavone stood at second position with the binding affinity value of -9.3 kcal/mol. This was found to react at three amino acid residues namely ARG 553, THR 556 and ASN 691. Ligands viz., Corilagin (*Terminalia bellirica* and *Terminalia chebula*), Agasthisflavone, 3-O-trans-caffeoyltormentic acid, Arjungenin (*Terminalia chebula*), Crateogolic acid (*Syzygium aromaticum*), 3,8’-biapigenin, cyanin (*Zingiber officinale*) were found to exhibit significant binding affinity (< -8.0 kcal/mol).

Spike Protein

X-ray crystal structure of spike glycoprotein (PDB ID: 6M71) was used chosen for performing virtual screening. Virtual screening was performed by choosing ACE interacting region as the binding site (Fig. 4a). 1,8-Dichloro-9,10-diphenylanthracene-9,10-diolfrom *Carica papaya* was found to exhibit significant binding affinity against spike glycoprotein (-8.2 kcal/mol). GLY 496 residue was found to be involved in the formation of hydrogen bond with the 1, 8-Dichloro-9, 10-diphenylanthracene-9,10-diol. Earlier, leaf extracts of *Carica papaya* was reported to have significant effect in combating dengue virus infection [26] and its exact role in increasing platelet counts is not clear. 1,8-Dichloro-9,10-diphenylanthracene-9,10-diol was found buried in the binding site of spike glycoprotein exhibiting hydrophobic interactions with residues such as LEU 39, TYR 41, TYR 449, TYR 453, TYR 495, PHE 497 and TYR 505 (Fig. 3e). This was followed by other small molecules viz., agasthisflavone, amentoflavone, ivermectin, agnuside (*Vitex negundo*), taraxerol (*Cissus quadrangularis*) and nimbinene (*Azadirachta indica*) exhibiting significantly higher level of binding affinity towards spike protein of nCOVID’19.

Non-Structural Proteins (NSPs)

Apart from the four major structural proteins (S, E, M and N proteins), non-structural proteins namely NSP3 (cleavage of N-terminal replicase poly protein), NSP9 (ssRNA binding) NSP10-NSP16 (co-factor in

activating replicating enzyme) and NSP15 involved in the transcription and replication of nCoVID'19 can also serve as potential targets for containing the virus using inhibitory herbal molecules[27].

Virtual screening of small molecules against NSP3 identified amentoflavone (*Mangifera indica*) as the top scored ligand with binding affinity of -7.5 kcal/mol. In the decreasing order of binding affinity, luteolin 7-O-(6"-malonylglucoside) (*Vitex negundo*), rubusic acid(*Pedalium murex*), acteoside (*Clerodendrum serratum*), ivermectin, taraxerol acetate (*Cissus_quadrangularis*), catechin 7-O-gallate, luteolin 7-O-beta-D-glucoside (*Vitex negundo*), agathisflavone (*Anacardium occidentale*) and luteolin-7-o-beta-d-glucopyranoside (*Vitex negundo)*were found to be placed next to amentoflavone. It was observed that three inhibitorsfrom *Vitex negundo*were reported with the highest binding scores. *Vitex negungo* belongs to *Verbenaceae* family known for its effects for ailments like ophthalmia, deafness, indigestion, piles and jaundice [28]. Results of earlier experiments conducted by Wu et al. [29] also revealed similar findings of vitexin from Vitex *negundo* exhibiting significant binding affinity towards NSP3.

Another small molecule friedelin from *Vitex negundo and Acorus calamus* was also found to exhibit significant binding affinity of -9.6 kcal/mol against NSP9 (Table 4). Eventhough, many hydrophobic interactions were observed, no hydrogen bond interaction was found in the binding site of NSP9. It is very interesting to observe that five out of the top ten inhibitors are from a single plant source *Solanum nigrum.* As evidenced from other studies, *Solanum nigrum* is one of the traditionally known medicinal plant known for its use in treatment ofseizure, pain, ulcer, inflammation, diarrhea, eye infections, jaundice and oxidative stresses [30-32].

Virtual screening against NSP15 identified oleonolic acid and urosolic acid as molecules exhibiting highest binding affinity values of -9.2 kcal/mol. Oleonolic acid and urosoic acid are known for their anticancerous and anti-inflammatory activities [33-35]. Both the phytochemicals are reported to be enriched in *Ocimum basilicum* and *Ocimum tenuiflorum* (Table 4).This was followed by crategolic acid (Syzygium aromaticum), arjungenin (*Terminalia chebula*), hederagenin (*Nigella sativa*), triterpenoid (*Abutilon indicum),* beta-amyrin (*Cissus quadrangularis),* friedelin (*Vitexnegundo, Acoruscalamus),* catechin 7-O-gallate (Camellia sinensis) and arjunolic acid (*Terminalia chebula).*

In the case of NSP-NSP16 protein complex, interface of the complex (Fig. 4b) was chosen as the binding site for performing virtual screening. Compounds such as amentoflavone, 10-methoxycamptothecin, 3,8'-biapigenin, taraxerol acetate (*Cissus_quadrangularis)*, corilagin (*Terminalia bellirica* and *Terminalia chebula)*, lupeol acetate (*Pedalium murex*), emetine, chlorogenin (*Solanum torvum)* and spirostan-3-ol (*Solanum torvum)* were ranked among the top 10 molecules exhibiting highest binding affinity.

Molecules exhibiting inhibitory activity against multiple protein targets of nCOVID'19

Phytochemicals exhibiting inhibitory activity against multiple targets of viruses are expected to confer durable protection to the patients. This will be more beneficial in situations where the virus is developing mutations in one of the targets. Small molecules namely, amentoflavone, agathisflavone, catechin-o-gallate and chlorogenin exhibited significant binding affinity towards multiple targets of nCOVID'19.

Amentoflavone showed inhibitory activity against RdRp (-9.3 kcal/mol), NSP9 (-8.3 kcal/mol), NSP3 (-7.4 kcal/mol), NSP10-NSP16 (-8.5 kcal/mol) and spike glycoprotein (-8.2 kcal/mol). In all the interactions, the target - ligand binding affinity was greater than -8.0 kcal/mol except NSP3. Protein-ligand interactions exhibited by the small molecules amentovlavone and agathisflavone is shown in the Fig. 5a-e and Table S3. Amentoflavone is a naturally occurring biflavanoid reported to be found in more than 120 plants[35]. Many of these plants have been used in traditional medicine for several thousand years in different parts of the world. Several studies have reported that amentoflavone possess anti-inflammatory, anti-oxidative, anti-diabetic, anti-tumor, anti-viral and anti-fungal activities [35]. Evidences have been reported for amentoflavone exhibiting anti-senescence activity in the cardiovascular and central nervous system[36]. Further, Amentoflavone isolated from *Torreyanucifera*was demonstrated to possess inhibitory activity against SARS-CoV3CL^{Pro} [37].

Similarly, agathisflavone was found to exhibit significant interaction against four different protein targets viz., RNA-dependent RNA polymerase (-8.9kcal/mol), SARS-CoV-2 main protease (-8.2kcal/mol), spike glycoprotein (-8.2 kcal/mol) and NSP 3 (-6.6kcal/mol) (Fig. 5f-l, Table. S3). Catechin-o-gallate was also found to possess significant binding affinity towards spike glycoprotein (-7.3 kcal/mol), NSP3 (-6.6 kcal/mol), RNA-dependent RNA polymerase (-7.9 kcal/mol) and NSP15 (-8.5 kcal/mol). Chlorogenin from *Solanum torvumb*exhibited binding affinity value of -8.2 kcal/mol (NSP9), -7.7 kcal/mol (M^{Pro}) and -7.6 kcalmol (NSP16-NSP10) of SARS-CoV-2.

Fifteen different compounds viz., 3,8'-biapigenin, 3-O-trans-caffeoyltormentic acid, agnuside, arjungenin, corilagin, crategolic acid, cyanin, friedelin, luteolin 7-O-(6"-, malonylglucoside), N-methylsolasodine, rubusic acid, solanocapsine, spirostan-3-ol, taraxerol and taraxerol acetate were found to possess significant interactions with at least two protein targets of SARS-CoV-2 (Table 5).

Effect of FDA approved drugs on SARS-CoV-2 protein targets

Hydroxycholoroquine,chloroquine and ivermectin drugs were selected as positive controls as they were reported to possess anti-viral activity[38, 39]. Hydroxychloroquine was reported to show promising inhibitory activity against nCOVID-19 spike protein [40, 41]. Our results revealed that hydroxycholorquine and chloroquine showed less binding affinity against all the 7 targets of nCOVID-19 compared to ivermectin (Table 6). Ivermectin exhibited significant binding affinity value of -9.4 kcal/mol and -8.2 kcal/mol against RNA - dependent RNA polymerase (RdRp) and spike protein respectively (Fig. 6). Ivermection also exhibited significant binding affinity against NSP9 (-7.5kcal/mol) and spike glycoprotein (-8.2 kcal/mol) (Table S4).

Analysis on top reported plants with best ligand hits

Among the 721 phytochemicals originating from of 37 plant species, 36 (5% approx.) phytochemicals from 22 plants (Fig. 7) were found to be the best hits with higher binding affinities against all the seven targets (Table 5). Among those 27 plants, 6 plants were found to be the ingredients of a traditional siddha herbal formulation namely “Kabasura kudineer” recommended by AYUSH Board of Government of

India for boosting immunity. *Vitex negundo* was reported to possess 34 different phytochemicals included in this study. Out of the 34, five different compounds namely, luteolin 7-O-beta-D-glucoside, luteolin 7-O-(6"-malonylglucoside), agnuside, luteolin-7-o-beta-d-glucopyranoside and friedelin were found to exhibit significant binding affinity against 5 different protein targets of SARS-CoV-2 namely, spike glycoprotein, SARS-CoV-2 main protease, NSP3, NSP9, NSP15 in the SARS-CoV-2.Followed by *Vitex negundo,* plants such as *Solanum nigrum* and *Pedalium murex* were found to possess 5 different antiviral compounds.

Solanocapsine, Spirostan-3-ol, N-methylsolasodine, Diosgenin and Solasodine are the phytochemicals reported in Solanum nigrum which effectively inhibited three SARS-CoV-2 targets (SARS CoV2 M^{Pro}, NSP9 and NSP16-NSP10).

Pedalium murex reported Diosgenin (NSP9), Lupeol acetate (NSP16-NSP10), Phytosterol (NSP9), Urosolic acid (NSP15) and Rubusic acid (SARS CoV2 M^{pro}, NSP3) as SARS CoV2 inhibitors. It is noteworthy that maximum of six targets have been inhibited by the compounds from *Pedalium Murex.* Inspite of its role as antiulcerogenic, nephroprotective, hypolipidemic, aphrodisiac, antioxidant, antimicrobial and insecticidal activities, *Pedalium Murex* has been traditionally used in treating ailments like cough and cold as a regular practice[42].

Medicinal plants namely, *Azadirachta indica, Terminalia chebula, Cissus quadrangularis, Clerodendrum serratum* and *Ocimum basilicum* were found to contain 4 different inhibitors. These herbal plants may be the potential targets for future research towards developing herbal formulations against SARS-CoV-2. Intensive genomics and proteomics research may lead to identification of novel drugs against this pandemic disease.

Generation of improved knowledge and understanding biochemical and molecular basis of herbals used in traditional Ayurveda and siddha medicine will accelerate development of effective drugs in controlling emerging diseases. In this study, comparative analysis of main proteases of MERS-CoV, SARS-CoV and SARS-CoV-2 revealed significant differences between the three homologs which were confirmed by differential binding affinity exhibited by 721 phytochemicals against the three main proteases. Cyanin from *Zingiber officinale* exhibited significant inhibitory activity against main proteases of all the three viruses. Table S5 provides details regarding the 2D diagram and binding affinity of cyanin with M^{Pro} of three CoVs. Popular fruit trees mango *(Mangifera indica)* and cashew nut (*Anacardium occidentale*) rich in amentoflavone and agathisflavone showing significant inhibitory activity against multiple targets of SARS-CoV-2. *Vitex negundo, Solanum nigrum, Pedalium Murex, Terminalia chebula, Azadirachta indica, Cissus quadrangularis, Clerodendrum serratum* and *Ocimum basilicum* were also found to contain phytochemicals showing inhibitory activity against SARS-CoV-2 proteins. More interestingly, this study has picked up *Carica Papaya* exhibiting inhibitory activity against spike glycoprotein and M^{Pro} of SARS-CoV-2 which was known for its protective role against dengue virus in humans [26]. Overall, this study has shortlisted potential phytochemicals having inhibitory activity against SARS-CoV-2 which can be taken for further testing and drug formulation studies.

Declarations

Competing interests: The authors declare no competing interests.

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Tables

Table 1 Protein targets with their PDB ID and the binding site volume

S. No	PDB ID	Protein Name	Binding site volume
1	2GZ9	SARS-CoV M^{Pro}	328.045
2	5C3N	MERS-CoV M^{Pro}	317.710
3	5R81	SARS-CoV2 M^{Pro}	308.363
4	6M0J	Spike glycoprotein	8071.179 (Interface of ACE and spike protein)
5	6M71	RNA-dependent RNA polymerase	4977.808
6	6W4H	NSP16 - NSP10	316.761
7	6W02	NSP3	242.332
8	6W01	NSP15	212.386
9	6W4B	NSP9	121.103

Table 2 List of herbal plants from which phytochemicals were screened *in silico*

S. No	Plant Name	S. No	Plant Name
1.	*Abutilon indicum (Thuthi)*	1.	*Nigella sativa (Fennel)*
1.	*Acorus calamus (Vasambu)*	1.	*Ocimum basilicum (Thiruneetrupachai)*
1.	*Aegle marmelos (Vilvam)*	1.	*Ocimum tenuiflorum (Tulsi)*
1.	*Anacyclus pyrethrum (Akara)**	1.	*Pedalium murex (Peru nerinji)*
1.	*Andrographis paniculata (Nilavembu)**	1.	*Pepper nigrum (Pepper)*
1.	*Azadirachta indica (Neem)*	1.	*Phyllanthus niruri (Keelanelli)*
1.	*Brassica oleracea (Cabbage)*	1.	*Piper betle (Vetrilai)*
1.	*Carica papaya (Papaya)*	1.	*Piper longum (Thippili)**
1.	*Cissus_quadrangularis_L (Perandai)*	1.	*Plectranthus amboinicus (Karppuravalli)*

1.	*Clerodendrum serratum (Kanduparangi)**	1.	*Salvia officinalis (Sage)*
1.	*Cocosnucifera (Coocnut Leaf Extract)*	1.	*Solanum nigrum (Manathakkali)*
1.	*Coriandrum sativum (Coriander)*	1.	*Solanum torvum (sundakai)*
1.	*Curcuma longa (Turmeric)*	1.	*Solanum trilobatum (Thoothuvalai)**
1.	*Cyprus rotundus (Korai kilangu)**	1.	*Syzygium aromaticum (Lavangam)**
1.	*Glycyrrhiza glabra (Liquo rice)*	1.	*Terminalia bellirica (Thanthrikai)**
1.	*Hygrophila auriculata (Mulli ver*)*	1.	*Terminalia chebula (Kadukai)**
1.	*Ipomea carnea (Neiveli Kaatamanakku)*	1.	*Vitex negundo (Nochi)*
1.	*Justicia adhatoda (Aada thoda)*	1.	*Zingiber officinale (Chukku)**
1.	*Malus domestica(apple)*		

*-Kabasura kudineer- plant compounds

Table 3 Binding site residues of CoV protein targets used for virtual screening

Protein Name	Binding site residues
Main Protease (SARS-CoV)	25 THR, 26 THR, 27 LEU, 41 HIS, 44 CYS, 45 THR, 46 ALA, 49 MET, 140 PHE, 141 LEU, 142 ASN, 143 GLY, 144 SER, 145 CYS, 163 HIS, 164 HIS, 165 MET, 166 GLU, 167 LEU, 168, PRO, 172 HIS, 188 ARG, 189 GLN, 190 THR, 192 GLN
Main Protease (MERS-CoV)	1 SER ,25MET,26THR, 27LEU, 41HIS, 42VAL, 44CYS, 46ALA, 49LEU , 143PHE, 144LEU, 145CYS, 146GLY, 148CYS, 166HIS, 167GLN, 168MET, 169GLU, 170LEU, 171, ALA, 175HIS, 190ASP, 191LYS, 192GLN, 193VAL, 194HIS, 195GLN, 196VAL
Main Protease (SARS-CoV-2)	24THR, 25THR, 26THR, 27LEU, 41HIS, 44CYS, 45THR, 46 SER, 49 MET, 140 PHE, 141 LEU, 142 ASN, 143 GLY, 144 SER, 145 CYS, 163 HIS, 164 HIS, 165 MET, 166 GLU, 167 LEU, 168 PRO, 187 ASP, 188 ARG, 189 GLN, 190THR, 192 GLN
Spike glycoprotein	**Chain E** - 403ARG, 405ASP, 406GLU, 408ARG, 409GLN, 415THR, 416GLY, 417LYS, 420ASP, 421TYR, 449TYR, 453TYR, 456PHE, 493GLN, 494SER, 495TYR, 496GLY, 497PHE, 501ASN, 502GLY, 503VAL, 504GLY, 505TYR
RNA-dependent RNA	164 ASP, 166 VAL, 167GLU, 429PHE, 430LYS, 431GLU, 436 GLU, 437 LEU, 438LYS, 439HIS, 440PHE, 441PHE, 442 PHE, 452 ASP, 455 TYR, 456TYR, 494 ILE, 496 ASN, 497 ASN, 499 ASP, 500 LYS, 501 SER ,503 GLY, 507 ASN, 511LYS, 540THR, 541GLN, 542MET, 543ASN, 544LEU,

polymerase NSP 12	545LYS, 546TYR, 547ALA, 548ILE, 549SER, 550ALA, 551LYS, 553ARG, 554ALA, 555ARG, 556THR, 557VAL, 558ALA, 559GLY, 565THR, 568ASN, 569ARG, 572HIS, 573GLN, 576LEU, 577LYS, 580ALA, 588VAL, 589ILE, 590GLY, 591THR, 592SER, 593LYS, 594PHE, 598TRP, 601MET, 602LEU, 616GLY, 617TRP, 618ASP, 619TYR, 620PRO, 621LYS, 622CYS, 623ASP, 624ARG, 665GLU, 667VAL, 676LYS, 680THR, 681SER, 682SER, 683GLY, 684ASP, 685ALA, 686THR, 687THR, 688ALA, 689TYR, 691ASN, 756MET, 758LEU, 759SER, 760ASP, 761ASP, 762ALA, 763VAL, 792VAL, 793PHE, 795SER, 797ALA, 798LYS, 799CYS, 800TRP, 810HIS, 811GLU, 812PHE, 813CYS, 814SER, 815GLN, 816HIS, 833ASP, 835SER, 836ARG, 837ILE, 840ALA, 841GLY, 843PHE, 844VAL, 845ASP, 847ILE, 848VAL, 854LEU, 855MET, 857GLU,858ARG, 859PHE, 861SER, 862LEU, 864ILE, 865ASP , C Chain - 3MET, 4SER, 7LYS, 40LEU, 41LEU, 43LYS
NSP16 - NSP10	6841 ASN, 6844 LYS, 6845 TYR, 6867HIS, 6868PHE, 6869 GLY, 6870ALA, 6871GLY, 6872SER, 6873ASP, 6878PRO, 6879 GLY, 6896SER, 6897ASP, 6898LEU, 6899ASN, 6900 ASP, 6901PHE, 6911GLY, 6912ASP, 6913CYS, 6928ASP, 6929 MET, 6930 TYR, 6931 ASP, 6932 PRO, 6933LYS, 6947 PHE, 6968 LYS
NSP3	6 PHE, 7 SER, 8 GLY, 10 LEU, 11 LYS, 12 LEU,18 ILE, 19 LYS, 20 ASN, 158 LYS, 161 TYR, 162 ASP, 165 VAL, 168 PHE
NSP15	69 GLU, 71 LYS, 90 LYS, 196 THR, 198 SER, 199 ARG, 200 ASN, 201 LEU, 202 GLN, 252 LEU, 255 LEU, 259 PHE, 266LEU, 268 ASP, 272 MET, 273 ASP. 274 SER, 275 THR, 277 LYS, 279 TYR, 295 VAL, 296 ILE, 297 ASP
NSP9	13 MET, 33 TYR, 38 GLY, 39 GLY, 40 ARG, 42 VAL, 57PHE, 58 PRO, 59 LYS, 60 SER, 66 ILE, 68 THR

Table 4 Shortlisted natural compounds for inhibition against various targets of coronaviruses

Protein Name	Binding Affinity Kcal/mol	Compound name	Plant Name
SARS COV Main Protease	-9.0	Rutin	*Terminalia chebula, Azadirachta indica and Ocimum basilicum*
	-8.7	Quercetin 3 gentiobioside	*Solanum nigrum*
	-8.6	3-O-trans-caffeoyltormentic acid	antiviral compounds
	-8.5	Epicatechin 3 o gallate	*Camellia sinensis*
	-8.5	Corilagin	*Terminalia bellirica*, *Terminalia chebula*
	-8.4	Quercetin galactoside	antiviral compounds
	-8.3	Quercetrin	*Azadirachta indica*
	-8.3	Isoquercetin	*Ocimum basilicum,Terminalia chebula*
	-8.3	Acetoside	*Clerodendrum serratum*
	-8.2	Cyanin	*Zingiber officinale*
SARS COV2 Main Protease	-8.2	Agathisflavone	*Anacardium occidentale*
	-8.1	Rubusic acid	*Pedalium murex*
	-7.9	Solanocapsine	*Solanum nigrum*
	-7.7	Chlorogenin	*Solanum torvum*
	-7.7	Lupeol	*Carica papaya* and *Azadirachta indica*
	-7.7	Cyanin	*Zingiber officinale*

	-7.7	3-O-trans-caffeoyltormentic acid	antiviral compound
	-7.7	Luteolin 7-O-(6''-malonylglucoside)	*Vitex negundo*
	-7.6	Agnuside	*Vitex negundo*
	-7.6	Luteolin 7-O-beta-D-glucoside	*Vitex negundo*
MERS Main Protease (MERS)	-8.6	Amentoflavone	*Mangifera indica*
	-8.4	Corilagin	*Terminalia bellirica*, *Terminalia chebula*
	-8.3	Cyanin	*Zingiber officinale*
	-8.2	Rutin	*Terminalia chebula, Azadirachta indica and Ocimum basilicum*
	-8.2	Betulinic acid	*Vitex negundo*
	-8.1	3,8'-biapigenin	*antiviral compound*
	-8.0	Epicatechingallate	*Camellia sinensis*
	-8.0	Cyanidin 3-O-glucoside	*Zingiber officinale*
	-8.0	Vicenin-2	*Ocimum basilicum*
	-8.0	Quercetin 3 gentiobioside	*Solanum nigrum*
RNA-dependent RNA polymerase	-9.4	Ivermectin	FDA Approved Drug
	-9.3	Amentoflavone	*Mangiferaindica*
	-9.0	Corilagin	*Terminalia bellirica, Terminalia chebula*
	-8.9	Agathisflavone	*Anacardium occidentale*
	-8.6	3-O-trans-caffeoyltormentic acid	antiviral compound
	-8.6	Ivermectin	FDA Approved Drug
	-8.2	Arjungenin	*Terminalia chebula*
	-8.2	Crateogolic acid	*Syzygium aromaticum*
	-8.1	3,8'-biapigenin	antiviral compound
	-8.0	Cyanin	*Zingiberofficinale*
	-7.9	Catechin 7-O-gallate	*Camellia sinensis*
NSP9	-9.6	Friedelin	*Vitex negundo, Acoruscalamus*
	-9.3	N-methylsolasodine	*Solanum nigrum*
	-8.9	Solasodine	*Solanum nigrum, Solanum torvum*
	-8.9	Diosgenin	*Solanum nigrum, Pedalium murex*
	-8.6	Spirostan-3-ol	*Solanum nigrum*
	-8.6	Solanocapsine	*Solanum nigrum*
	-8.5	Taraxerol	*Cissus quadrangularis*
	-8.3	Amentoflavone	*Mangifera indica*
	-8.2	Chlorogenin	*Solanum torvum*
	-8.0	Campesterol	*Ocimumbasilicum, Solanum trilobatum,Clerodendrum serratum, Curcuma longa*
			Ocimum basilicum, Acorus calamus, Abutilon indicum, Solanum trilobatum, Terminalia bellirica, Cyprus rotundus, Clerodendrum serratum, Azadirachta indica, Cissus quadrangularis, Curcuma longa, Ipomoea carnea

	-8.0	Phytosterol	*Cissus quadrangularis, Curcuma longa, Ipomea carnea, Pedalium murex, Terminalia chebula*
NSP15	-9.2	Oleanolic acid	*Ocimum basilicum, Cyprus rotundus, Clerodendrum serratum, Ocimum tenuiflorum*
	-9.2	Ursolic-acid	*Ocimum basilicum, Pedalium murex, Malus domestica, Ocimum tenuiflorum*
	-8.9	Crategolic acid	*Syzygium aromaticum*
	-8.8	Arjungenin	*Terminalia chebula*
	-8.7	Hederagenin	*Nigella sativa*
	-8.6	Triterpenoid	*Abutilon indicum*
	-8.6	Beta-Amyrin	*Cissus quadrangularis*
	-8.6	Friedelin	*Vitex negundo, Acoruscalamus*
	-8.5	Catechin 7-O-gallate	*Camellia sinensis*
	-8.5	Arjunolic acid	*Terminalia chebula*
NSP3	-7.4	Amentoflavone	*Mangifera indica*
	-7.1	Luteolin 7-O-(6''-malonylglucoside)	*Vitex negundo*
	-6.8	Rubusic acid	*Pedalium murex*
	-6.7	Acteoside	*Clerodendrum serratum*
	-6.7	Ivermectin	FDA Approved Drug
	-6.7	Taraxerol acetate	*Cissus quadrangularis*
	-6.6	Catechin 7-O-gallate	antiviral compound
	-6.6	Luteolin 7-O-beta-D-glucoside	*Vitex negundo*
	-6.6	Agathisflavone	*Anacardium occidentale*
	-6.6	luteolin-7-o-beta-d-glucopyranoside	*Vitex negundo*
NSP10-NSP16	-8.5	Amentoflavone	*Mangifera indica*
	-8.3	10-methoxycamptothecin	antiviral compound
	-8.2	3,8'-biapigenin	antiviral compound
	-8.0	Taraxerol acetate	*Cissus quadrangularis*
	-7.8	Corilagin	*Terminalia bellirica,Terminalia chebula*
	-7.8	Lupeol acetate	*Pedalium murex*
	-7.6	Emetine	FDA Approved Drug
	-7.6	Chlorogenin	*Solanum torvum*
	-7.6	Spirostan-3-ol	*Solanum nigrum*
	-7.6	5,7-Dihydroxy-4'-methoxy-8,3'-di-C-prenylflavanone	*Azadirachta indica*
Spike Glycoprotein	-8.2	1,8-Dichloro-9,10-diphenylanthracene-9,10-diol	*Carica papaya*
	-8.2	Agathisflavone	*Anacardium occidentale*
	-8.2	Amentoflavone	*Mangifera indica*
	-8.2	Ivermectin	FDA Approved Drug
	-8.1	3 o caffeoyltormentic acid	antiviral compound
	-7.8	Ivermectin	FDA Approved Drug
	-7.5	Agnuside	*Vitex negundo*
	-7.4	Taraxerol	*Cissus quadrangularis*

	-7.1	Yuruxoflor	*Cissus quadrangularis*
	-7.3	Nimbinene	*Azadirachta indica*
	-7.3	Catechin 7 O gallate	antiviral compound

Table 5 Ligands screened as inhibitors of SARS COV2 targets

. No	Compound name	Plant Name	Protein targets
	Acetoside	*Clerodendrum serratum**	NSP3
	Agathisflavone	*Anacardium occidentale*	SARS CoV2 M^{pro} RdRp NSP3 Spike glycoprotein
	Agnuside	*Vitex negundo*	SARS CoV2 M^{pro} Spike glycoprotein
	Amentoflavone	*Mangifera indica*	RdRp NSP9 NSP3 NSP10-NSP16 Spike glycoprotein
	Arjungenin	*Terminalia chebula**	RdRp NSP15
	Arjunolic acid	*Terminalia chebula**	NSP15
	Beta-Amyrin	*Cissus quadrangularis*	NSP15
	Campesterol	*Ocimum basilicum, Solanum trilobatum*, Clerodendrum serratum*, Curcuma longa*	NSP9
	Catechin 7-O-gallate	*Camellia sinensis*	RdRp NSP15 NSP3 Spike glycoprotein
	Chlorogenin	*Solanum torvum*	SARS CoV2 M^{pro} NSP9 NSP10-NSP16
	Corilagin	*Terminalia bellirica*, Terminalia chebula**	RdRp NSP10-NSP16
	Crategolic acid	*Syzygium aromaticum**	NSP15 RdRp
	Cyanin	*Zingiber officinale**	SARS CoV2 M^{pro} RdRp

			RdRp
	Diosgenin	*Solanum nigrum, Pedalium murex*	NSP9
	Emetine	FDA Approved Drug	NSP10-NSP16
	Friedelin	*Vitex negundo, Acorus calamus*	NSP9 NSP15
	Hederagenin	*Nigella sativa*	NSP15
	Ivermectin	FDA Approved Drug	RdRp NSP3 Spike glycoprotein
	Lupeol	*Carica papaya* and *Azadirachta indica*	SARS CoV2 M^{pro}
	Lupeol acetate	*Pedalium murex*	NSP10-NSP16
	Luteolin 7-O-(6''-malonylglucoside)	*Vitex negundo*	SARS CoV2 M^{pro} NSP3
	Luteolin 7-O-beta-D-glucoside	*Vitex negundo*	SARS CoV2 M^{pro} NSP3
	Luteolin-7-o-beta-d-glucopyranoside	*Vitex negundo*	NSP3
	Nimbinene	*Azadirachtaindica*	Spike glycoprotein
	N-methylsolasodine	*Solanum nigrum*	NSP9
	Oleanolic acid	*Ocimum basilicum, Cyprus rotundus, Clerodendrum serratum*, Ocimum tenuiflorum*	NSP15
	Phytosterol	*Ocimum basilicum, Acorus calamus, Abutilon indicum, Solanum trilobatum*, Terminalia bellirica*, Cyprus rotundus, Clerodendrum serratum*, Azadirachta indica, Cissus_quadrangularis_L, Curcuma longa, Ipomeacarnea, Pedalium murex, Terminalia chebula**	NSP9
	Rubusic acid	*Pedalium murex*	SARS CoV2 M^{pro} NSP3
	Solanocapsine	*Solanum nigrum*	SARS CoV2 M^{pro} NSP9
	Solasodine	*Solanum nigrum, Solanum torvum*	NSP9
	Spirostan-3-ol	*Solanum nigrum*	NSP9 NSP10-NSP16
	Taraxerol	*Cissus quadrangularis*	NSP9 Spike glycoprotein
	Taraxerol acetate	*Cissus quadrangularis*	NSP3 NSP10-NSP16
	Triterpenoid	*Abutilon indicum*	NSP15
	Ursolic-acid	*Ocimum basilicum, Pedalium murex, Malus domestica, Ocimum tenuiflorum*	NSP15

1.	5,7-Dihydroxy-4'-methoxy-8,3'-di-C-prenylflavanone	*Azadirachta indica*	NSP10-NSP16
1.	1,8-Dichloro-9,10-diphenylanthracene-9,10-diol	*Carica papaya*	Spike glycoprotein
	3 O caffeoyltormentic acid	antiviral compound	Spike glycoprotein
	10-methoxycamptothecin	antiviral compound	NSP10-NSP16
	3,8'-biapigenin	antiviral compounds	RdRp NSP10-NSP16
	3-O-trans-caffeoyltormentic acid	antiviral compound	SARS CoV2 M^{pro} RdRp

*-Kabasura kudineer- plant compounds

Table 6 Binding affinity value of Hydroxychloroquine, Cholroquine and Ivermectin against SARS COV2 targets

SARS CoV2 **Protein Target**	**Hydroxychloroquine** Binding affinity (kcal/mol)	**Chloroquine** Binding affinity (kcal/mol)	**Ivermectin** Binding affinity (kcal/mol)
SARS CoV2 M^{Pro}	-5.5	-4.9	-7.3
RdRp	-5.6	-5.4	-9.4
NSP3	-4.5	-4.2	-6.7
NSP9	-5.7	-5.2	-7.5
NSP16 - NSP10	-5.7	-5.3	-1.9
NSP15	-5.5	-5.4	-6.4
Spike glycoprotein	-5.3	-5.2	-8.2

Figures

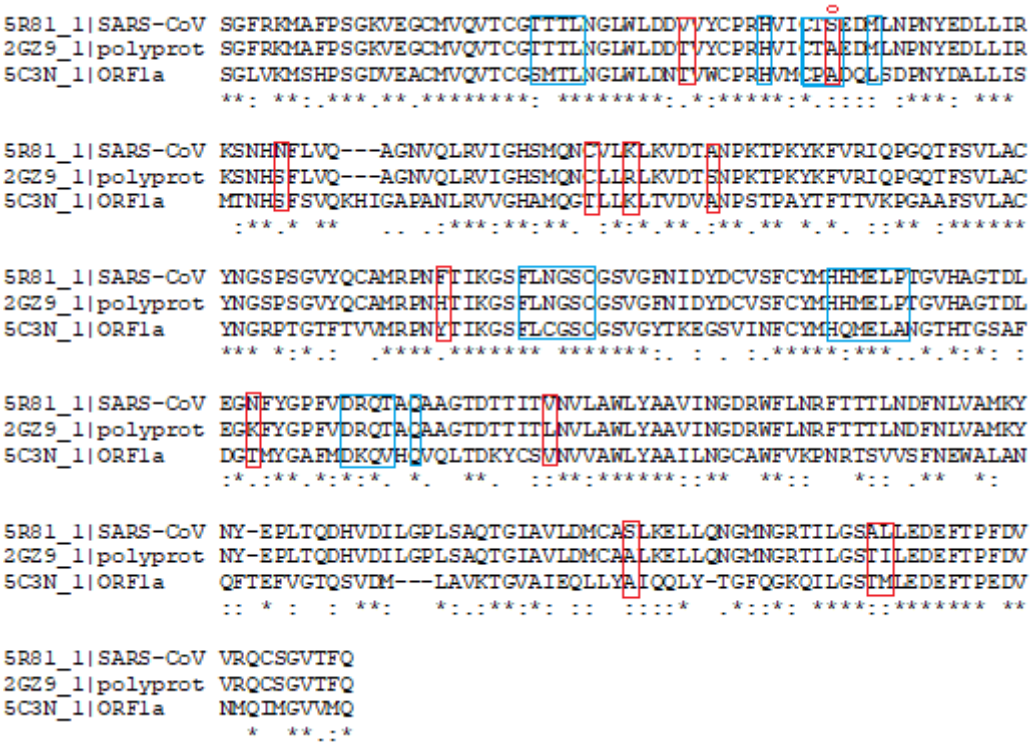

Figure 1

Multiple sequence alignment of SARS CoV2 (PDB ID: 58R1), SARS CoV (PDB ID: 2GZ9) and MERS CoV (PDB ID: 5C3N) main proteases. Binding site residues are marked with blue box. Red marked residues indicate the change observed between SARS CoV2 and SARS CoV. Serine to Alanine change at the 45th position observed in the binding site is marked with circle.

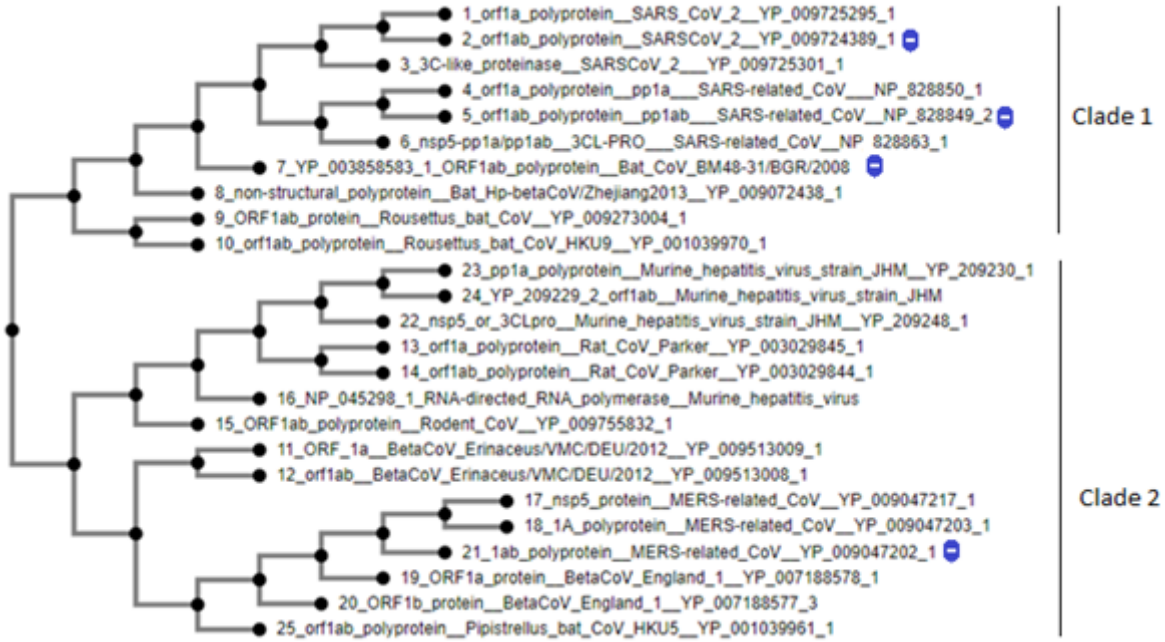

Figure 2

Phylogenetic analysis of SARS CoV2 MPRO with other CoV MPRO proteins

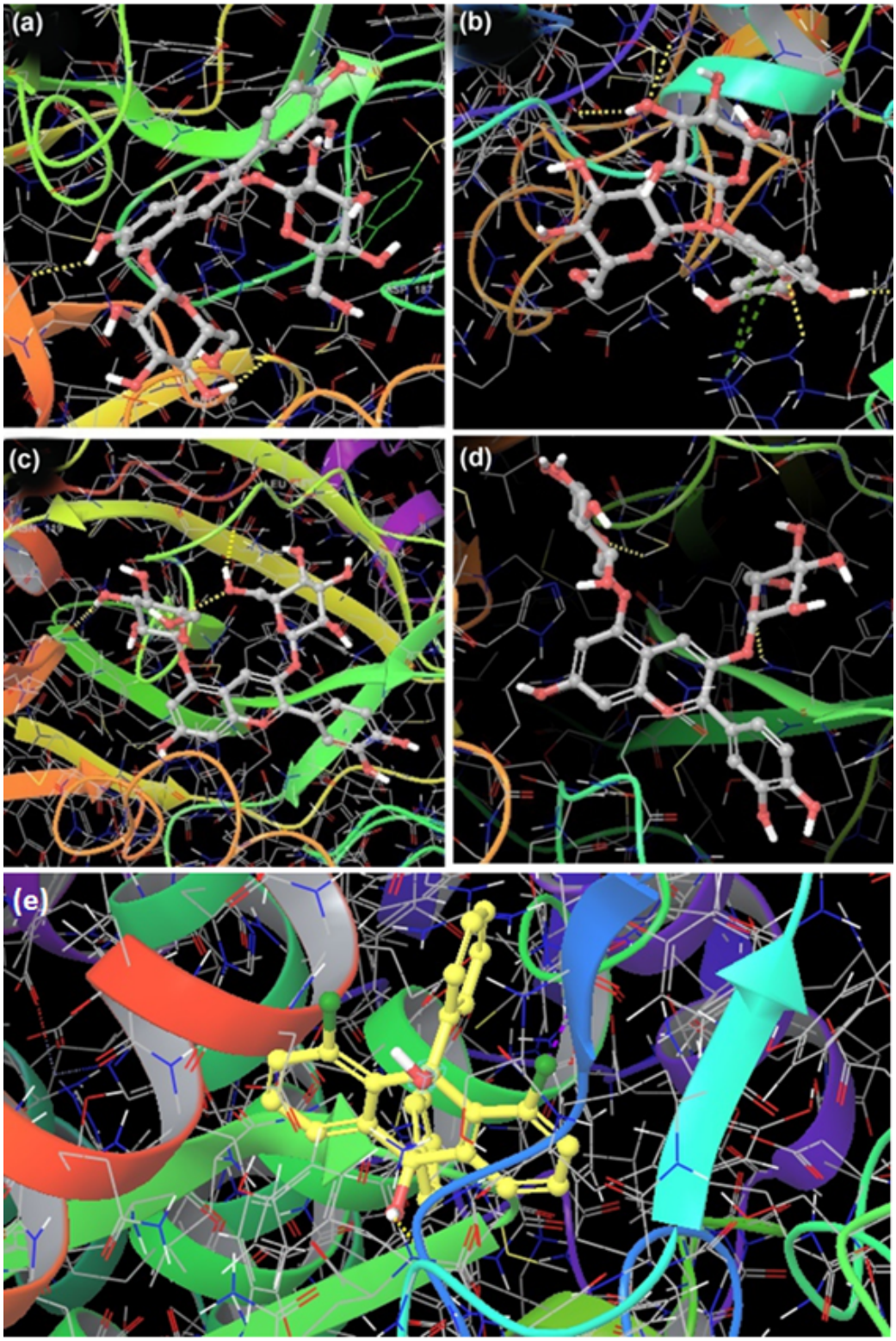

Figure 3

Complex structure of Cyanin with (a) SARS-CoV-2 MPro, (b) SARS-CoV-2 RdRp(c) SARS-CoVMPro (d) MERS-CoVMPro and (e) SARS-CoV-2 spike protein complex with 1,8-Dichloro-9,10-diphenylanthracene-9,10-diol.

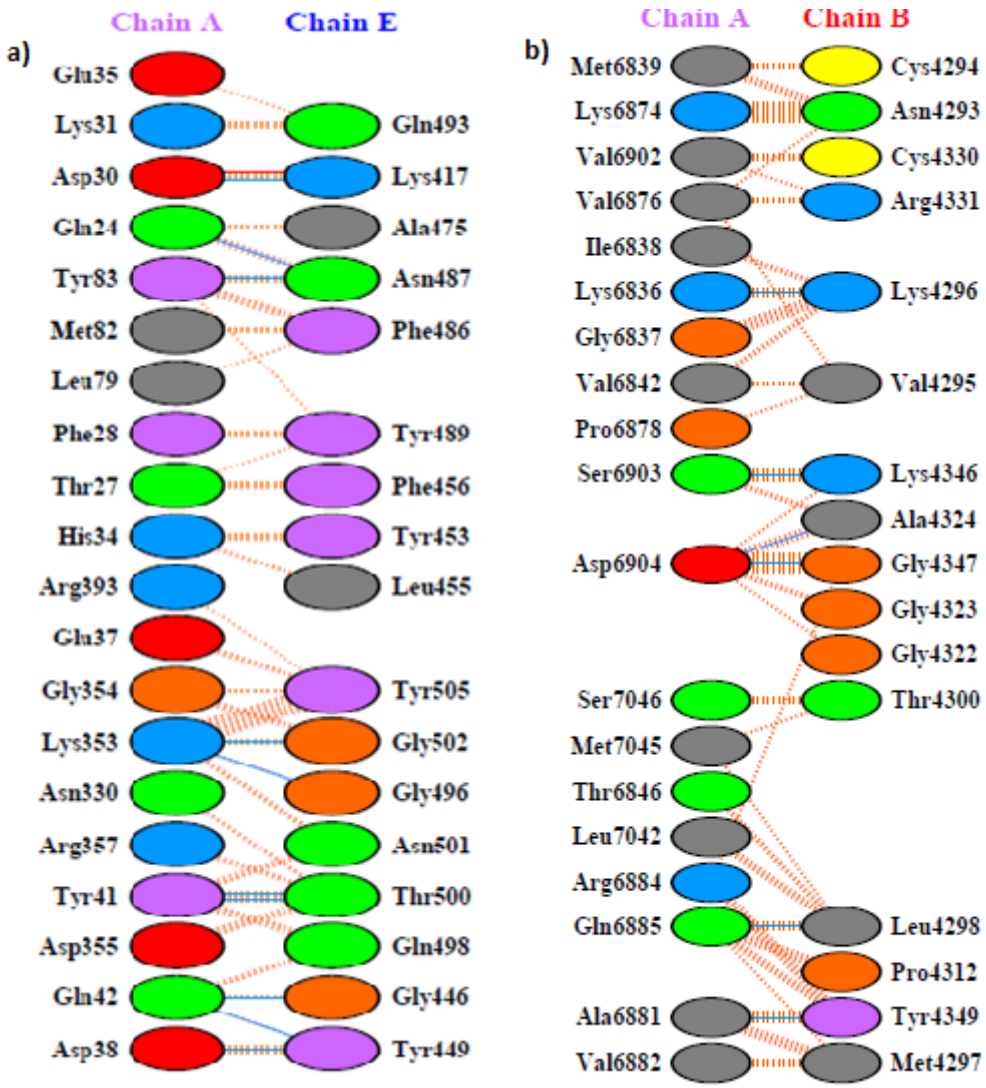

Figure 4

a) PDB ID: 6M0J - Residue interaction between ACE-2 (chain A) and spike glycoprotein (chain E). b) PDB ID: 6W4H - Interface of NSP10-NSP16 complex. Residues are colored based on their physiochemical properties.

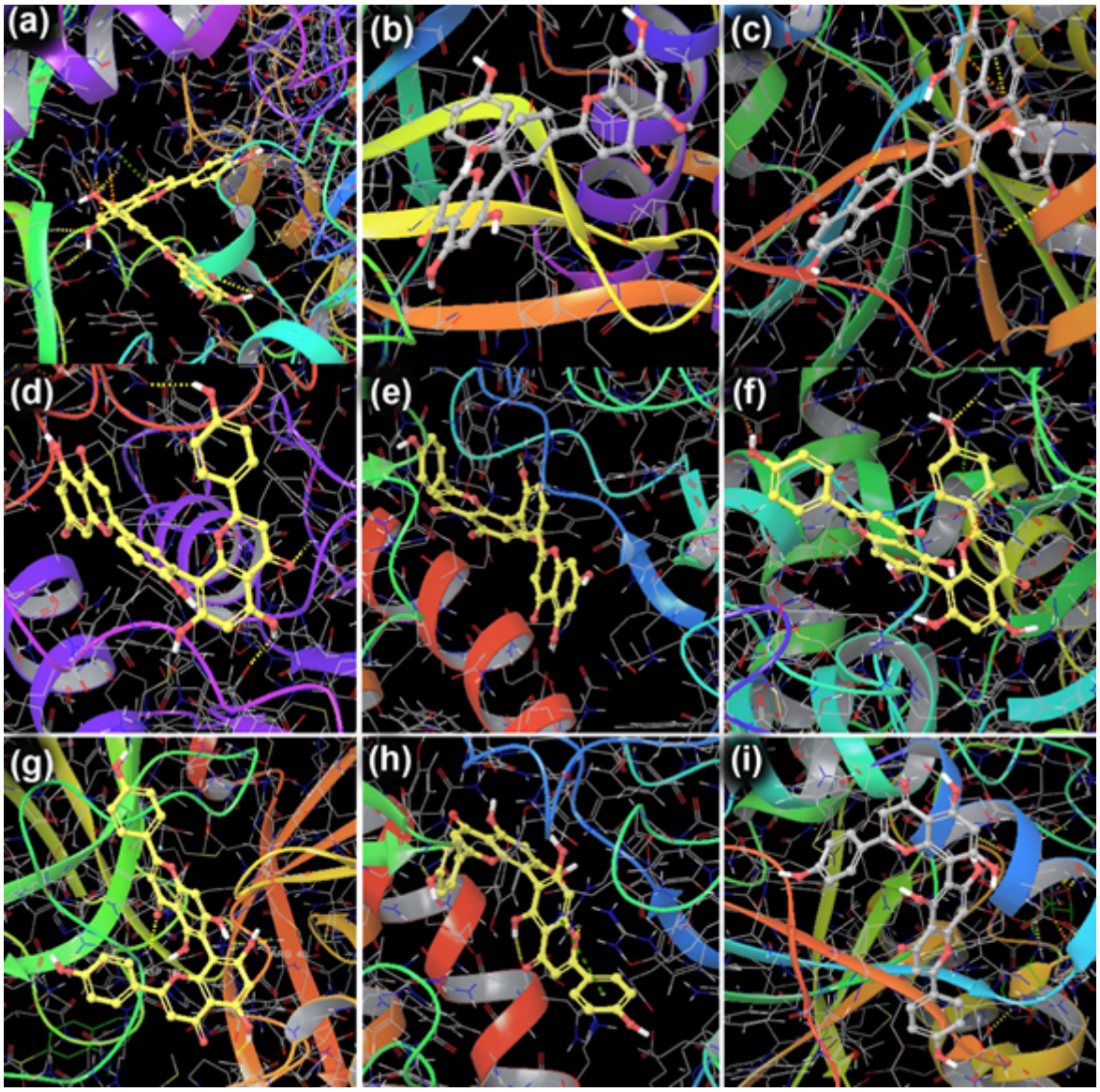

Figure 5

SARS-CoV-2 complex structure of amentoflavone with (a) RdRp, (b) NSP9, (c)NSP3, (d) NSP10-NSP16, (e) spike protein and agathisflavone with (f) RdRp, (g) MPro, (h) spike protein and (i) NSP3

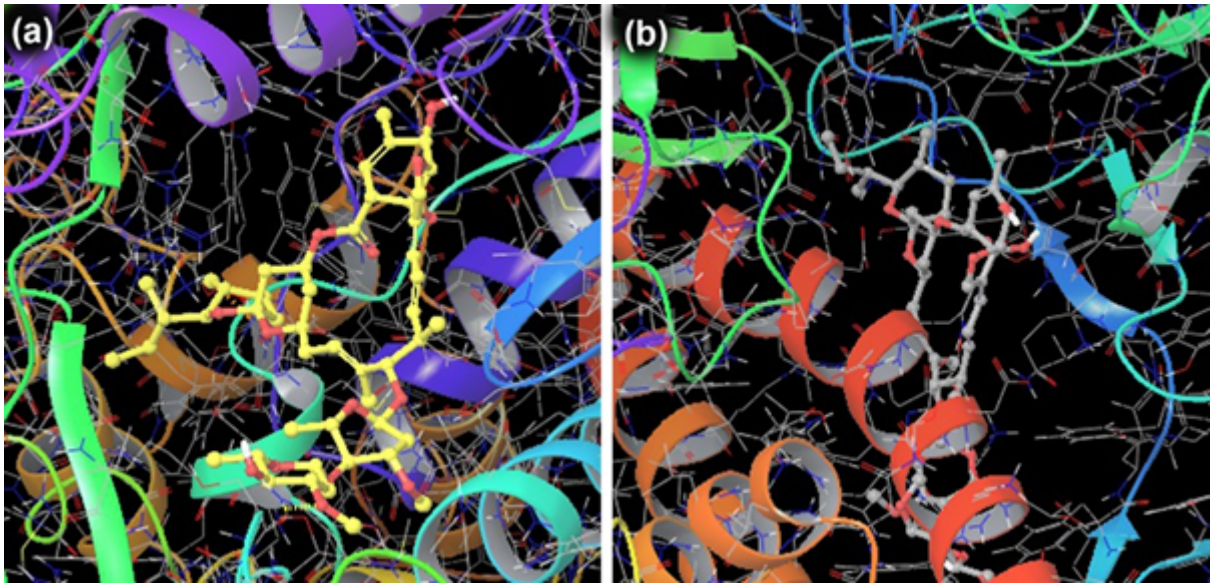

Figure 6

SARS-CoV-2 complex structure of ivermectin with (a) RdRp and (b) Spike protein

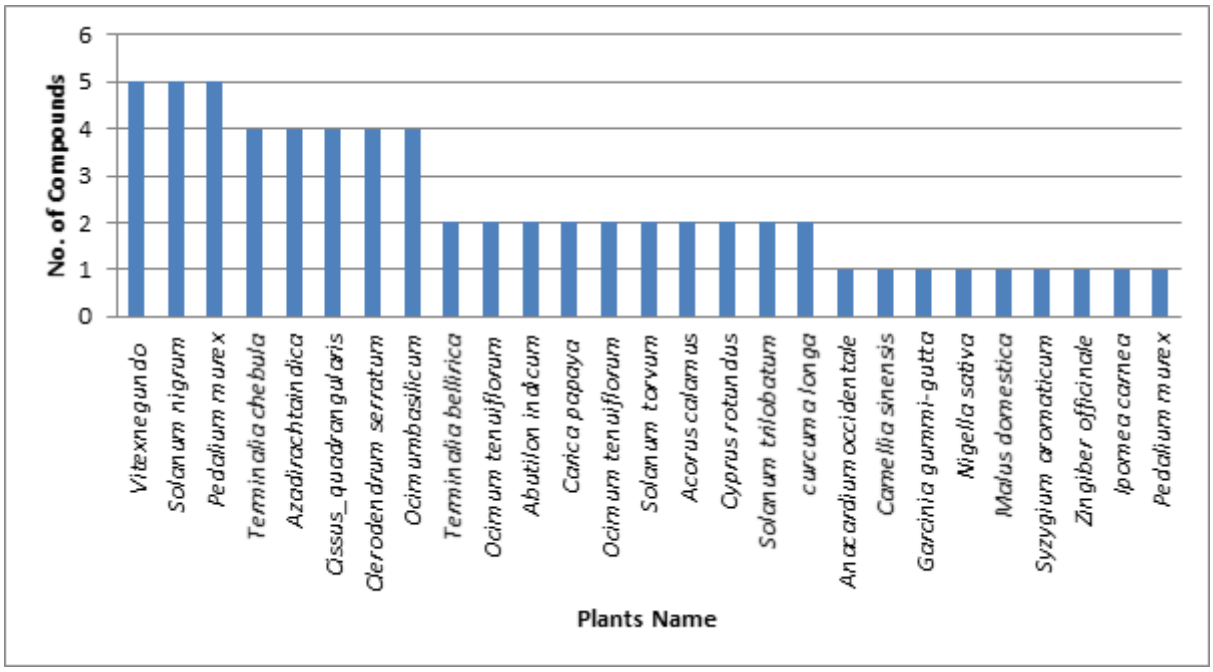

Figure 7

Numbers of phytochemicals in plant species that possess inhibitory activity

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryTableS1.docx
- SupplementaryTableS2S5.docx