Exploring Phytochemicals of Traditional Medicinal Plants Exhibiting Inhibitory Activity Against Main Protease, Spike Glycoprotein, RNA-dependent RNA Polymerase and Non-Structural Proteins of SARS-CoV-2 Through Virtual Screening

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Severe Acute Respiratory Syndrome Corona Virus 2 (SARS-CoV-2) being a causative agent for global pandemic disease nCOVID’19, has acquired much scientific attention for the development of effective vaccines and drugs. 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 605 phytochemicals from 37 plant species (of which 14 plants were endemic to India) and 139 antiviral molecules (Pubchem and Drug bank) in inhibiting SARS-CoV-2 multiple protein targets through a virtual screening approach. Results of our experiments revealed that SARS-CoV-2 MPro shared significant disimilarities against SARS-CoV MPro and MERS-CoV MPro indicating the need for discovering novel drugs. This study has screened the phytochemical cyanin (Zingiber officinale) which may exhibit broad-spectrum inhibitory activity against main proteases of SARS-CoV-2, SARS-CoV and MERS-CoV with binding energies of (−) 8.3 kcal/mol (−) 8.2 kcal/mol and (−) 7.7 kcal/mol respectively. Amentoflavone, agathisflavone, catechin-7-o-gallate and chlorogenin were shown to exhibit multi-target inhibitory activity. Further, Mangifera indica, Anacardium occidentale, Vitex negundo, Solanum nigrum, Pedalium murex, Terminalia chebula, Azadirachta indica, Cissus quadrangularis, Clerodendrum serratum and Ocimum basilicum reported as potential sources of phytochemicals for combating nCOVID’19. More interestingly, this study has highlighted the anti-viral properties of the
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. nCOVID-19 is the third life threatening virus in the SARS family of viruses after SARS-CoV occurred during 2002–03 and MERS-CoV during 2012 (Lee et al., 2003; Cheng et al., 2007; Zaki et al., 2012; De Groot et al., 2013). 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%) (Xu et al., 2020). Its unique genetic makeup has made it not responsive to available medical treatments and 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 short listing 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 (3CLpro)/papain-like protease (PLpro); 2) RNA-dependent RNA polymerase (RdRp) and 3) spike glycoprotein (S protein) as potential targets for drug designing (Elfiky, 2020; Sampangi-Ramaiah et al., 2020).

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 (Elfiky, 2020). Ineffectiveness of existing anti-viral drugs have made the doctors to resort using traditional medicines in nCOVID-19 treatments (Yang et al., 2014). 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 modeling and docking studies (Colson et al., 2020; Dong et al., 2020; Touret and de Lamballerie, 2020). Elfiky (2020) made an attempt to screen 27 different ligands present in commonly used herbs of Indian cuisines against SARS-CoV-2 Main protease and identified 15 different ligands effective in binding the viral protease. Yang et al. (2014) 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 of ligands which may hinder discovery of effective viral inhibitor in the herbal gene pool. In this context, short listing 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 744 ligands including small molecules and phytochemicals from 37 different Indian medicinal plants against seven 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 (PDB ID: 5R81) was used for PSI BLAST (NCBI) (Altschul et al., 1997) 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 (Katoh et al., 2019).

Virtual Screening of Herbal Ligands Against Potential Targets of nCOVID-19

Protein Targets

Coronavirus genome was reported to encode for 29 proteins, out of which the 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). Another part of the genome encodes for envelope and coat proteins. Availability of X-ray crystal structures for most of the proteins in the coronavirus two genome facilitates virtual screening to search for potential inhibitors. We have performed molecular docking of 744 ligands against seven
TABLE 1 | Protein targets with their PDB ID and the binding site volume.

| S. No | PDB ID | Protein name                  | Binding site volume (Å) units |
|-------|--------|-------------------------------|-------------------------------|
| 1     | 2GZ9   | SARS-CoV MPro                 | 328.045                      |
| 2     | 5C3N   | MERS-CoV MPro                 | 317.710                      |
| 3     | 5R81   | SARS-CoV2 MPro                | 308.363                      |
| 4     | 6MUJ   | Spike glycoprotein            | 8,071.179 (interface of ACE and spike protein) |
| 5     | 6M71   | RNA-dependent RNA polymerase  | 4,977.808                    |
| 6     | 6W4H   | NSP16 - NSP10                 | 316.761                      |
| 7     | 6W02   | NSP3                         | 242.332                      |
| 8     | 6W01   | NSP15                        | 212.386                      |
| 9     | 6W4B   | NSP9                         | 121.103                      |

different target proteins of the nCOVID'19 genome (Table 1). In addition, virtual screening was also performed against MPro of SARS CoV (PDB ID: 2GZ9) and MERS CoV (PDB ID: 5C3N) for identifying inhibitors 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 (1992–2016), PubChem (Kim et al., 2019) and DrugBank (Wishart et al., 2018). From the DrugBank database, 99 chemical structures of drugs approved for the treatment of respiratory diseases and compounds exhibiting antiviral activity were collected. Forty chemical compounds with COVID 19 antiviral property retrieved from pubchem database were also included in the ligand dataset. Information regarding the origin, traditional use and protocol were obtained from literature, Indian medicinal plants database (http://www.medicalplants.in/) and Indian Medicinal Plants, Phytochemistry and Therapeutics (https://cb.imsc.res.in/imppat/home) database. Structures of 605 phytochemicals belonging to 37 different herbals and spices used in South Indian Traditional Medicine were also used for virtual screening (Supplementary Table S1). Among the 37 herbals, 14 herbs were found native to India (Table 2) namely, Abutilon indicum (Thuthi), Ocimum tenuiflorum (Tulsi), Pedaliun murex (Peru nerini), Anacystis pyrethrum (Akara), Androgaphis paniculata (Nilavembu), Pepper nigrum (Pepper), Azadirachta indica (Neem), Phyllanthus niruri (Keelanelli), Cissus quadrangularis L (Perandai), Plecranthus amboinicus (Karpuravalli), Clerodendrum serratum (Kanduparangili), Solanum torvum (sundakai), Solanum trifolatum (Thoohuthavali) and Terminalia chebula (Kadukai). Phytochemicals from different parts of the plant such as Root, Rhizome, pericarp leaves, Flower, Seed essential oil, Stem and Fruits was curated in order to find bioactive molecule that may have role in the inhibition of SARS CoV two targets.

Known active ingredients of eight 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 744 small molecules/ligands (Supplementary Table S1: a-c) were used for virtual screening against seven different protein targets. Interactions of phytochemicals were compared with drugs such as hydroxychloroquine (Pubchem ID: 3652), chloroquine (Pubchem ID: 2719) and ivermectin (Pubchem ID: 6427057) retrieved from pubchem database.

Virtual Screening

Virtual screening was performed using Python Prescription Virtual Screening tool (PyRx 0.8) containing AutoDock Vina module (Dallakyan and Olson, 2015). Protein structure was prepared using SWISS PDB Viewer by adding hydrogen atoms and energy minimization. Prepared protein structure was fed into the PyRx tool along with the structure of 744 ligands. Both the ligands and protein molecules were converted to pdbqt file using the AutoDock module of PyRx tool. Inhibitors are expected to bind in the active site/binding site of the protein to inhibit the function of the protein target. In the present study, binding sites were predicted using CASTP server (Tian et al., 2018) and the predicted sites were used for setting grid (XYZ dimensions: 25*25*25) in the AutoDock Vina for virtual screening experiment with the exhaustiveness value of 8. Predicted binding sites were also verified with protein-ligand binding sites of experimental structures for accuracy. Furthermore, phylogenetic analysis of SARS-CoV-2 MPro was carried out using PSI-BLAST (NCBI) (Altschul et al., 1997) and MAFFT server (Katoh et al., 2019). Top 10 ligand hits against each of the seven protein targets were taken for further analysis. 2D and 3D interactions between the protein-ligand were analyzed using Schrodinger Maestro visualizer and BIOVIA discovery studio visualizer 2020 (Accelrys Inc. San Diego, CA, United States) software. ADME properties of top 10 ligands screened against individual protein targets were predicted using SWISSADME server (http://www.swissadme.ch) (Supplementary Table S2).

RESULTS AND DISCUSSION

Phylogenetic Analysis on Coronavirus Main Proteases

Main protease (MPro, also called 3CLPro) is considered as one of the important molecular targets for designing novel drugs against coronaviruses (Anand et al., 2003). 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
| S. No | Plant name/Tamil name          | Geographical distribution of plants                                                                 | Plant part                             | Medicinal use                                                                 | References       |
|-------|--------------------------------|-------------------------------------------------------------------------------------------------------|----------------------------------------|--------------------------------------------------------------------------------|------------------|
| 1.    | Abutilon indicum (thuthi)      | Native to tropic and subtropical regions, found in Karnataka and Tamil nadu                            | Root, flower, leaf                     | Anti-oxidant, anti-bacterial, analgesic, anti-inflammatory, anti-cancer, hepatoprotective, immunomodulatory and larvicidal activities | Abat et al. (2017) |
| 2.    | Acorus calamus (vasambu)       | Throughout asia                                                                                       | Leaves and rhizome                     | Anti-oxidant, anti-inflammatory, anti-ulcer, anti-diabetic, anti-microbial, wound-healing, antiriaproductive, pesticidal and insecticidal properties | Sharma et al. (2020) |
| 3.    | Aegle marmelos (silvam)        | Indian sub continent and southeast asia                                                                | Leaves and fruits                      | Antidiabetic, anticancerous, antifertility, antimicrobial, immunogenic, and insecticidal activities | Pathirana et al. (2020) |
| 4.    | Anacyclus pyrethrum (akara)*   | Native to mediterranean europe and parts of north africa, but also naturalized in other parts of europe, India and Pakistan | Root                                   | Antidiabetic, sialagogue, aphrodisiac, immunostimulant, antidepressant, antimicrobial, insecticide, anesthetic, anti-inflammatory Anticonvulsant, antioxidant | Pandey et al. (2018) |
| 5.    | Andrographis paniculata (nilavembu)* | Native to India and Sri Lanka                                                                         | Plant                                  | Anticancer, anti diarrheal, antihypertensive, anti-hiv, antihyperglycemic, anti-inflammatory, antifungal, antibacterial, and antitumour activities | Hossain et al. (2014) |
| 6.    | Azadirachta indica (neem)      | Indian sub continent and throughout south asia                                                          | Leaves                                 | Ant-inflammatory, antiarthritic, antipyretic, hypoglycemic, anti gastric ulcer, antifungal, antibacterial, and antitumour activities | Alzohairy, (2016) |
| 7.    | Brassica oleracea (cabbage)    | Originated from europe and distributed in caribbean countries, Indonesia, Malaysia and India          | Root, shoot, stem, leaves, leaf buds, flower buds, florets, landraces, sprouts, inflorescence, seeds, seed oil, and callus | Antimicrobial, antibacterial, antidiabetic, antimalarial, antiaging, antilulcer, antihyperglycemic, anti-hyperlipidemic, anti-proliferative, neuroprotective, antidiabetic, anti-genotoxic and antioxidant activities | Chen et al. (2016) |
| 8.    | Carica papaya (papaya)         | Originated from mesoamerica and distributed in other tropical and subtropical regions of the world     | Leaves, root, fruit, seeds             | Antiparasitic, antiseptic, antiparasitic, antifungal, antibacterial, and antitumour activities | Vj and Prashar, (2015) |
| 9.    | Cissus quadrangularis_f (perandai) | India, Bangladesh and Srilanka                                                                      | Stem                                   | Anti-ulcer, anti-bacterial, anolytic, antipyretic, antidiabetic, bone healing, antioxidant and anti-inflammatory properties | Rex and Ravi, (2020) |
| 10.   | Clerodendrum serratum (kanduparangi)* | Bengal, Odisha and Peninsular India                                                                  | Plant, root and leaves                 | Anti-oxidant, anti-carogenic, hepatoprotective, wound healing, anti-allergic | Kumar and Nishwaswar, (2013) |
| 11.   | Coccosucucfera (coconut leaf extract) | South East Asia                                                                                       | Leaves, root, coconut oil, shell, fruit | Antihelminthic, anti-inflammatory, antinociceptive, antioxidant, antifungal, antimicrobial, and antitumor activities | DebMandal and Mandal, (2011) |
| 12.   | Coriandrum sativum (coriander) | Western Asia and Southern Europe                                                                     | Fruits, leaves, plant                  | Antibacterial, antifungal and anti-oxidative activities | Mandal and Mandal, (2015) |
| 13.   | Curcuma longa (turmeric)       | Indian subcontinent and South East Asia                                                               | Root                                   | Anti-inflammatory, antioxidant, antimitagenic, anti-diabetic, antibacterial, hepatoprotective, expectorant and anticancerous | Krup et al. (2013) |
| 14.   | Cypruss rotundus (floral kilangu)* | Southern Asia, Africa, Southern and Central Europe                                                    | Root, rhizome, essential oil and tuber | Antandrogenic, antibacterial, anticancerous, anticonvulsant, antidiabetic, antidiarrheal, antinotoxine, anti-inflammatory, antipiddemic, antimalarial, antimitagenic, anticoexsity, antioxidant, anti-uropathogenic, | Peerzada et al. (2015) |
| S. No. | Plant name/Tamil name | Geographical distribution of plants | Plant part | Medicinal use | References |
|-------|----------------------|-------------------------------------|------------|---------------|------------|
| 15.   | Glycyrrhiza glabra (liquorice) | Western Asia, Northern Africa and Eurasia | Liquorice extract, root | hepatoprotective, cardioprotective, neuroprotective, and nootropic agent | Kaur et al. (2013a) |
| 16.   | Hygrophila auriculata (mullivera) | Tropical Asia and Africa | Root, stem | Anti-nociceptive, antitumor, antioxidant, hepatoprotective, hypoglycemic, hematocrit, diuretic, free radical scavenging, antihypertensive, antinflammatory, antipyretic, anabolic and androgenic activities | Hussain et al., 2010 |
| 17.   | Ipomea carnea (neivilkaatamanakku) | South America and found in Tamilnadu | Plant extract | Glycosidase inhibitory activities, antioxidant antiinflammatory, antidiabetic, wound healing antimicrobial, immunomodulatory, cardiovascular, abortifacient, antifungal properties | Saxena, Tyagi, kumar, singh |
| 18.   | Justicia adhatoda (aadathoda) | Asia | Plant, flower, leaf | Antitussive, abortifacient, antimicrobial cardiovascular protection, anticholinesterase, antinflammatory | Kaur et al. (2013b) |
| 19.   | Malus domestica (apple) | Central Asia and Europe and cultivated worldwide | Fruit | Antioxidant, antiproliferative, antidepresant, antinflammatory, antimicrobial | Lobo and Satish, 2019 |
| 20.   | Nigella sativa (fennel) | South West Asia, Southern Europe, North Africa | Seed | Antidiabetic, anticancer, immunomodulator, analgesic, antimicrobial, anti-inflammatory, spasmolytic, bronchodilator, hepatoprotective, renal protective, gastroprotective, antioxidant properties | Ahmad et al. (2013) |
| 21.   | Ocimum basilicum (thiruneetrupachai) | South East Asia and Central Africa | Leaves | Anti-cancer, radioprotective, antimirolibical, anti-inflammatory effects, immunomodulatory, anti-stress, anti-diabetic, anti-pyretic, anti-artheritic, anti-inflammatory, as a prophylactic agent and in cardiovascular disease | Shahrabian et al., 2020 |
| 22.   | Ocimum tenuiflorum (tulsi) | India and South East Asia | Leaves, stem, flower, root, seeds and even whole plant | Antifertility, anticancer, antidiabetic, antifungal, antimicrobial, hepatoprotective, cardioprotective, antinflammatory, aspasmodytic, analgesic, adaptogenic and diaphoretic actions | Prakash and Gupta, 2005 |
| 23.   | Pedalium murex (paru nari) | Southern part and deccan region of India and Ceylon | Whole plant, root, seed, leaf | Antihypertensive and antiplatelets, antioxidant, antitumor, antithromatics, antipyretic, analgesic, antinflammatory, anti-diareh, antispasmodic, anxiolytic, antidepressants, hepatoprotective, immunno-modulatory, antibacterial, antifungal, anti-thyroids, antiapoptotic, anti-metastatic, antimutagenic, antimetastatic, anticon toalin toxin, insecticidal and larvicial activities | Damanhouri and Ahmad, 2014 |
| 24.   | Pepper nigrum (pepper) | South Asia (India, southern Thailand, Malaysia) | Fruit, seed, seed essential oil | Antiviral, antibacterial, antiplasmodial, anti-inflammatory, antimalarial, anticholinesterase, antidiabetic, hypolipidemic, antioxidant | Patel et al., 2011a |
| 25.   | Phyllanthus niruri (keelanelli) | India tropical and coastal areas | Leaves, whole plant | Antinflammatory and antiplatelets, antioxidant, antitumor, antithromatics, antipyretic, analgesic, antinflammatory, anti-diareh, antispasmodic, anxiolytic, antidepressants, hepatoprotective, immunno-modulatory, antibacterial, antifungal, anti-thyroids, antiapoptotic, anti-metastatic, antimutagenic, antimetastatic, anticon toalin toxin, insecticidal and larvicial activities | Patel et al., 2011b |

(Continued on following page)
alignment identified 12 significant differences between main proteases of SARS-CoV and SARS-CoV-2 (Figure 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 of SARS-CoV2M Pro with other CoV M Pro sequences sharing >50 percentage similarity revealed its significant genetic relatedness with SARS CoV (96.08% similarity) and bat CoV (76.84% similarity) (Figure 2). Also 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 744 ligands belonging to small molecules and active compounds from 37 medicinal herbs against seven major protein targets of nCOVID’19 predicted probable SARS-CoV-2 inhibitors. Information regarding the binding site residues predicted using CASTp server is provided in Table 3. Binding affinity of drugs such as hydroxychloroquine, chloroquine and ivermectin are provided in Table 4 which was used as reference for comparison of phytochemicals. Top 10 hits reported with higher binding affinity for each target protein is considered for downstream analysis (Table 5). Seven molecular targets of SARS-CoV-2 include, Main protease (PDB ID: 5R81), RNA-dependent RNA polymerase (RdRp) (PDB ID: 5M71), NSP3 (PDB ID: 6W01), NSP10-NSP16 (PDB ID: 6W02), NSP9 (PDB ID: 6W4B), NSP10-NSP16 (PDB ID: 6W4H), NSP15 (PDB ID: 6W01) and Spike protein (PDB ID: 6M0J).

Small Molecules/Herbal Compounds Exhibiting Significant Binding Affinity Against Main Protease

Despite of significant structural (RMSD: 0.71 Å) and binding site volume similarity of MPro between SARS-CoV (PDB ID: 2GZ9) and SARS-CoV-2 (PDB ID: 5R81), they showed differential binding affinity against different inhibitors (Table 1). Virtual screening of small molecules against MPro identified agathisflavone as the best hit exhibiting the binding affinity value of –8.2 kcal/mol.

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 CY5, 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, 26 THR, 27 LEU, 41 HIS, 42 VAL, 44 CY5, 46 ALA, 49 LEU, 143 PHE, 144 LEU, 145 CYS, 166 HIS, 167 GLU, 168 MET, 169 GLU, 170 LEU, 171 ALA, 175 HIS, 190 ASP, 191 LYS, 192 GLN, 193 VAL, 194 HIS, 195 GLN, 196 VAL |
| Main protease (SARS-CoV-2)    | 24 THR, 25 THR, 26 THR, 27 LEU, 41 HIS, 44 CY5, 45 THR, 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, 172 THR, 187 ASP, 188 ARG, 189 GLN, 190 THR, 192 GLN |
| Spike glycoprotein            | Chain E - 403 ARG, 405 ASP, 406 GLU, 408 ARG, 409 GLN, 415 THR, 416 GLY, 417 LYS, 420 ASP, 421 TYR, 449 TYR, 453 TYR, 456 PHE, 493 GLN, 494 SER, 495 TYR, 496 GLY, 497 PHE, 501 ASN, 502 GLY, 503 VAL, 504 GLY, 505 TYR |
| RNA-dependent RNA polymerase NSP 12 | 164 ASP, 166 VAL, 167 GLU, 429 PHE, 430 LYS, 431 GLU, 436 GLU, 437 LEU, 438 CY5, 439 HIS, 440 PHE, 441 PHE, 452 ASP, 455 TYR, 456 TYR, 494 ILE, 496 ASN, 497 ASN, 499 ASP, 500 LYS, 66,501 SER, 503 LYS, 507 ASN, 511 LYS, 540 THR, 541 GLN, 542 MET, 543 LYS, 545 LYS, 546 TYR, 547 ALA, 548 LYS, 549 SER, 550 ALA, 551 LYS, 552 ARG, 554 ALA, 555 ARG, 556 THR, 557 VAL, 558 ALA, 559 GLY, 560 THR, 568 ASN, 569 ARG, 572 HIS, 573 GLN, 574 LEU, 577 LYS, 580 ALA, 588 VAL, 589 ILE, 590 GLY, 591 THR, 592 SER, 593 LYS, 594 PHE, 598 LYS, 601 MET, 602 LEU, 616 GLY, 617 TRP, 618 ASP, 619 THR, 620 PRO, 621 LYS, 622 CY5, 623 ASP, 624 ARG, 666 GLU, 667 GLY, 668, LYS, 680 THR, 681 SER, 682 SER, 683 GLY, 684 ASP, 685 ALA, 686 THR, 687 THR, 688 ALA, 689 TYR, 691 ASN, 756 MET, 758 LEU, 759 SER, 760 ASP, 761 ASP, 762 ALA, 763 VAL, 792 VAL, 793 PHE, 795 SER, 797 ALA, 798 LYS, 799 CY5, 800 TRP, 810 HIS, 811 GLU, 812 PHE, 813 CY5, 814 SER, 815 GLN, 816 HIS, 818 ASP, 835 SER, 836 ARG, 837 ILE, 840 ALA, 841 GLY, 843 PHE, 844 VAL, 845 ASP, 847 ILE, 848 VAL, 854 LEU, 856 MET, 857 GLU, 858 ARG, 859 PHE, 861 SER, 862 LEU, 864 ILE, 865 ASP C chain - 3MET, 4SER, 7LYS, 40LEU, 41LEU, 43LYS |
| NSP16 - NSP10                 | 6814 ASN, 6844 LYS, 6845 TYR, 6867 HIS, 6888 PHE, 6889 GLY, 6870 ALA, 6871 GLY, 6872 SER, 6873 ASP, 6878 PRO, 6879 GLY, 6896 SER, 6897 ASP, 6898 LEU, 6899 ASN, 6900 ASP, 6901 PHE, 6911 GLY, 6912 ARG, 6913 CY5, 6914 ASP, 6915 ASP, 6916 MET, 6930 TYR, 6931 ASP, 6932 PHE, 6933 GLY, 6947 PHE, 6968 LYS |
| NSP3                         | 6 PHE, 7 SER, 8 GLY, 10 LEU, 11 LYS, 12 LEU, 14 LEU, 19 LYS, 20 ASN, 158 LYS, 161 TYR, 162 ASP, 165 VAL, 168 PHE |
| NSP15                        | 13 MET, 33 TYR, 38 GLY, 39 GLY, 40 ARG, 42 VAL, 57 PHE, 58 PRO, 59 LYS, 60 SER, 68 ILE, 68 THR |

Table 4: Binding affinity value of Hydroxychloroquine, Chloroquine and Ivermectin against SARS-CoV-2 targets.

| SARS-CoV-2 Protein target | Hydroxychloroquine (pubchem ID: 3652) binding affinity | Chloroquine (pubchem ID: 2719) binding affinity | Ivermectin (pubchem ID: 6427057) binding affinity |
|---------------------------|---------------------------------------------------------|-----------------------------------------------|---------------------------------------------------|
|                           | (kcal/mol)                                              | (kcal/mol)                                    | (kcal/mol)                                        |
| SARS-CoV-2MPro            | –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                                             |
TABLE 5 | Details of shortlisted top 10 natural compounds/small molecules screened against various targets of coronaviruses.

| Protein name | Binding affinity kcal/mol | Compound name | Puchem ID | Chemical structure | Bioavailability score | Solubility score/Class | Plant name |
|--------------|---------------------------|---------------|-----------|--------------------|-----------------------|------------------------|------------|
| SARS COV main protease (PDB ID: 2GZ9) | -9.0 | Rutin (ZINC4096848) | 5,280,805 | ![Chemical structure](image1) | 0.17 | 0.29/Soluble | Terminalia chebula, Azadirachta indica and Ocimum basilicum |
| | -8.7 | Quercetin 3 gentiobioside (ZINC49783852) | 5,320,834 | ![Chemical structure](image2) | 0.17 | 0.29/Soluble | Solanum nigrum |
| | -8.6 | 3-O-trans-caffeoyltormentic acid | 44,584,640 | ![Chemical structure](image3) | 0.56 | -5.99/ moderately soluble | Antiviral compounds |
| | -8.5 | (-)Epicatechin 3 o gallate (epicatechin) | 107,905 | ![Chemical structure](image4) | 0.55 | -3.09/soluble | Camellia sinensis |
| | -8.5 | Corilagin (ZINC4098612) | 73,568 | ![Chemical structure](image5) | 0.17 | -0.51/soluble | Terminalia bellirica, Terminalia chebula |
| | -8.4 | Quercetin galactoside (ZINC3973253) | 5,281,643 | ![Chemical structure](image6) | 0.17 | -1.51/soluble | Antiviral compounds |
| | -8.3 | Quercetin (ZINC4175638) | 5,280,459 | ![Chemical structure](image7) | 0.17 | -2.08/soluble | Azadirachta indica |
| | -8.3 | Isoquercetin (ZINC4096845) | 5,280,804 | ![Chemical structure](image8) | 0.17 | -1.51/soluble | Ocimum basilicum, Terminalia chebula |
| | -8.3 | Acetoside (ZINC8234351) | 5,281,800 | ![Chemical structure](image9) | 0.17 | -0.22/soluble | Clerodendrum serratum |
| | -8.2 | Cyanin (ZINC4097727) | 441,688 | ![Chemical structure](image10) | 0.17 | 0.86/soluble | Zingiber officinale |
| SARS-COV-2 main protease (PDB ID: 5R81) | -8.2 | Agathisflavone (ZINC40986505) | 5,281,599 | ![Chemical structure](image11) | 0.17 | -8.7/Poorly soluble | Anacardium occidentale |

(Continued on following page)
| Protein name                          | Binding affinity kcal/mol | Compound name                  | Puchem ID      | Chemical structure | Bioavailability score | Solubility score/Class | Plant name |
|--------------------------------------|---------------------------|--------------------------------|----------------|--------------------|-----------------------|------------------------|------------|
| Rubusic acid                         | −8.1                      | 101,297,651                    |                |                    | 0.56                  | −5.3/moderately soluble | Pedalium murex       |
| Solanocapaine                        | −7.9                      | 73,419                         |                |                    | 0.55                  | −4.2/moderately soluble | Solanum nigrum       |
| Chlorogenin (ZINC84668707)           | −7.7                      | 12,303,065                     |                |                    | 0.55                  | −3.69/soluble           | Solanum torvum      |
| Lupeol (ZINC4081455)                 | −7.7                      | 259,846                        |                |                    | 0.55                  | −6.74/poorly soluble    | Carica papaya and Azadirachta indica |
| Cyanin (ZINC4097727)                 | −7.7                      | 441,688                        |                |                    | 0.17                  | 0.86/soluble             | Zingiber officinale |
| 3-O-trans-caffeoyltormentic acid     | −7.7                      | 44,584,640                     |                |                    | 0.56                  | −5.99/moderately soluble | Antiviral compound  |
| Luteolin 7-O-6''-malonyl(glucoside)  | −7.7                      | 5,281,669                      |                |                    | 0.11                  | −2.05/soluble             | Vitex negundo      |
| Agnuside (ZINC4098330)               | −7.6                      | 442,416                        |                |                    | 0.17                  | 0.66/soluble             | Vitex negundo      |
| Luteolin 7-O-beta-D-glucoside        | −7.6                      | 5,280,637                      |                |                    | 0.17                  | −2.1/soluble             | Vitex negundo      |
| MERS main protease (MERS)            | −8.6                      | 5,281,600                      |                |                    | 0.17                  | −8/poorly soluble        | Mangifera indica    |

(Continued on following page)
TABLE 5 | (Continued) Details of shortlisted top 10 natural compounds/small molecules screened against various targets of coronaviruses.

| Protein name | Binding affinity kcal/mol | Compound name | Puchem ID | Chemical structure | Bioavailability score | Solubility score/Class | Plant name |
|--------------|---------------------------|---------------|-----------|--------------------|----------------------|------------------------|------------|
| RNA-dependent RNA polymerase (PDB ID: 6M71) | -9.4 | Ivermectin (ZINC238808778) (DB00602) | 6,427,057 | ![Chemical structure](image) | 0.17 | -3.89/soluble | FDA approved drug |
| -8.4 | Corilagin (ZINC4098612) | 73,568 | ![Chemical structure](image) | 0.17 | -0.51/soluble | Terminalia bellirica, Terminalia chebula |
| -8.3 | Cyanin (ZINC4097727) | 441,688 | ![Chemical structure](image) | 0.17 | 0.86/soluble | Zingiber officinale |
| -8.2 | Rutin (ZINC4096846) | 5,280,805 | ![Chemical structure](image) | 0.17 | -0.29/soluble | Terminalia chebula, Azadirachta indica and Ocimum basilicum |
| -8.2 | Betulinic acid (ZINC4097714) | 64,971 | ![Chemical structure](image) | 0.85 | 5.7/moderately soluble | Vitex negundo |
| -8.1 | 3,5'-biapigenin (ZINC17545581) | 10,414,856 | ![Chemical structure](image) | 0.17 | -8.7/poorly soluble | antiviral compound |
| -8.0 | (-)-Epicatechin-3-o-gallate (ZINC3978503) | 107,905 | ![Chemical structure](image) | 0.55 | -3.09/soluble | Camellia sinensis |
| -8.0 | Chrysanthemin (ZINC4097706) | 441,667 | ![Chemical structure](image) | 0.17 | -0.93/soluble | Zingiber officinale |
| -8.0 | Vicenin-2 (ZINC4098604) | 442,664 | ![Chemical structure](image) | 0.17 | -0.27/soluble | Ocimum basilicum |
| -8.0 | Quercetin 3 gentiobioside (ZINC49783852) | 5,320,834 | ![Chemical structure](image) | 0.17 | 0.29/soluble | Solanum nigrum |

(Continued on following page)
TABLE 5 | (Continued) Details of shortlisted top 10 natural compounds/small molecules screened against various targets of coronaviruses.

| Protein name | Binding affinity kcal/mol | Compound name (ZINCID) | Puchem ID | Chemical structure | Bioavailability score | Solubility score/Class | Plant name |
|--------------|---------------------------|------------------------|-----------|--------------------|-----------------------|------------------------|------------|
| −9.3         | Amentoflavone (ZINC3984030) | 5,281,600              | ![Chemical structure](image1) | 0.17 | -8.7/poorly soluble | Mangifera indica |
| −9.0         | Corilagin (ZINC4098612)    | 73,568                 | ![Chemical structure](image2) | 0.17 | -0.51/soluble | Terminalia bellirica, Terminalia chebula |
| −9.0         | Agathisflavone (ZINC4098505) | 5,281,599             | ![Chemical structure](image3) | 0.17 | -8.7/poorly soluble | Anacardium occidentale |
| −8.6         | 3-O-trans-cafeoyltormentic acid | 44,584,640         | ![Chemical structure](image4) | 0.56 | -5.99/moderately soluble | Antiviral compound |
| −8.2         | Arjungenin (ZINC38143755)  | 6,321,424             | ![Chemical structure](image5) | 0.17 | -3.89/soluble | Terminalia chebula |
| −8.2         | Crateogolic acid (ZINC4273446) | 12,444,386          | ![Chemical structure](image6) | 0.56 | -3.9/soluble | Syzygium aromaticum |
| −8.1         | Maslinic acid (ZINC4273446) | 73,659                | ![Chemical structure](image7) | 0.56 | -5.3/moderately soluble | Antiviral compound |
| −8.0         | 3.5′-biapigenin (ZINC17545,581) | 10,414,856          | ![Chemical structure](image8) | 0.17 | -8.7/Poorly soluble | Zingiber officinalis |
| −7.9         | Cyanin                  | 441,688               | ![Chemical structure](image9) | 0.17 | 0.86/soluble | Camellia sinensis |
| −9.6         | Friedelin (ZINC4097720)   | 91,472                | ![Chemical structure](image10) | 0.55 | -7.88/poorly soluble | Vitex negundo, Acoruscalamus |

(Continued on following page)
TABLE 5 | (Continued) Details of shortlisted top 10 natural compounds/small molecules screened against various targets of coronaviruses.

| Protein name | Binding affinity kcal/mol | Compound name | Puchem ID | Chemical structure | Bioavailability score | Solubility score/Class | Plant name |
|--------------|---------------------------|---------------|-----------|-------------------|----------------------|------------------------|------------|
| NSP15 (PDB ID: 6W01) | −9.2 | Oleanolic acid (ZINC3785416) | 10,494 | ![Chemical structure](image) | 0.85 | -6.12/poorly soluble | Ocimum basilicum, Cyprus rotundus, Clerodendrum serratum, Ocimum tenuiflorum |
| −9.3 | N-methylsolasodine | 21,573,751 | ![Chemical structure](image) | 0.55 | -4.46/moderately soluble | Solanum nigrum |
| −8.9 | Solasodine (ZINC8214722) | 442,985 | ![Chemical structure](image) | 0.55 | -4.8/moderately soluble | Solanum nigrum, Solanum torvum |
| −8.9 | Diosgenin (ZINC6857614) | 99,474 | ![Chemical structure](image) | 0.55 | -4.49/moderately soluble | Solanum nigrum, Pedalium murex |
| −8.6 | Spirostan-3-ol (ZINC253504500) | 3,035,446 | ![Chemical structure](image) | 0.55 | Moderately soluble | Solanum nigrum |
| −8.6 | Solanocapsine | 73,419 | ![Chemical structure](image) | 0.55 | -4.22/moderately soluble | Solanum nigrum |
| −8.5 | Taraxerol (ZINC4082498) | 92,097 | ![Chemical structure](image) | 0.55 | -7.16/Poorly soluble | Cissus quadrangularis |
| −8.3 | Amentoflavone (ZINC3984030) | 5,281,600 | ![Chemical structure](image) | 0.17 | -8.7/Poorly soluble | Mangifera indica |
| −8.2 | Chlorogenin (ZINC846668707) | 12,303,065 | ![Chemical structure](image) | 0.55 | -3.69/soluble | Solanum torvum |
| −8.1 | Agathisflavone (ZINC4098505) | 5,281,599 | ![Chemical structure](image) | 0.17 | -8.7/poorly soluble | Anacardium occidentale |

(Continued on following page)
| Protein name  | Binding affinity kcal/mol | Compound name | Puchem ID | Chemical structure | Bioavailability score | Solubility score/Class | Plant name |
|---------------|---------------------------|---------------|-----------|--------------------|-----------------------|------------------------|------------|
| NSP3 (PDB ID: 6W02) | −7.4 | Amentoflavone (ZINC3984030) | 5,281,600 | 0.17 | −8.7/poorly soluble | Mangifera indica |
| −9.2 | Ursolic-acid (ZINC3978827) | 64,945 | | 0.85 | −5.67/moderately soluble | Ocimum basilicum, Pedalium murex, Malus domestica, Ocimum tenuiflorum |
| −8.9 | Orategolic acid (ZINC4273446) | 73,659 | | 0.56 | −5.3/Moderately soluble | Syzygium aromaticum |
| −8.8 | Arjunerinin (ZINC38143755) | 12,444,386 | | 0.56 | −3.9/soluble | Terminalia chebula |
| −8.7 | Hederagenin (ZINC3946009) | 73,299 | | 0.56 | −5.55/moderately soluble | Nigella sativa |
| −8.6 | Triterpenoid (ZINC40164454) | 451,674 | | 0.56 | −5.12/moderately soluble | Abutilon indicum |
| −8.6 | Beta-amyrin (ZINC3978270) | 73,145 | | 0.55 | −7.16/poorly soluble | Cissus quadrangularis |
| −8.5 | Catechin 7-O-gallate | 471,393 | | 0.55 | −3.09/soluble | Camellia sinensis |
| −8.5 | Arjunolic acid (ZINC31158038) | 73,641 | | 0.56 | −4.72/moderately soluble | Terminalia chebula |
| −8.5 | Amento flavone (ZINC3984030) | 5,281,600 | | 0.17 | −8.7/poorly soluble | Mangifera indica |
### TABLE 5 (Continued) Details of shortlisted top 10 natural compounds/small molecules screened against various targets of coronaviruses.

| Protein name | Binding affinity kcal/mol | Compound name | Puchem ID | Chemical structure | Bioavailability score | Solubility score/Class | Plant name |
|--------------|---------------------------|---------------|-----------|--------------------|-----------------------|------------------------|------------|
|              | −7.1                      | Luteolin 7-O-(6''-malonyl(glucoside) | 5,281,669 | ![Chemical Structure](image) | 0.11                  | −2.05/soluble            | Vitex negundo |
|              | −6.8                      | Rubusic acid  | 101,297,651 | ![Chemical Structure](image) | 0.56                  | −5.3/moderately soluble | Pedalium murex |
|              | −6.7                      | Acteoside (ZINC8234351) | 5,281,800 | ![Chemical Structure](image) | 0.17                  | −0.22/soluble            | Clerodendrum serratum |
|              | −6.7                      | Ivermectin (ZINC238808778) (DB00602) | 6,427,057 | ![Chemical Structure](image) | 0.17                  | −3.89/soluble            | FDA approved drug |
|              | −6.7                      | Taraxerol acetate (ZINC31334747) | 94,225 | ![Chemical Structure](image) | 0.55                  | −7.77/poorly soluble     | Cissus quadrangularis |
|              | −6.6                      | Catechin 7-O-gallate | 471,393 | ![Chemical Structure](image) | 0.55                  | −3.09/soluble            | Antiviral compound |
|              | −6.6                      | Luteolin 7-O-beta-D-glucoside (ZINC4096258) | 5,280,637 | ![Chemical Structure](image) | 0.17                  | −2.1/soluble            | Vitex negundo |
|              | −6.6                      | Agathisflavone (ZINC4098505) | 5,281,599 | ![Chemical Structure](image) | 0.17                  | −8.7/poorly soluble     | Anacardium occidentale |
|              | −6.6                      | Luteolin-7-o-beta-d-glucopyranoside | 5,291,488 | ![Chemical Structure](image) | 0.17                  | −2.1/soluble            | Vitex negundo |

(Continued on following page)
TABLE 5 | Details of shortlisted top 10 natural compounds/small molecules screened against various targets of coronaviruses.

| Protein name          | Binding affinity kcal/mol | Compound name                   | Puchem ID | Chemical structure | Bioavailability score | Solubility score/Class | Plant name                          |
|-----------------------|---------------------------|---------------------------------|-----------|-------------------|-----------------------|------------------------|-------------------------------------|
| NSP10-NSP16 (PDB ID: 6W4H) | −8.5                      | Amentoflavone (ZINC3984030)     | 5,281,600 | ![Chemical structure](image) | 0.17                  | −8.7/poorly soluble         | Mangifera indica                    |
|                       |                           | 10-Methoxycamptothecin (ZINC4823972) | 97,283   | ![Chemical structure](image) | 0.55                  | −5.93/moderately soluble    | Antiviral compound                  |
|                       |                           | 3.8'-biapigenin (ZINC17545581)  | 10,414,856 | ![Chemical structure](image) | 0.17                  | −8.7/poorly soluble         | Antiviral compound                  |
|                       |                           | Taraxerol acetate (ZINC31334747) | 94,225    | ![Chemical structure](image) | 0.55                  | −7.77/poorly soluble        | Cissus quadrangularis               |
|                       |                           | Corilagin (ZINC4098612)         | 73,568    | ![Chemical structure](image) | 0.17                  | −0.15/soluble                | Terminalia bellirica, Terminalia chebula |
|                       |                           | Lupeol acetate (ZINC4097722)    | 92,157    | ![Chemical structure](image) | 0.55                  | −7.35/poorly soluble        | Pedalium murex                      |
|                       |                           | Emetine (ZINC0000003830747)     | 10,219    | ![Chemical structure](image) | 0.55                  | −7.7/poorly soluble          | FDA approved drug                   |
|                       |                           | Chlorogenin (ZINC846668707)     | 12,303,065 | ![Chemical structure](image) | 0.55                  | −3.68/soluble                | Solanum torvum                     |
|                       |                           | Spirostan-3-ol (ZINC253504500)   | 14,492,795 | ![Chemical structure](image) | 0.55                  | −6.56/poorly soluble        | Solanum nigrum                     |
|                       |                           | 5,7-Dihydroxy-4'-methoxy-8,3' di-C-prenyllavone | 3,035,446 | ![Chemical structure](image) | 0.55                  | −4.51/moderately soluble    | Azadirachta indica                  |

(Continued on following page)
TABLE 5 (Continued) Details of shortlisted top 10 natural compounds/small molecules screened against various targets of coronaviruses.

| Protein name                                      | Binding affinity kcal/mol | Compound name                                      | Puchem ID | Chemical structure | Bioavailability score | Solubility score/Class | Plant name            |
|---------------------------------------------------|---------------------------|---------------------------------------------------|-----------|---------------------|------------------------|------------------------|-----------------------|
| Spike glycoprotein (PDB ID: 6M0J)                 | −8.2                      | 1,8-Dichloro-9,10-diphenylanthracene-9,10-diol    | 320,066   | ![Chemical structure](image1) | 0.55                   | -10.45/insoluble       | Carica papaya         |
|                                                   | −8.2                      | Agathisflavone (ZINC4098505)                       | 5,281,599 | ![Chemical structure](image2) | 0.17                   | -8.7/poorly soluble    | Anacardium occidentale |
|                                                   | −8.2                      | Amentoflavone (ZINC3984030)                        | 5,281,600 | ![Chemical structure](image3) | 0.17                   | -8.7/poorly soluble    | Mangifera indica      |
|                                                   | −8.2                      | Ivermectin (ZINC238808778)                        | 6,427,057 | ![Chemical structure](image4) | 0.17                   | -3.89/soluble          | FDA approved drug      |
|                                                   | −8.1                      | 3 o caffeoyltormentic acid                        | 44,584,640| ![Chemical structure](image5) | 0.56                   | -5.99/moderately soluble | Antiviral compound    |
|                                                   | −7.5                      | Agruside (ZINC4098330)                            | 442,416   | ![Chemical structure](image6) | 0.17                   | 0.66/soluble           | Vitex negundo         |
|                                                   | −7.4                      | 1,8-Dichloro-9,10-diphenylanthracene-9,10-diol    | 320,066   | ![Chemical structure](image7) | 0.55                   | -10.45/insoluble       | Cissus quadrangularis |
|                                                   | −7.3                      | Taraxerol (ZINC4082498)                           | 92,097    | ![Chemical structure](image8) | 0.55                   | -7.16/poorly soluble   | Azadirachta indica    |
|                                                   | −7.3                      | Nimbinene                                         | 44,715,635| ![Chemical structure](image9) | 0.55                   | -5.66/moderately soluble | Antiviral compound    |
|                                                   | −7.3                      | β-amyrin (ZINC13298265)                           | 92,156    | ![Chemical structure](image10) | 0.55                   | -7.77/poorly soluble   | Cissus quadrangularis |
Out of 744 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 $-9.0\text{ kcal/mol}$. In case of MERS-CoV main protease (PDB ID: 5C3N), amentoflavone predominantly found in *Mangifera indica* and *Garcinia* species showed the maximum binding affinity value of $-8.6\text{ kcal/mol}$. Interestingly, a cytotoxic biflavonoid agathisflavone found in cashew nut (*Anacardium occidentale*) was shown to exhibit significant binding affinity with $-8.0\text{ kcal/mol}$ against the SARS-CoV-2 MPro (*Supplementary Table S3*). Agathisflavone is a biflavonoid derived from plant source and has been found to possess several biological activities (De Amorim et al., 2018). 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 (Islam et al., 2019). Also, Agathisflavones have been reported for their cytotoxicity against malignant cell lines in earlier studies (Konan et al., 2012). This compound was found to involve in the formation of three hydrogen bonds with residues such as ASP 187, PRO 52 and ARG 40. This was followed by Rubusic acid ($-8.1\text{ kcal/mol}$) (*Pedalium murex*), solanocapsine ($-7.9\text{ kcal/mol}$) (*Solanum nigrum*), chlorogenin ($-7.7\text{ kcal/mol}$) (*Solanum torvum*), Lupeol ($-7.7\text{ kcal/mol}$) (*Carica papaya* and *Azadirachta indica*), Cyanin ($-7.7\text{ kcal/mol}$) (*Zingiber officinale*), 3-O-trans-cafeoyltormentic acid ($-7.7\text{ kcal/mol}$) (antiviral compound), Luteolin-7-O-(6″-malonylglucoside) ($-7.7\text{ kcal/mol}$) (*Vitex negundo*), Agnuside ($-7.6\text{ kcal/mol}$) (*Vitex negundo*) and Luteolin 7-O-beta-D-glucoside ($-7.6\text{ kcal/mol}$) (*Vitex negundo*) exhibiting significant binding affinity in the decreasing order against MPro.

The phytochemical cyanin found in *Zingiber officinale* was found to have higher binding affinity with the main proteases of all three coronaviruses (*Figures 3A–C*) and placed in the top 10 hits list. It showed a binding affinity value of $-8.3\text{ kcal/mol}$, $-8.2\text{ kcal/mol}$ and $-7.7\text{ kcal/mol}$ against SARS-CoV-2 (PDB ID: 5R81), SARS CoV (PDB ID: 2GZ9) and MERS CoV (PDB ID: 5C3N) main proteases respectively. Hydrogen bond interactions for SARS-CoV-2 (THR 26, SER 46 and GLU 166), SARS CoV (THR 26, LEU 141 and CYS 145) and MERS CoV (THR 26, CYS 149 and GLU 169) were observed in the binding sites respectively.

**Effect of Suggested FDA Drugs on SARS-CoV-2 Protein Targets**

Hydroxychloroquine, chloroquine and ivermectin drugs were selected as positive controls to compare the binding interaction of phytochemicals for the assessment of anti-viral activity (Caly et al., 2020; Principi and Esposito, 2020). Hydroxychloroquine was reported to show promising inhibitory activity against nCOVID’19 spike protein (Gautret et al., 2020; Liu et al., 2020). Our results revealed that hydroxychloroquine and chloroquine showed less binding affinity against all the seven targets of nCOVID’19.
FIGURE 4 | Docked complex structures of spike protein with (A) ivermectin, (B) agasthisflavone, (C) amentoflavone, and RdRp with (D) ivermectin (E) agasthisflavone (F) amentoflavone.
FIGURE 5 | Docked complex structures of NSP3 protein with (A) ivermectin (B) agasthisflavone (C) amentoflavone, and NSP9 with (D) ivermectin (E) agasthisflavone (F) amentoflavone.
compared to ivermectin (Table 4). Ivermectin exhibited significant binding affinity value of $-9.4 \text{ kcal/mol}$ and $-8.2 \text{ kcal/mol}$ against RNA - dependent RNA polymerase (RdRp) (PDB ID: 6M71) and spike protein (PDB ID: 6M0J) respectively (Figures 4A,D). Ivermectin also exhibited significant binding affinity against NSP3 (PDB ID: 6W02) ($-6.7 \text{ kcal/mol}$) and NSP9 (PDB ID: 6W4B) ($-7.5 \text{ kcal/mol}$) (Figures 5A–D) (Supplementary Table S4). Analysis of hydrogen bond interaction with ivermectin, showed three hydrogen bonds for RdRp target (ARG 403, TYR 453 and TYR 489) and two hydrogen bonds for spike protein (ASN 497 and SER 814), each one hydrogen bond for NSP3 (TYR 161) and NSP9 (ARG 40) respectively. It is also important to note that the ivermectin was reported as best hits for the above mentioned targets compared to hydroxychloroquine and chloroquine against SARS-CoV-2 targets. In the following section, binding of ivermectin was compared with amentoflavone and agasthisflavone phytochemicals as they were predicted to inhibit multi-target SARS-CoV-2 proteins based on the docking score.

**Spike Protein**

X-ray crystal structure of spike glycoprotein (PDB ID: 6M0J) was chosen for performing virtual screening. Virtual screening was performed by choosing ACE interacting region as the binding site.
### TABLE 6 | Phytochemicals having strong interaction based on the top 10 hits screened for SARS-CoV-2 targets.

| S. No | Compound name | Plant name | Protein targets |
|-------|---------------|------------|-----------------|
| 1.     | Acetoside     | Clerodendrum serratum<sup>a</sup> | NSP3, SARS-CoV-2M<sup>pro</sup>, RdRp, NSP3, Spike glycoprotein |
| 2.     | Agathisflavone| Anacardium occidentale | SARS-CoV-2M<sup>pro</sup>, NSP3, Spike glycoprotein |
| 3.     | Arjunolic acid | Terminalia chebula<sup>a</sup> | NSP15, NSP9, NSP10-NSP16, Spike glycoprotein |
| 4.     | Beta-amyrin   | Cissus quadrangularis | NSP15, spike glycoprotein |
| 5.     | Catechin 7-O-gallate | Camellia sinensis | RdRp, NSP15, NSP3, Spike glycoprotein |
| 6.     | Chlorogenicin | Solarum torvum | SARS-CoV-2M<sup>pro</sup>, NSP9, NSP10-NSP16 |
| 7.     | Corilagin     | Terminalia bellica<sup>a</sup>, Terminalia chebula<sup>a</sup> | RdRp, NSP10-NSP16 |
| 8.     | Crategolic acid| Syzygium aromaticum<sup>a</sup> | NSP15, NSP10-NSP16, RdRp |
| 9.     | Cyanin        | Zingiber officinale<sup>a</sup> | SARS-CoV-2M<sup>pro</sup>, NSP9, NSP3, Spike glycoprotein |
| 10.    | Diosgenin     | Solarum nigrum, Pedalium murex | NSP10-NSP16 |
| 11.    | Emetine       | FDA approved drug | NSP10-NSP16 |
| 12.    | Friedelin     | Vitex negundo, Acorus calamus | NSP15 |
| 13.    | Hederagenin   | Nigella sativa | NSP15, NSP9 |
| 14.    | Ivermectin    | FDA approved drug | RdRp, NSP3, Spike glycoprotein |
| 15.    | Lupeol        | Carica papaya and Azadirachta indica | SARS-CoV-2M<sup>pro</sup>, NSP10-NSP16 |
| 16.    | Lupeol acetate| Pedalium murex | NSP10-NSP16 |
| 17.    | Luteolin 7-O-(6'-malonylglucoside) | Vitex negundo | SARS-CoV-2M<sup>pro</sup>, NSP3, Spike glycoprotein |
| 18.    | Luteolin 7-O-beta-D-glucoside | Vitex negundo | SARS-CoV-2M<sup>pro</sup>, NSP3 |
| 19.    | Luteolin 7-o-beta-D-glucopyranoside | Vitex negundo | Spke glycoprotein |
| 20.    | Nimbinene     | Azadirachta indica | NSP9 |
| 21.    | N-methylsolasodine | Solarum nigrum | NSP9 |
| 22.    | Oleaenic acid | Ocimum basilicum, Cyprus rotundus, Clerodendrum serratum<sup>a</sup>, Ocimum tenuiflorum | SARS-CoV-2M<sup>pro</sup>, NSP3, NSP15 |
| 23.    | Rubasic acid  | Pedalium murex | SARS-CoV-2M<sup>pro</sup>, NSP3 |
| 24.    | Solanocapsine | Solarum nigrum | SARS-CoV-2M<sup>pro</sup>, NSP9 |
| 25.    | Solasodine    | Solarum nigrum, Solarum torvum | NSP9 |
| 26.    | Spirostan-3-ol | Solarum nigrum | NSP9, NSP10-NSP16 |
| 27.    | Taraxerol     | Cissus quadrangularis | NSP9 |
| 28.    | Taraxerol acetate | Cissus quadrangularis | NSP3, NSP10-NSP16 |
| 29.    | Triterpenoid   | Abutilon indicum | NSP15, NSP10-NSP16 |
| 30.    | Ursolic-acid  | Ocimum basilicum, Pedalium murex, Malus domestica, Ocimum tenuiflorum | NSP15 |
|        | 5,7-Dihydroxy-4’-methoxy-8,3’-di-C-prenylflavanone | Azadirachta indica | NSP10-NSP16 |

(Continued on following page)
1,8-Dichloro-9,10-diphenylanthracene-9,10-diol from 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 a hydrogen bond with the 1, 8-Dichloro-9, 10-diphenylanthracene-9,10-diol. Earlier, leaf extracts of Carica papaya were reported to have significant effect in combating dengue virus infection (Rajapakse et al., 2019) 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 (Figure 7). This was followed by other small molecules viz., agasthisflavone (−8.2 kcal/mol) (Figure 4B), amentoflavone (−8.4 kcal/mol) (Figure 4C), ivermectin (−8.2 kcal/mol), three o caffeoyltormentic acid (−8.1 kcal/mol), agnuside (−7.5 kcal/mol) (Vitex negundo), taraxerol (−7.3 kcal/mol) (Cissus quadrangularis), nimbine (−7.3 kcal/mol) (Azadirachta indica) and β-amyrin (−7.3 kcal/mol) (Cissus quadrangularis) exhibiting significantly higher level of binding affinity toward spike protein of nCovid’19. Comparison of hydrogen bond interactions at the binding site for ivermectin (3 hydrogen bonds), amentoflavone (4 hydrogen bonds) and agasthisflavone (3 hydrogen bonds) showed higher number of interactions for amentoflavone involving residues TYR 449, SER 494, GLY 496 and ASP 30 respectively.

Residues are colored based on their physiochemical properties.

**Ribonucleic Acid-dependent Ribonucleic Acid Polymerase (RdRp)**

RdRp (PDB ID: 6M71) is responsible for replication of COVID-19 genome inside the host. Among the ligands tested, ivermectin (Figure 4D) showed the higher binding affinity value of −9.4 kcal/mol compared to the 744 small molecules screened in the present study. Amentoflavone stood at second position with the binding affinity value of −9.3 kcal/mol (Figure 4F). This was found to have interaction with three amino acid residues namely ARG 553, THR 556 and ASN 691. Ligands viz., Corilagin (−9.0 kcal/mol) (Terminalia bellirica and Terminalia chebula), Agasthisflavone (−8.9 kcal/mol) (Anacardium occidentale) (Figure 4E), 3-O-trans-cafeoyltormentic acid (−8.6 kcal/mol), Arjungenin (−8.2 kcal/mol) (Terminalia chebula), Crateogolic acid (−8.2 kcal/mol) (Syzygium aromaticum), 3,8-biapigenin (−8.0 kcal/mol), cyanin (−7.9 kcal/mol) (Zingiber officinale) were found to exhibit significant binding affinity. Interaction analysis showed higher number of vander waals interactions for ivermectin compared to hydrogen bond interactions. Comparison of hydrogen bond interactions exhibited toward ivermectin (3 hydrogen bonds), agasthisflavone (4 hydrogen bond interactions) and amentoflavone (6 hydrogen bond interactions−ARG 553, ARG 555, THR 556, SER 759, ASN 691 and THR 687).

**Non-structural Proteins**

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) (PDB ID: 6W02), NSP9 (ssRNA binding) (PDB ID: 6W4B), NSP10-NSP16 (co-factor in activating replicating enzyme) (PDB ID: 6W4H) and NSP15 (PDB ID: 6WO1) involved in the transcription and replication of nCoVID’19 can also serve as potential targets for containing the virus using inhibitory herbal molecules (Prajapat et al., 2020).

Virtual screening of small molecules against NSP3 identified amentoflavone (Mangifera indica) as the top scored ligand with binding affinity of −7.5 kcal/mol (Figure 5C). In the decreasing order of binding affinity, luteolin 7-O-(6’-malonylglucoside) (−7.1 kcal/mol) (Vitex negundo), rubusic acid (−6.8 kcal/mol) (Pedalia murex), acteoside (−6.7 kcal/mol) (Clerodendrum serratum), ivermectin (−6.7 kcal/mol), taraxerol acetate (−6.7 kcal/mol) (Cissus quadrangularis), catechin 7-O-gallate (−6.6 kcal/mol), luteolin 7-O-beta-D-glucoside (−6.6 kcal/mol) (Vitex negundo), agasthisflavone (−6.6 kcal/mol) (Anacardium occidentale) (Figure 5B) and luteolin-7-o-beta-D-glucopyranoside (−6.6 kcal/mol) (Vitex negundo) were found to have higher binding affinity next to amentoflavone. It was observed that three inhibitors from Vitex negundo were reported with the highest binding scores. Vitex negundo belongs to Verbenaceae family known for its effects for ailments like ophthalmia, deafness, indigestion, piles and jaundice (Venkateswarlu, 2012). Results of earlier experiments conducted by Wu et al. (2020) also revealed similar findings of vitexin from Vitex negundo exhibiting significant binding affinity toward 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 (PDB ID: 6W4B) (Table 5). Even though, 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 plants known for its use in treatment of seisure, pain, ulcer, inflammation, diarrhea, eye infections, jaundice and oxidative stresses (Jain et al., 2011; Javed et al., 2011; Wang et al., 2013; Zaidi et al., 2014).

Virtual screening against NSP15 (PDB ID:6W01) identified oleonolic acid and usorolic acid as molecules exhibiting highest binding affinity values of −9.2 kcal/mol. Oleonolic acid and usorolic acid are known for their anti-cancerous and anti-inflammatory activities (Arshad Qamar et al., 2010; Kashyap et al., 2016; Yu et al., 2017). Both the phytochemicals are reported to be enriched in Ocimum basilicum and Ocimum tenuiflorum (Table 5). This was followed by crategolic acid (−8.9 kcal/mol) (Syzygium aromaticum), arjungenin (−8.8 kcal/mol) (Terminalia chebula), hedergenin (−8.7 kcal/mol) (Nigella sativa), triterpenoid (−8.6 kcal/mol) (Abutilon indicum), beta-amyrin (−8.6 kcal/mol) (Cissus quadrangularis), friedelin (−8.6 kcal/mol) (Vitex negundo, Acorus calamus), catechin 7-O-gallate (−8.5 kcal/mol) (Camellia sinensis) and arjunolic acid (−8.5 kcal/mol) (Terminalia chebula).

In the case of NSP10-NSP16 (PDB ID: 6W4H) protein complex, interface of the complex (Figure 6B) was chosen as the binding site for performing virtual screening. Compounds such as amentoflavone (−8.5 kcal/mol), 10-methoxycamptothecin (−8.3 kcal/mol), 3,8-biapigenin (−8.2 kcal/mol), taraxerol acetate (−8.0 kcal/mol) (Cissus quadrangularis), corilagin (−7.8 kcal/mol) (Terminalia bellirica and Terminalia chebula), lupeol acetate (−7.8 kcal/mol) (Pedalia murex), emetine (−7.6 kcal/mol), chlorogenin (−7.6 kcal/mol) (Solanum torvum), spirostan-3-ol (−7.6 kcal/mol) (Solanum torvum) and 5,7-Dihydroxy-4’-methoxy-8’-di-C-prenyllavonone (−7.6 kcal/mol) (Azadirachta indica)
were ranked among the top 10 molecules exhibiting highest binding affinity.

**Molecules Exhibiting Inhibitory Activity Against Multiple Protein Targets of nCOVID’19**

Phytochemicals showing strong interactions 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 toward multiple targets of nCOVID’19.

Amentoflavone showed docking score of RdRp (−9.3 kcal/mol) (Figure 4C), NSP9 (−8.3 kcal/mol) (Figure 5F), NSP3 (−7.4 kcal/mol) (Figure 5C), NSP10-NSP16 (−8.5 kcal/mol) and spike glycoprotein (−8.2 kcal/mol). In all the docked complexes, the target-ligand binding affinity was greater than (−8.0 kcal/mol) except NSP3. 3D and 2D Protein-ligand interactions exhibited by the small molecules amentoflavone and agathisflavone are shown in Figure 4, Figure 5 and Supplementary Table S3. Molecular interactions such as hydrogen bond, vander waals interaction, pi interaction were observed to be higher for amentoflavone in comparison with the drug ivermectin. Amentoflavone is a naturally occurring biflavonoid reported to be found in more than 120 plants (Yu et al., 2017). 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 (Yu et al., 2017). Evidences have been reported for amentoflavone exhibiting anti-senescent activity in the cardiovascular and central nervous system (Park et al., 2011). Further, Amentoflavone isolated from Torreya nucifera was demonstrated to possess inhibitory activity against SARS-CoV3CL<sup>Pro</sup> (Ryu et al., 2010).

Similarly, agathisflavone was found to exhibit significant interaction against four different protein targets viz., RNA-dependent RNA polymerase (−8.9 kcal/mol), SARS-CoV-2 main protease (−8.2 kcal/mol), spike glycoprotein (−8.2 kcal/mol) and NSP 3 (−6.6 kcal/mol) (Supplementary Table S3). Catechin-o-gallate was also found to possess significant binding affinity toward 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). Role of catechins for antiviral activity against the number of viruses due to the increased affinity for cellular membranes has been reported in many articles (Kaihatsu et al., 2018). Chlorogenin from Solanum torvum exhibited binding affinity value of −8.2 kcal/mol (NSP9), −7.7 kcal/mol (M<sup>Pro</sup>) and −7.6 kcal/mol (NSP16-NSP10) of SARS-CoV-2. Solanum torvum is well known for its pharmacological properties for the treatment of human ailments and its phytocnsituent chlorogenin also has antiviral effect on influenza viruses (Agrawal et al., 2010; Lim, 2013; Shi et al., 2020).

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 6) (Figure 8). Among the above reported 15 compounds, cyanin targeted all the three Coronavirus M<sup>Pro</sup>. 2D representation of M<sup>Pro</sup>-cyanin interaction of all the coronaviruses is provided in the Supplementary Table S5.

**DISCUSSION**

Among the 605 phytochemicals originating from 37 plant species, 33 (6% approx.) phytochemicals from 22 plants (Figure 8) were found to be the best hits with higher binding affinities against all the seven targets (Table 5). Among those 22 plants, four 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 32 different phytochemicals that were included in this study. Of the 33, 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 five different protein targets of SARS-CoV-2 namely, spike glycoprotein, SARS-CoV-2 main protease, NSP3, NSP9, NSP15 in the SARS-CoV-2.

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-CoV-2M<sup>Pro</sup>, NSP9 and NSP16-NSP10).

Pedalium murex was reported to have Diosgenin (NSP9), Lupeol acetate (NSP16-NSP10), Urosolic acid (NSP15) and Rubusic acid (SARS-CoV-2 M<sup>Pro</sup>, NSP3) which might be probable SARS-CoV-2 inhibitors. It is noteworthy that maximum of five targets were predicted to be inhibited by the compounds from Pedalium murex. In spite of its role as anti-ulcerogenic, nephroprotective, hypolipidemic, aphrodisiac, anti-oxidant, anti-microbial and insecticidal activities, Pedalium murex has been traditionally used in treating ailments like cough and cold as a regular practice (Patel et al., 2011a).

Medicinal plants namely, Azadirachta indica, Terminalia chebula, Cassia quadrangularis, Clerodendrum serratum and Ocimum basilicum reported more than two phytochemicals which effectively inhibited SARS-CoV-2 targets. Intensive genomics and proteomics research may lead to identification of novel drugs against this pandemic disease.

Knowledge of the phytochemical composition in the reported plants is important to understand the role of bioactive molecule in the traditional medicine. Phytochemical composition of the some of the screened plants quantified using various analytical techniques are given as follows: Cyanin (Zingiber officinale) (20%) (Ghasemzadeh et al., 2010), amentoflavone (Mangifera indica) (90.92 ± 4.91 mg/100 g) (Ma et al., 2011), agathisflavone...
Antiviral activity of the plants highlighted in this study, whose phytochemicals are predicted to have inhibitory effect on SARS-CoV-2 targets have proven antiviral role on many other human pathogenic viruses. Antiviral activity of *Vitex negundo L.* against Chikungunya virus and HIV-1 Reverse transcriptase (RT) have been previously reported by Kothandan and Swaminathan (2014) and Nair and Ramachandran (2012) (Nair, 2012; Kothandan and Swaminathan, 2014). *Solanum nigrum* was reported to have antiviral effect against Hepatitis C Virus chronic infection (Javed et al., 2011). Similarly, the antiviral and virucidal effect of neem leaves was studied regarding its activity and possible mechanism of action against Coxsackie B group of viruses (Badam et al., 1999). *Swagami* and *Sailaja 2021* (*Swagami* and *Sailaja, 2021*) have reported the antiviral activity of *Terminalia chebula* (0.11%) (Kumar et al., 2010), Rutin (*Ocimum basilicum*) (425.71 ± 2.15 μg/g) (Vlase et al., 2014) and Isoquercetin (*Ocimum basilicum*) (179.19 ± 1.93 μg/g) (Vlase et al., 2014).

Antiviral activity of the plants highlighted in this study, whose phytochemicals are predicted to have inhibitory effect on SARS-CoV-2 targets have proven antiviral role on many other human pathogenic viruses. Antiviral activity of *Vitex negundo L.* against Chikungunya virus and HIV-1 Reverse transcriptase (RT) have been previously reported by Kothandan and Swaminathan (2014) and Nair and Ramachandran (2012) (Nair, 2012; Kothandan and Swaminathan, 2014). *Solanum nigrum* was reported to have antiviral effect against Hepatitis C Virus chronic infection (Javed et al., 2011). Similarly, the antiviral and virucidal effect of neem leaves was studied regarding its activity and possible mechanism of action against Caxsackie B group of viruses (Badam et al., 1999). *Swagami* and *Sailaja 2021* (*Swagami* and *Sailaja, 2021*) have reported the antiviral activity of *Clerodendrum serratum* against yellow fever virus. Likewise, antiviral effect of *Terminalia chebula* on swine influenza A virus infection has also been proved (Ma et al., 2010). Phytochemicals like apigenin and ursolic acid from *Ocimum basilicum* has shown broad-spectrum of antiviral activity against many of the DNA and RNA viruses (Chiang et al., 2005). *Zingiber officinale* has been proved to have antiviral activity against human respiratory syncytial virus (Chang et al., 2005). Also, the plants like *Mangifera indica* and *Anacardium occidentale* L. inhibits Herpes simplex virus two and influenza virus neuraminidase respectively (Parvez, 2016; de Freitas et al., 2020).

ADME properties are important to understand the pharmacokinetics and physio-chemical properties of phytochemicals which further provides insight on drug design and formulation research. Analysis on solubility and bioavailability based on the SWISSADME prediction for the screened inhibitors showed different level of classifications as given in Supplementary Table S2. According to the Silicos-IT LogSw score, 41% of the screened ligands were classified soluble, 31% (moderately soluble), 27% (poorly soluble) and one molecule (1,8-Dichloro-9,10-diphenylanthracene-9,10-diol) alone as insoluble. Bioavailability score predicted on the probability of a compound to have at least 10% oral bioavailability (Martin, 2005) in rat has been reported in which one compound (utelog 7-O-(6’-malonylglyceride)) has less score (0.11 score), 17 compounds have (0.17 score), 27 compounds have (0.55 score) and three compounds (ursolic acid, betulinic acid and oleanolic acid) with 0.85 score respectively.

CONCLUSION

Generation of improved knowledge and understanding biochemical and molecular basis of herbs 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 (PDB ID: 5C3N), SARS-CoV (PDB ID: 2GCZ9) and SARS-CoV-2 (PDB ID: 5R81) revealed significant differences between the three homologs which were confirmed by differential binding affinity exhibited by 744 phytochemicals/small molecules against the three main proteases. Cyanin from *Zingiber officinale* was screened as the best phytochemical with highest binding energy against main proteases of all the three viruses. Popular fruit tree, mango (*Mangifera indica*) and, cashew nut (*Anacardium occidentale*) rich in amento flavone and agathis flavone were showing possible 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 that may have possible inhibitory activity against SARS-CoV-2 proteins. More interestingly, this study has picked up *Carica Papaya* that may possess inhibitory activity against spike glycoprotein and MPro of SARS-CoV-2 which was known for its protective role against dengue virus in humans (Rajapakse et al., 2019). Overall, this study has shortlisted potential phytochemicals that may have inhibitory activity against SARS-CoV-2 which could be taken for further testing, formulation and discovery of novel drugs.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/Supplementary Material.

AUTHOR CONTRIBUTIONS

SN, JM, CR, KA, and BN performed experimental, data analysis, and drafting of original manuscript. KN, GR, RM, MS, and KN conceptualized the experiment, refined the manuscript, funded the project, and helped in revising the manuscript. All authors read and approved the final version of the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2021.667704/full#supplementary-material
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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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