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Targeting non-structural proteins and 3CL\textsuperscript{pro} in SARS-CoV-2 virus using phytochemicals from medicinal plants - In-silico approach

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\textbf{ABSTRACT}

In the present study, the main protease 3CL\textsuperscript{pro} and non-structural protein (NSP-12 with co-factors 7 and 8) trimer complex are used to study the protein-drug interactions with the phytochemicals from \textit{Ocimum Sanctum}, \textit{Tinospora Cordifolia}, \textit{Glycyrrhiza Glabra}, and \textit{Asadachtra Indica}. Which can give insight to be used as potent antiviral drugs against SARS-CoV-2. Twenty phytochemicals, five from each plant species, known for their wide range of biological activities were chosen from the literature. The in-silico study was carried out using virtual screening tools and the top five, which showed the least binding energies, were selected. Molecular docking tools revealed that gedunin and epoxy azadiradione proved to be excellent inhibitors for 3CL\textsuperscript{pro} and so did Tinosporide for non-structural-protein complex. Further, the best-hit phytochemicals with respect to structure similarities with FDA drugs and investigatory drugs, were considered for comparative study. Molecular docking was done to check the drug-protein interactions and to check the inhibitory responses of these drugs against the viral protein. The analyses showed that the phytochemicals had similar responses on the protein complex but with exceptionally higher inhibitory responses hence which may be taken for further clinical study.

1. Introduction

Coronaviruses have become the major pathogens of emerging respiratory disease outbreaks. They are a large family of single-stranded RNA viruses that can be isolated in different animal species. They possess a crown-like appearance under an electron microscope due to the presence of spike glycoprotein on the envelope \cite{1}. SARS-CoV-2 is a \textit{Beta coronavirus} like the SARS and MERS human coronaviruses \cite{2,3}. The occurrence of the illness ranged from mild to severe. SARS-CoV-2 propagates through RNA replication using RNA-dependent RNA polymerase \cite{4}. SARS-CoV-2 releases its genomic material in the cytoplasm and becomes translated into the nuclei. The genomic material released by this virus is mRNA that is ready to be translated into protein. In its genome range, this virus is complemented by about 14 open reading frames (ORFs), each of which encodes a variety of proteins, both structural and non-structural that play a role in its survival as well as virulence power \cite{5}. The viral 3-chymotrypsin-like cysteine protease (3CL\textsuperscript{pro}) enzyme controls coronavirus replication and is essential for its life cycle. SARS-CoV 3CL\textsuperscript{pro} and SARS-CoV-2 3CL\textsuperscript{pro} share a 99.02\% sequence similarity with 12-point mutations \cite{6}. Usually, beta-coronaviruses produce an 800 kDa polypeptide upon transcription of the genome. This polypeptide is proteolytically cleaved to generate various proteins. The proteolytic processing is mediated by papain-like protease (PL\textsuperscript{pro}) and 3-chymotrypsin-like protease (3CL\textsuperscript{pro}). The 3CL\textsuperscript{pro} cleaves the polypeptide at 11 distinct sites to generate various non-structural proteins that are important for viral replication \cite{7}.

The proteins of SARS-CoV consist of two large polypeptides: ORF1a and ORF1ab (that proteolytically cleaved to form 16 non-structural proteins), four structural proteins: spike (S), envelope (E), membrane (M), and nucleocapsid (N), and eight accessory proteins: ORF3a, ORF3b, ORF6, ORF7a, ORF7b, ORF8a, ORF8b, and ORF9b \cite{8}. Nsp12 is the RNA dependent RNA polymerase which is vital for viral replication. Nsp7 forms a hexadecameric complex with nsp8, which acts as a processivity clamp for nsp12. This shows that nsp7 can act as potential drug targets \cite{9,10}. In COVID-19, it is thought that the drugs keep the virus out of host cells by blocking the glycosylation of host receptors and breaking
down the production of viral proteins. But Hydroxychloroquine shows no significant reduction in deaths related to COVID-19. Other drugs include Ritonavir,洛匹那韦, Napamostat, Mafotamide, and Cortisosteroid and nowadays, convalescent plasma is also used to treat COVID-19 patients. Since many Indian medicinal plants exhibit anti-viral, anti-inflammatory, and antioxidant properties, it may be favorable to consider them for the treatment of COVID-19. Standard clinical trials should be carried out to scientifically prove its efficacy [11]. The amalgamation of medicinal plants as potential drugs for COVID-19 is very much possible, provided there are more research and funding in clinical trials.

Human coronaviruses generally are positive-sense and very long (30,000 bp) single-stranded RNA viruses. Two groups of protein characterize Human Coronaviruses-structural, such as Spike (S), Nucleocapsid (N) Matrix (M), and Envelope (E), and non-structural proteins such as RNA dependent RNA polymerase (RdRp) also called nsp12. Among the different Non-Structural Proteins mentioned, the NSP12 subunit is the essential RdRp (RNA-dependent RNA polymerase) of the coronavirus replication machinery, which was even able to extend a homopolymeric primer-template substrate by a few dozen nucleotides in vitro [12]. The amino acid sequence alignment revealed that the NSP12 of SARS-CoV-2 shared 96.3% similarity with the NSP12 of SARS (Ruan et al., 2020). NSP12 is the RNA-dependent RNA polymerase that copies viral RNA. NSP12 exhibits poor processivity in RNA synthesis that is the presence of NSP7 and NSP8 lowers the dissociation rate of NSP12 from RNA [13]. Since the sequence similarity of SARS-CoV and SARS-CoV-2 is approximately the same, and nsp12 is very important for viral replication, inhibiting it is very important. Any drug molecule that can inhibit nsp12 and stop the replication, can stop the development of serious symptoms relating to SARS-CoV and SARS-CoV-2. Hence, one of the proteins we chose has the PDB ID: 7BV2 which is NSP12-NSP7-NSP8 complex bound to the template-primer RNA and triphosphate form of Remdesivir.

The viral 3-chymotrypsin-like cysteine protease (3CL^pro) enzyme controls coronavirus replication and is essential for its life cycle. 3CL^pro is a proven drug discovery target in the case of severe acute respiratory syndrome coronavirus (SARS-CoV) and Middle East respiratory syndrome coronavirus (MERS-CoV). Recent studies revealed that the genome sequence of SARS-CoV-2 is very similar to that of SARS-CoV [6]. The SARS-CoV main protease (M^pro), also called 3-Chymotrypsin like protease (3CL^pro), plays a key role in proteolytic processing of viral polyproteins, essential proteins for viral replication and function, is considered as a key drug target. Lopinavir and Ritonavir were tested as potential inhibitors of 3CL^pro. The other two proteins of our choice have PDB ID’s – 6XMK and 6M2N. 6XMK refers to the 1.70 Å resolution structure of SARS-CoV-2 3CL protease in complex with an inhibitor. 6M2N SARS-CoV-2 3CL protease (3CL pro) in complex with a novel inhibitor with a resolution of 2.20 Å. Hence, inhibitors that block the function of 3CL^pro and nsp12 with nsp-7 and nsp-8 as the cofactors can be expected to inhibit virus replication, making this enzyme one of the best targets for the treatment of COVID-19.

The plants of choice for this work include Ocimum sanctum, Glycer rhiza glabra, Asadhrachta indica, and Tinospora cordifolia. All the phytochemicals were chosen after thorough research. Active phytoconstituents of ayurvedic medicinal plants like T. cordifolia and O. sanctum are predicted to significantly hinder the main protease (M^pro or 3CL^pro) of SARS-CoV-2. O. sanctum has been proven to possess properties of anti fertility, anticaen, anti diabetic, anti fungal, anti microbial, anti apoptotic, cardioprotective, anti neoplastic, anti spasmodic, and analgesic actions [14]. T. cordifolia has a wide array of bioactive principles as well as it has been proven medicinally important plant, and have not received considerable scientific attention. The therapeutic properties of medicinal plants are attributed owing to the presence of active substances such as alkaloids, flavonoids, glycosides, vitamins, tannins, and coumarins [15]. Compounds like berberine, choline, Pal matine show anti-viral infections, antitancer, anti diabetic, inflammation, neurological, immunomodulatory actions [16]. G. glabra has many phytochemicals that have varied functions. Glycyrrhizic acid and glabridin which are triterpenoid saponin have anti ulcer, anti oxidative, corticosteroid, anti viral, anti HIV functions. They also possess immunostimulant actions. Isoliquiritigenin which is a flavonoid has anti microbial functions [16]. A. indica possesses maximum useful non wood products (leaves, bark, flowers, fruits, seed, gum, oil, and neem cake) than any other tree species. These non-wood products are known to have antiallergenic, anti dermatic, anti feadent, anti fungal, anti inflammatory, anti pyretic, antiscab, cardiac, diuretic, insecticidial, larvicidal, nematicidial, and spermicidal and other biological activities. Because of these activities’ neem has found enormous applications making it a green treasure. Based on exhaustive literature survey we have chosen phytochemicals from Ocimum sanctum, Tinospora Cordi folia, Glycer rhiza Glabra, and Azadirachta Indica. Also, alkaloid based phytochemicals derived from marine plants such as tetradrine, lyc orine, berberine, ergotamine, cepharanthine, palmatine, quinine and noscapine, showed prominent effect against SARS-CoV-2.

In our study, in silico studies of SARS-CoV-2 proteins: 3CL^pro and NSP12 trimer complex against phytochemicals of selected medicinal plants were conducted. 5 phytochemicals were noted from every plant and virtual screening was performed. The Top 5 phytochemicals from the docking result of each protein were obtained. The top 2 results from Auto Dock were recorded and FDA-approved/under investigational drug comparison studies were performed.

2. Methodology

2.1. Molecular Docking

2.1.1. Macromolecule and ligands preparation

The three-dimensional structures of SARS-CoV-2 proteins: 3CL^pro (PDB ID: 6M2N and 6XMK) and NSP12-NSP7-NSP8 complex (PDB ID: 7BV2) were retrieved from RCSB Protein Data Bank in pdb format. Water, ligand molecules, and the extra chains were removed using Discovery Studio Visualizer version 20. Protein energy minimization was carried out in AutoDockTools 1.5.6 [17] by adding polar hydrogen and Kollman charges to these proteins. Phytochemicals of A. indica, O. sanctum, T. cordifolia, and G. glabra, listed in Table 1, were searched and sorted based on the highest activity in Dr. Duke’s phytochemical and ethnobotanical databases. All the phytochemicals were downloaded from PubChem in SDF format. But 3D structures of two phytochemicals: 1-Octacosanol and tinosporide, were generated using ChemDraw as it was not available in PubChem. The SDF files were converted to pdb files in Open Babel software [18]. Energy minimization of ligands was done using an auto-optimization tool in Avogadro software [19] with force field UFF. Minimization process was continued until Δ E = 0, and ligands were saved in pdb format.

2.1.2. Virtual screening

PyRx 0.8 (Dallakyan and Olson, 2015) was used to virtually screen 20 phytochemicals against the three proteins. Vina wizard is the docking algorithm used for virtual screening. In PyRx, a grid box to cover complete protein was created after loading the protein and all the 20 ligands [Table 2]. The exhaustiveness was set to 50 for the complete process. The top 5 best hits, after the virtual screening, were ranked based on minimum binding energy with upper and lower RMSD equal to zero. The top 5 best hits out of 20 phytochemicals, obtained for each protein, were further analyzed by docking in AutoDockTools 1.5.6 [17].

2.1.3. Docking studies

Molecular Docking studies for the top 5 best hits against SARS-CoV-2 3CL^pro (PDB ID: 6M2N and 6XMK) and NSP12-NSP7-NSP8 (PDB ID: 7BV2) complex was performed using AutoDock Tools [17]. Prepared pdb file of protein and minimized ligand was used for docking. The grid parameter file was prepared by creating a grid box (dimensions: 55 Å
2.3. ADMET study

The drug-like properties of top compounds, which showed a considerable binding affinity against SARS-CoV-2 3CL\textsuperscript{pro} and NSP12-NSP7-NSP8 complex, were analyzed in two database servers: SWISS-ADME [20] and pkCSM [20]. ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) study is important in analyzing pharmacokinetic properties of all ligands that can be used as a drug. SWISS-ADME was used for predicting physicochemical properties, drug-likeness, and medicinal chemistry. pkCSM was used for predicting ADMET properties. Lipinski’s rule of five [21] determines the drug-likeness property of ligands.

3. Results and discussion

The protein 6M2N and 6XMK, belonging to the 3CL protease group (3-chymotrypsin-like protease), is known for cleaving the polyprotein at 11 distinct sites to generate non-structural proteins that are vital for viral replication. The protein 7BV2, belonging to the trimer NSP12, is the RNA-dependent RNA polymerase that copies viral RNA. NSP12 makes a complex with an NSP7-NSP8 heterodimer and an NSP8 monomer to increase the processivity of NSP12. Fig. 1 depicts the three-dimensional structures of proteins SARS CoV-2 3CL\textsuperscript{pro} (PDB ID: 6M2N and 6XMK) and NSP12-NSP7-NSP8 complex (PDB ID: 7BV2), after removing water molecules, ligand molecules, and chains that are not under study.

3.1. Virtual screening analysis

A virtual screening of selected twenty phytochemicals was performed against the three proteins selected in PyRx. The top 5 best hits were selected for each protein with minimum binding energy and upper and lower RMSD equal to zero and were taken for further docking analysis. Top 5 phytochemicals against 6M2N, 6XMK and 7BV2 are: 11-deoxoglycyrrhetinic acid from yashtimadhu, nimocinol, gedunin from Neem, glabridin from yashtimadhu, and epoxy azadiradione from Neem with the least binding energies and represented in Table 2. A graphical representation of the least binding energy of the different models was done in Fig. 2.

3.2. Molecular docking analysis

Molecular docking was carried out for the best 5 phytochemicals against the prepared protein separately. The docked poses for each ligand with ten different conformations (ranked by energy) were analyzed from the .dlg file. Results of binding affinity, reference RMSD, inhibition constant, ligand efficiency, (vdW + H-bond + desolv) energy were tabulated for three proteins respectively in Table 3. From the theoretical study it has been observed that, the phytochemical-epoxy azadiradione (from Neem plant) can be a better inhibitor for protein SARS-CoV-2 3CL\textsuperscript{pro} (PDB: 6M2N) from Table 3, phytochemical- Gedunin (from Neem plant) can be a better inhibitor for protein SARS-CoV-2 3CL protease (PDB: 6XMK) and phytochemical-tinosporide (from Guduchi plant) can be a better inhibitor for protein SARS-CoV-2 NSP 12 bound to NSP 7 and NSP 8 (PDB: 7BV2), with better binding affinities, ligand efficiencies, and inhibition constants, compared to the other phytochemicals. This suggests that use of this plants in the regular food practices may help in controlling the growth of SARS-CoV-2 3CL.

### Table 1
List of phytochemicals of various medicinal plants.

| Plant Phytochemicals | Ocimum Sanctum (Tulsi) | Tinospora Cordifolia (Guduchi) | Asparagus Glabra (Yashtimadhu) |
|----------------------|------------------------|-------------------------------|-------------------------------|
| Eugenol              |                        | berberine                     | nimbol                        |
| α-farnesene          |                        | palmatine                     | nimbol                        |
| methyl isoeugenol    |                        | choline                       | quercetin                     |
| cyclohexane,1,2,4-triethyl |                | tinosporide                   | epoxy azadiradione            |
| Caryophyllene        |                        | -1 octacosanol                | gedunin                       |
|                      |                        |                               | kaempferol                    |

### Table 2
Binding affinities of phytochemicals against SARS CoV-2 3CL\textsuperscript{pro}.

| Plant | Phytochemicals | Binding energy (kcal/mol) in PDB ID: 6M2N | Binding energy (kcal/mol) in PDB ID: 6XMK | Binding energy (kcal/mol) in PDB ID: 7BV2 |
|-------|----------------|-------------------------------------------|-------------------------------------------|-------------------------------------------|

*55 Å *55 Å-for PDB ID: 6M2N and 7BV2; and 70 Å*60 Å*60 Å for PDB ID: 6XMK* around active site pockets of protein. The docking parameter file was prepared for Auto-Dock using the Lamarckian genetic algorithm. The docked structure ranked by minimum binding energy for each ligand with different conformations, were analyzed. The top 2 best hits from docking were further studied with FDA approved/under investigation drugs. Visualization of the protein-ligand complex was done using Discovery Studio Visualizer version 20. Some of the results that were analyzed: 3D structure of protein-ligand complex, 2-D diagram of bonding interactions between protein and ligand in the complex, and representation of different surfaces of ligand that acts as donor/acceptor of the hydrogen bond.

2.2. Comparison between the best phytochemicals and FDA approved/under investigation drugs

The top 2 best phytochemicals against each protein obtained from docking studies were taken for further analysis. FDA approved/under investigation drugs, that were structurally similar to the top 2 best hits for each protein, was identified by an extensive literature survey. Docking studies were conducted for FDA approved/under investigational drugs using AutoDockTools-1.5.6.

2.3. ADMET study

The drug-like properties of top compounds, which showed a considerable binding affinity against SARS-CoV-2 3CL\textsuperscript{pro} and NSP12-NSP7-NSP8 complex, were analyzed in two database servers: SWISS-ADME [20] and pkCSM [20] ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) study is important in analyzing pharmacokinetic properties of all ligands that can be used as a drug. SWISS-ADME was used for predicting physicochemical properties, drug-likeness, and medicinal chemistry. pkCSM was used for predicting ADMET properties. Lipinski’s rule of five [21] determines the drug-likeness property of ligands.
3.3. Visualization

The protein and ligand complexes were opened in Discovery Studio Visualizer version 20 and the three different types of images shown below are the Surface Ligand Interaction, the 2D structure depicting the different types of bonds, and the 3D structure of the protein-ligand complex. Fig. 3 depicts the visualization of the complex of SARS-CoV-2 3CL\textsuperscript{pro} (PDB ID: 6M2N)-Epoxy Azadiradione (Neem). Fig. 4 depicts the visualization of the complex of SARS-CoV-2 3CL\textsuperscript{pro} (PDB ID: 6XMK)-Gedunin (Neem). Fig. 5 depicts the visualization of the complex of SARS-CoV-2 Nsp12 bound to Nsp7 and Nsp8 (PDB ID: 7BV2) - Tinosporide (Guduchi).

3.4. Comparison between the best phytochemicals and FDA approved/under investigation drugs

Form the docking studies it has been observed that two phytochemicals from the selected medicinal plants showed best results on SARS-CoV-2 3CL hence they are compared with the FDA approved/under investigation drugs with similar structures also have the same effect as the ones extracted from plants. The comparison was done between the phytochemicals found in the medicinal plants and the drugs approved by the FDA/under investigation, that have similar structures that have been used for different purposes. For protein 6M2N, the best

Table 3

| Plant   | Phytochemical            | Reference RMSD (Å) | Binding Energy (kcal/mol) | Ligand Efficiency | The inhibition constant $K_i$ (μM) | vdW + H-bond + desolv energy (kcal/mol) | PDB ID |
|---------|--------------------------|--------------------|---------------------------|-------------------|-----------------------------------|-----------------------------------------|--------|
| Neem    | epoxy azadiradione       | 78.79              | -8.64                     | -0.25             | 0.466                             | -9.37                                   | 6M2N   |
| Yashtimadhu | Glabridin            | 82.21              | -8.49                     | -0.35             | 0.596                             | -9.22                                   | 6M2N   |
| Neem    | Nimbocinol             | 81.51              | -8.38                     | -0.25             | 0.721                             | -9.59                                   | 6M2N   |
| Neem    | Gedunin                | 79.84              | -8.34                     | -0.24             | 0.769                             | -9.28                                   | 6M2N   |
| Yashtimadhu | 11-deoxoglycyrrhetinic acid | 79.48              | -7.89                     | -0.24             | 1.66                              | -8.75                                   | 6M2N   |
| Neem    | Gedunin                | 34.25              | -8.52                     | -0.24             | 0.5641                            | -9.35                                   | 6XMK   |
| Guduchi | Berberine              | 35.01              | -8.33                     | -0.33             | 0.788                             | -8.92                                   | 6XMK   |
| Guduchi | Tinosporide            | 35.67              | -8.19                     | -0.3              | 0.995                             | -8.78                                   | 6XMK   |
| Yashtimadhu | Kaempferol          | 33.89              | -8.16                     | -0.39             | 1.04                              | -9.33                                   | 6XMK   |
| Neem    | Quercetin              | 33.87              | -8.04                     | -0.37             | 1.28                              | -9.53                                   | 6XMK   |
| Yashtimadhu | 11-deoxoglycyrrhetinic acid | 37.15              | -7.29                     | -0.23             | 4.52                              | -8.27                                   | 6XMK   |
| Guduchi | Tinosporide            | 163.23             | -7.69                     | -0.28             | 2.32                              | -7.71                                   | 7BV2   |
| Yashtimadhu | 11-deoxoglycyrrhetinic acid | 165.36             | -7.66                     | -0.23             | 2.44                              | -6.47                                   | 7BV2   |
| Neem    | Gedunin                | 163.96             | -7.26                     | -0.21             | 4.74                              | -7.82                                   | 7BV2   |
| Neem    | epoxy azadiradione     | 160.61             | -7.23                     | -0.21             | 5.03                              | -7.78                                   | 7BV2   |
| Neem    | Quercetin              | 168.1              | -6.81                     | -0.31             | 10.11                             | -8.41                                   | 7BV2   |
Fig. 3. SARS CoV-2 3CL\textsuperscript{pro} (PDB ID: 6M2N)-epoxy azadiradione (Neem): (A) H-bond interaction between Epoxy Azadiradione and 3CL\textsuperscript{pro}. (B) 3-D structure of 3CL\textsuperscript{pro}. Epoxy Azadiradione complex. (C) 2-D diagram of binding interactions of the ligand in the active site pockets of protein.

Fig. 4. SARS CoV-2 3CL\textsuperscript{pro} (PDB ID: 6XMK) - Gedunin (Neem): (A) H-bond interaction between Gedunin and 3CL\textsuperscript{pro}. (B) 3-D structure of 3CL\textsuperscript{pro}-Gedunin complex. (C) 2-D diagram of binding interactions of the ligand in active site pockets of protein.

Fig. 5. SARS CoV-2 Nsp12-Nsp7-Nsp8 complex (PDB ID: 7BV2) - Tinosporide (Guduchi): (A) H-bond interaction between tinosporide and Nsp12-Nsp7-Nsp8 complex. (B) 3-D structure of Nsp12-Nsp7-Nsp8 complex-tinosporide complex. (C) 2-D diagram of binding interactions of the ligand in active site pockets of protein.
two inhibitors were: Epoxy Azadiradione and Glabridin. The drugs with structural similarities concerning Gedunin and berberine were Berberinic acid and Myristinin A respectively. Epoxy Azadiradione is a limonoid which is a class of phytochemicals belonging to the pentacyclic triterpenoid class. Berberinic acid is also a pentacyclic triterpenoid. From the literature survey, we found out Berberinic acid, which is structurally similar and with biological activities like antiviral, anti-inflammatory, analgesic, and antitumoral, and is under investigation. Glabridin is a hydroxysisoflavans. From the literature survey, we found out Myristinin A, which is structurally similar and with biological activities like antiviral and antitumoral and is under investigation.

For protein 6XMK, the best two inhibitors were: Gedunin and Berberine. The drugs with structural similarities for gedunin and berberine were corosolic acid and papaverine respectively. Gedunin is a triterpenoid tetracyclic which belongs to the class of phytochemicals extracted from Neem. The FDA drug with an almost similar structure belongs to the class of compounds is corosolic acid which is a pentacyclic triterpene acid. From the literature, we found out Corosolic acid is well-known for many biological activities like antidiabetic, anti-inflammatory, antiproliferative, and protein kinase-C inhibition activity. Testing has been done on cancer cell lines. The next best phytochemical is berberine. It is a quaternary ammonium salt from the protobERRibeRINE group of benzylisoquinoline alkaloids. The direct parent of Papaverine is Benzylisoquinoline. Papaverine is a direct-acting smooth muscle relaxant used in the treatment of impotence and as a vasodilator, especially for cerebral vasodilation. It is a drug approved by the FDA.

For protein 7BV2, the best two inhibitors were: Tinosporide and 11-Deoxoglycyrrhetinic Acid. For tinosporide, the drug chosen was Salvinorin A. Salvinorin A is considered a dissociative hallucinogen. It is approved by the FDA as a vasodilator, especially for cerebral vasodilation. It is a drug approved in the treatment of peptic, esophageal and oral ulceration and is under investigation.

Docking analysis of these selected drugs was conducted. Results of docking analysis of phytochemicals and FDA approved/under investigation drugs were compared and tabulated in Table 4 for each protein respectively. The structures of phytochemicals and the FDA drugs with similar structures are depicted below (Figs. 6, 7 and 8).

Docking analysis of these selected drugs was conducted. Results of docking analysis of phytochemicals and FDA approved/under investigation drugs were compared and tabulated in Table 4. For protein 6M2N, it can be observed that all the active amino acids in both Epoxy Azadiradione and the drug Berberinic acid match except for GLY143. The ligand efficiency is also similar. Though the binding energies are not the exactly same, it can be seen that berberine acid can act as a potential inhibitor based on the comparison with the phytochemical epoxy azadiradione. Similarly, it can be observed that 12 active amino acids in both the phytochemical glabridin and the drug Myristinin –A match. Myristinin A, which is structurally similar to glabridin and with biological activities like antitumoral and is under investigation. Hence, it might be a potent inhibitor against SARS-CoV-2. Also, it can be used for further study on different types of SARS viruses.

For protein 6XMK, from Table 4, it can be seen that 11 active amino acids in both the phytochemical –gedunin and the drug Corosolic acid match. The binding efficiency, ligand efficiency is very close. Hence, the activity might be similar to that of gedunin. Corosolic acid has shown biological activities like antidiabetic, anti-inflammatory, anti-proliferative, and protein kinase C inhibition activity. Similarly, 10 active amino acids in both berberine and the drug Papaverine match. The binding efficiency, ligand efficiency is similar, albeit not the same. Hence, the activity might be similar to that of berberine. Papaverine is approved to treat spasms of the gastrointestinal tract, bile ducts, and ureter and for use as a cerebral and coronary vasodilator in subarachnoid hemorrhage (combined with balloon angioplasty and coronary artery bypass surgeries). Hence papaverine might have the ability to act

### Table 4

Docking results between Phytochemical and FDA approved/under investigation drugs against SARS-CoV-2 3CL^pro^.

| Ligand                  | Reference RMSD (Å) | Binding Energy (kcal/mol) | Ligand Efficiency | Inhibition Constant Ki (µM) | PDB ID  | Active Amino Acid Residues                                                                 |
|------------------------|--------------------|---------------------------|------------------|----------------------------|---------|-------------------------------------------------------------------------------------------|
| Epoxy Azadiradione     | 78.79              | –8.64                     | –0.25            | 0.466                      | 6M2N    | GLY 143, LEU 141, ASN 142, SER 144, CYS 145, HIS 163, MET 165, GLU 166, LEU 167, PRO 168, ARG 188, GLN 189, THR 190, GLN 192 |
| Betulinic acid         | 77.91              | –7.43                     | –0.23            | 3.59                       | 6M2N    | CYS 44, ASP 48, LEU 50, PRO 52, TYR 54, PHE 140, LEU 141, SER 144                        |
| Glabridin              | 82.21              | –8.49                     | –0.35            | 0.596                      | 6M2N    | GLY 143, HIS 145, SER 164, GLU 166, GLN 192                                              |
| Myristinin A           | 78.41              | –6.98                     | –0.17            | 7.63                       | 6M2N    | SER 144, HIS 163, LEU 141, PHE 140, HIS 172, GLN 192                                    |
| Gedunin                | 34.25              | –8.52                     | –0.24            | 0.5641                     | 6XM2    | THR 24, THR 25, THR 26, LEU 27, HIS 41, ASN 142, ARG 188                               |
| Corosolic acid         | 35.67              | –7.61                     | –0.22            | 2.63                       | 6XM2    | HIS 146, MET 165, GLY 143, CYS 145, ASN 142, PRO 168, LEU 167, ALA 191, GLN 189, GLU 166, THR 190, |
| Berberine              | 35.01              | –8.33                     | –0.33            | 0.788                      | 6XM2    | THR 45, THR 52                                                                           |
| Papaverine             | 36.64              | –7.28                     | –0.29            | 4.63                       | 6XM2    | ASP 760, CYS 622, LYS 621, TYR 619, LYS 798, SER 95, PHE 793, PRO 620, MET 794, ASP 618, LYS 551 |
| Tinosporide            | 163.23             | –7.69                     | –0.28            | 2.32                       | 7BV2    | ASP 761, TRP 617, ASP 623 PRO 620, ARG 624                                             |
| Salvinorin A           | 163.01             | –9.06                     | –0.29            | 0.2297                     | 7BV2    | ASP 441, PHE 442, PHE 440, ASN 552, LYS 551, GLN 444                                   |
| 11-deoxy glycyrrhetinic acid | 163.36             | –7.66                     | –0.23            | 2.44                       | 7BV2    | PRO 620, ALA 554, PHE 441, PHE 442, PHE 440, ASN 552, LYS 551, GLN 444                 |
| Carbenoxolone          | 164.15             | –9.1                      | –0.22            | 0.2153                     | 7BV2    | ASP 760, LYS 621, PHE 793, PRO 620, MET 794, ASP 618, LYS 551                           |
as a potential antiviral agent too.

For protein 7BV2, from Table 4 it can be observed that all active amino acids in both the phytochemical tinosporide and the Salvinorin A drug match. The binding efficiency is very high and ligand efficiency is similar. Salvinorin A is considered a dissociative hallucinogen. It is under investigation as a CNS depressant. But it is showing good promise in computational studies. Hence it will be interesting to check whether such a drug can have varied effects. Similarly, it can be observed that 10 active amino acids in both the phytochemical 11-deoxoglycyrrhetic acid and the drug Carbenoxolone match. The binding efficiency is very high (~9.1 kcal/mol) and ligand efficiency are the same. Carbenoxolone is an FDA approved drug used for the treatment of peptic, esophageal and oral ulceration and inflammation. Also, Carbenoxolone drug showed good results in treating Herpis Simplex. Hence our docking
study also suggests that Carbenoxolone has a tendency to inhibit SARS-CoV-2 virus. Further the ADMET tests were run for both the FDA drugs to compare with the top six phytochemicals. All the parameters were similar to the best-hit phytochemicals. This indicates that the phytochemicals used in the present study may have their potential in inhibition of SARS-CoV-2 virus and hence these compounds may be used for the testing to combat Covid-19.

4. Conclusion

Traditional Indian medicines are known for their excellent therapeutic values since times immemorial. In our study, we have examined various phytochemicals with four different plant species to check their efficacy towards combating SARS-CoV-2 protein. Summarizing the virtual screening and docking results, we found out that epoxy azadiradione (binding energy $-8.64$ kcal/mol, Ligand efficiency 25%) from *A. indica* against 6M2N, gedunin (binding energy $-8.52$ kcal/mol, Ligand efficiency 24%) from *A. indica* against 6XMK and tinosporide (binding energy $-7.69$ kcal/mol, Ligand efficiency 28%) from *T. cordifolia* against 7BV2 proved to be the best drug candidates with excellent inhibitory responses. Also, these phytochemicals which shared commonality in structures with FDA approved drugs Myristinin-A, Corosolic acid, Carbenoxolone and Salvinorin A showed almost similar protein-drug interactions with exceptionally higher inhibitory responses. This proves that epoxy azadiradione, gedunin from *A. indica*, and tinosporide from *T. cordifolia* have excellent roles in inhibition of SARS-CoV-2 viral protein. And hence use of these plant phytochemicals in regular food habit may help in combating SARS-CoV-2. Also, these phytochemicals can be taken for the further study towards the treatment of Covid-19.

Declaration of competing interest

Authors are does not have any conflict of interest.

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