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Journal of the Indian Chemical Society 99 (2022) 100488

Contents lists available at ScienceDirect

Journal of the Indian Chemical Society

journal homepage: www.journals.elsevier.com/journal-of-the-indian-chemical-society

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

Haritha Rajaram [a], N. Harshitha [a], Shweta A. Ram [a], Swarna M. Patra [b], Vidya Niranjan [c], K.A. Vishnumurthy [b,*]

[a] Department of Chemical Engineering, R V College of Engineering, Bengaluru, 560059, India
[b] Department of Chemistry, R V College of Engineering, Bengaluru, 560059, India
[c] Department of Biotechnology, R V College of Engineering, Bengaluru, 560059, India

ARTICLE INFO

Keywords:
SARS-CoV-2
Medicinal plants
In-silico study
3CL^{pro}
Non-structural proteins
Phytochemicals

ABSTRACT

In the present study, the main protease 3CL^{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 *Ocimum Sanctum, Tinospora Cordifolia, Glycyrrhiza Glabra,* and *Azadirachta 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^{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 [1]. SARS-CoV-2 is a *Beta coronavirus* like the SARS and MERS human coronaviruses [2,3]. The occurrence of the illness ranged from mild to severe. SARS-CoV-2 propagates through RNA replication using RNA-dependent RNA polymerases enzyme [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 [5]. The viral 3-chymotrypsin-like cysteine protease (3CL^{pro}) enzyme controls coronavirus replication and is essential for its life cycle. SARS-CoV 3CL^{pro} and SARS-CoV-2 3CL^{pro} share a 99.02% sequence similarity with 12-point mutations [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^{pro}) and 3-chymotrypsin-like protease (3CL^{pro}). The 3CL^{pro} cleaves the polyprotein at 11 distinct sites to generate various non-structural proteins that are important for viral replication [7].

The proteins of SARS CoV consist of two large polyproteins: 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 [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 nsp's can act as potential drug targets [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

* Corresponding author.
E-mail address: vishnumurthyka@rvce.edu.in (K.A. Vishnumurthy).

https://doi.org/10.1016/j.jics.2022.100488
Received 21 May 2021; Received in revised form 1 January 2022; Accepted 16 April 2022
Available online 25 April 2022

down the production of viral proteins. But Hydroxychloroquine shows no significant reduction in deaths related to COVID-19. Other drugs include Ritonavir, Lopinavir, Camostat, Nafamostat, Famotidine, and Corticosteroid and nowadays, convalescent plasma is also used to treat COVID-19 patients. Since many Indian medicinal plants exhibit antiviral, 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 96.35% 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 proteinase (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 nsp-12 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, Glycyrrhiza glabra, Azadirachta 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 antifertility, anticancer, antidiabetic, antifungal, antimicrobial, hepatoprotective, cardioprotective, antiemetic, antispasmodic, 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, Palmatine show anti-viral infections, anticancer, 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, antioxidant, 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, antidermatic, antifeedant, antifungal, anti-inflammatory, antipyretic, antiscab, cardiac, diuretic, insecticidal, larvicidal, nematicidal, 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 Cordifolia, Glycyrrhiza Glabra, and Azadirachta Indica. Also, alkaloid-based phytochemicals derived from marine plants such as tetrandrine, lycorine, 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) was 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 $\Delta E = 0$, 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 Auto-Dock 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

Table 1
List of phytochemicals of various medicinal plants.

Plant	*Ocimum Sanctum* (Tulsi)	*Tinospora Cordifolia* (Guduchi)	*Azadirachta Indica* (Neem)	*Glycyrrhiza Glabra* (Yashtimadhu)
Phytochemicals	Eugenol	berberine	nimocinol	glabridin
	α –farnesene	palmatine	nimbiol	11-deoxoglycyrrhetinic acid
	methyl isoeugenol	choline	quercetin	isoliquiritigenin
	cyclohexane,1,2,4-triethenyl	tinosporide	epoxy azadiradione	liqcoumarin
	Caryophyllene	1-octacosanol	gedunin	kaempferol

Table 2
Binding affinities of phytochemicals against SARS Cov-2 $3CL^{pro}$.

Plant	Phytochemicals	Binding energy (kcal/mol) PDB ID: 6M2N	Binding energy (kcal/mol) in PDB ID: 6XMK	Binding energy (kcal/mol) PDB ID: 7BV2
Tulsi	1,2,4-trivinylcyclohexane	−5.1	−5.4	−5.8
Yashtimadhu	11-deoxoglycyrrhetinic acid	−8.5	−8.7	−9
Tulsi	alpha-farnesene	−5.5	−5.7	−5.2
Guduchi	Berberine	−7.6	−8.2	−7.2
Tulsi	Caryophyllene	−6.3	−6.1	−6.8
Guduchi	Choline	−3.4	−3.5	−3.9
Neem	epoxy azadiradione	−8.1	−7.6	−8.3
Tulsi	Eugenol	−5.2	−5.7	−5.8
Neem	Gedunin	−8.3	−8	−8.1
Yashtimadhu	Glabridin	−8.3	−7.8	−7.9
Yashtimadhu	Isoliquiritigenin	−6.7	−7.6	−6.9
Yashtimadhu	Kaempferol	−7.5	−7.9	−7.9
Yashtimadhu	Liqcoumarin	−6.4	−6.7	−6.8
Tulsi	methyl isoeugenol	−5	−5.5	−5.5
Neem	Nimbiol	−7.4	−7.4	−7.8
Neem	Nimocinol	−8.4	−7.7	−7.7
Guduchi	octacosan-1-ol	−5	−4.2	−4.5
Guduchi	Palmatine	−7.2	−7.5	−7.2
Neem	Quercetin	−7.8	−8	−8
Guduchi	Tinosporide	−8	−7.9	−8.2

*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: 3-D 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-likeness properties of top compounds, which showed a considerable binding affinity against SARS CoV-2 $3CL^{pro}$ 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 $3CL^{pro}$ (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 protease (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.

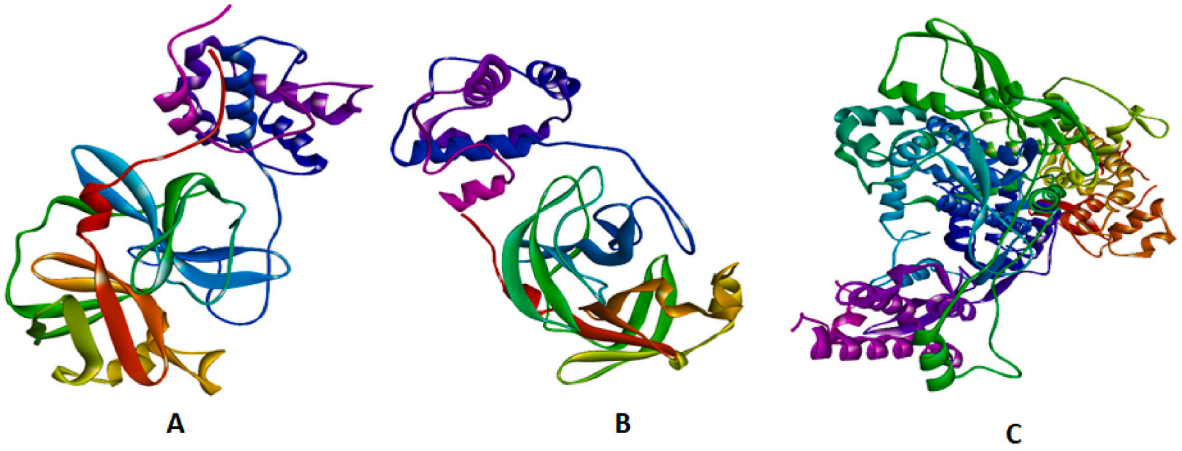

Fig. 1. 3-D structure of prepared protein: (A) SARS CoV-2 $3CL^{pro}$ (PDB ID: 6M2N) with chain A retained, (B) SARS CoV-2 $3CL^{pro}$ (PDB ID: 6XMK), with chain A retained, (C) SARS CoV-2 NSP12-NSP7-NSP8 complex (PDB ID: 7BV2) with chain A retained.

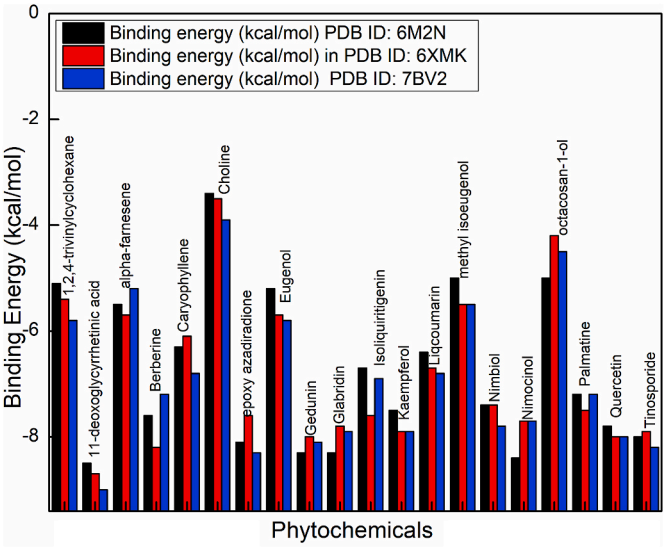

Fig. 2. Binding energies obtained from PDB IDs 6M2N, 6XMK and 7BV2.

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 $3CL^{pro}$ (PDB ID: 6M2N)-Epoxy Azadiradione (Neem). Fig. 4 depicts the visualization of the complex of SARS CoV-2 $3CL^{pro}$ (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
Docking analysis against SARS-CoV-2 $3CL^{pro}$.

Plant	Phytochemical	Reference RMSD (Å)	Binding Energy (kcal/mol)	Ligand Efficiency	The inhibition constant Ki (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	Nimocinol	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	−0.22	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

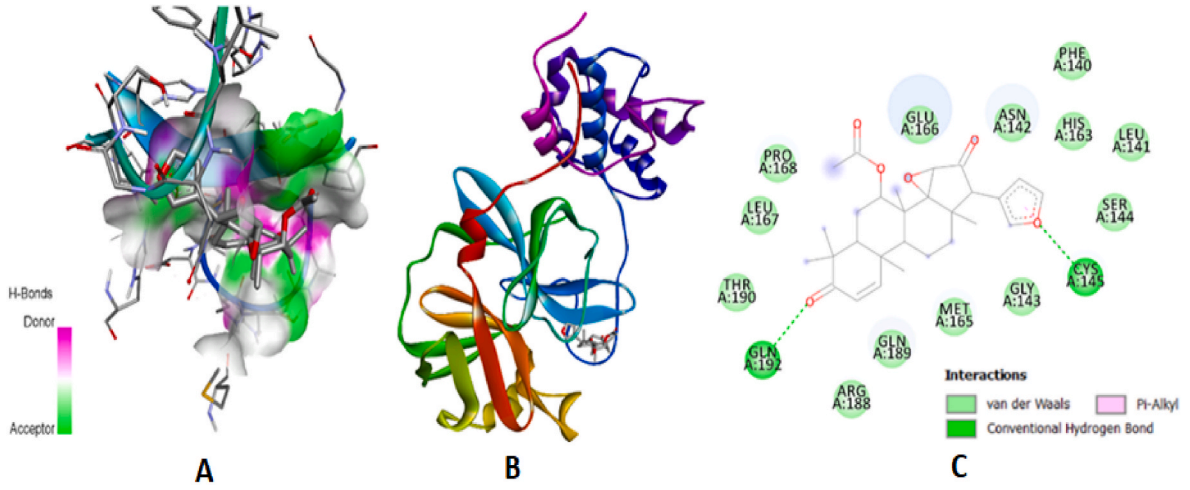

Fig. 3. SARS CoV-2 $3CL^{pro}$ (PDB ID: 6M2N)-epoxy azadiradione (Neem):(A) H-bond interaction between Epoxy Azadiradione and $3CL^{pro}$. (B) 3-D structure of $3CL^{pro}$-Epoxy Azadiradione complex. (C) 2-D diagram of binding interactions of the ligand in the active site pockets of protein.

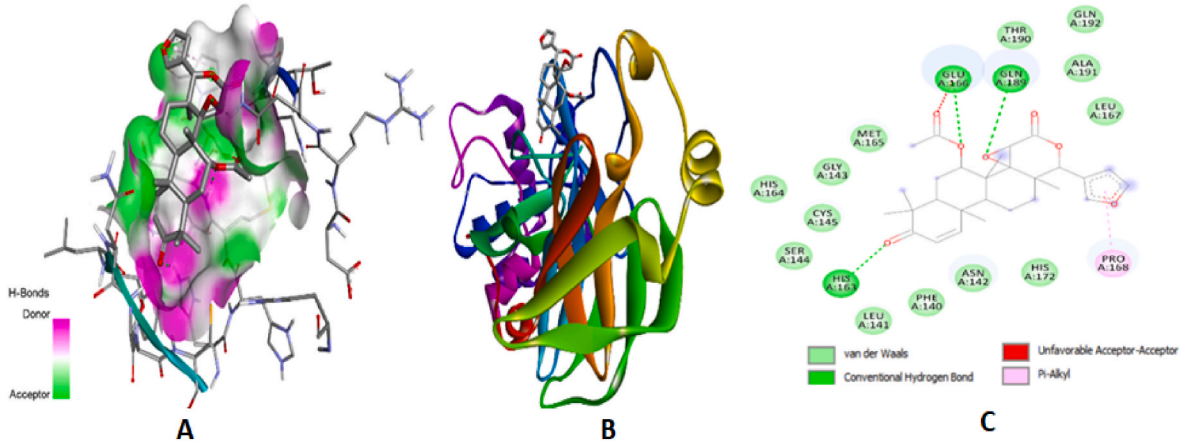

Fig. 4. SARS CoV-2 $3CL^{pro}$ (PDB ID: 6XMK) - Gedunin (Neem):(A) H-bond interaction between Gedunin and $3CL^{pro}$. (B) 3-D structure of $3CL^{pro}$-Gedunin complex. (C) 2-D diagram of binding interactions of the ligand in active site pockets of protein.

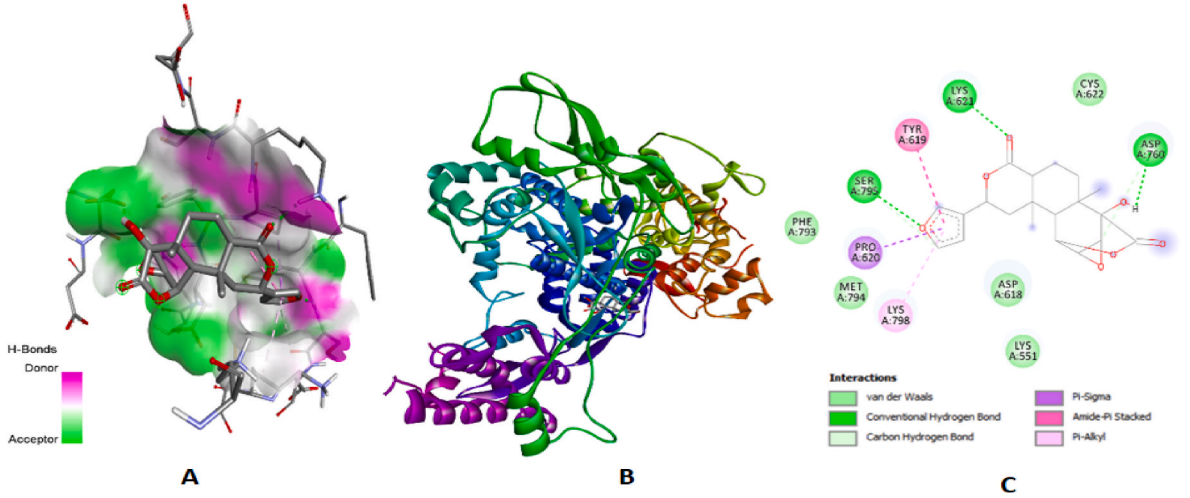

Fig. 5. SARS CoV-2 Nsp12-Nsp7-Nsp8 complex (PDB ID: 7BV2) - Tinosporide (Guduchi): (A) H-bond interaction between tinosporide and Nsp12-Nsp7-Nsp8 complex. (B) 3-D structure of Nsp12-Nsp7-Nsp8 complex-tinosporide complex. (C) 2-D diagram of binding interactions of the ligand in active site pockets of protein.

two inhibitors were: Epoxy Azadiradione and Glabridin. The drugs with structural similarities concerning Gedunin and berberine were Betulinic acid and Myristinin A respectively. Epoxy Azadiradione is a limonoid which is a class of phytochemicals belonging to the pentacyclic triterpenoid class. Betulinic acid is also a pentacyclic triterpenoid. From the literature survey, we found out Betulinic acid, which is structurally similar and with biological activities like antiviral, anti-inflammatory, analgesic, and antitumoral, and is under investigation. Glabridin is a hydroxyisoflavans. 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 tetranortriterpenoid which belongs to the class of phytochemicals extracted from Neem. The FDA drug with an almost similar structure belonging to the same 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 protoberberine 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 and for 11- deoxoglycyrrhetinic acid is carbenoxolone. Tinosporide belongs under the class of diterpenoid furanolactones, whereas Salvinorin A is a diterpene lactone. From the literature survey, we found out Salvinorin A, which is structurally similar to Tinosporide. It is considered a dissociative hallucinogen. It is under investigation as a CNS depressant. Research has focused on discovering a new analog of SA that can induce analgesia and reduce inflammation with a long-lasting effect but without the hallucinatory component. 11-deoxoglycyrrhetinic acid is a triterpenoid saponin glycoside. Carbenoxolone (CBX) is a glycyrrhetinic acid derivative with a steroid-like structure, similar to substances found in the root of the licorice plant. Carbenoxolone is used for the treatment of peptic, esophageal and oral ulceration and inflammation.

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 Betulinic 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 betulinic 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, antiproliferative, 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 (A°)	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	PHE 140, 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		
Glabridin	82.21	−8.49	−0.35	0.596	6M2N	CYS 44, ASP 48, LEU 50, PRO 52, TYR 54, PHE 140, LEU 141, SER 144	
Myristinin A	78.41	−6.98	−0.17	7.63	6M2N	LEU 167, PRO 168, THR 190, ALA 191, GLN 192	
Gedunin	34.25	−8.52	−0.24	0.5641	6XMK	SER 144, HIS 163, LEU 141, PHE 140, HIS 172, GLN 192	HIS 164, MET 165, GLY 143, CYS 145, ASN 142, PRO 168, LEU 167, ALA 191, GLN 189, GLU 166, THR 190,
Corosolic acid	35.67	−7.61	−0.22	2.63	6XMK	THR 24, THR 25, THR 26, LEU 27, HIS 41, ASN 142, ARG 188	
Berberine	35.01	−8.33	−0.33	0.788	6XMK	SER 144, LEU 141, ASN 142, CYS 145, GLY 143, HIS 163, HIS 172, MET 49, PRO 168	
Papaverine	36.64	−7.28	−0.29	4.63	6XMK	THR 45, THR 25, ALA 191, THR 190, GLU 166, HIS 164, ASP 187, MET 49	
Tinosporide	163.23	−7.69	−0.28	2.32	7BV2		ASP 760, CYS 622, LYS 621, TYR 619, LYS 798, SER7 95, PHE 793, PRO 620, MET 794, ASP 618, LYS 551
Salvinorin A	163.01	−9.06	−0.29	0.2297	7BV2	ASP 761, TRP 617, ASP 623	
11-deoxy glycyrrhetinic acid	165.36	−7.66	−0.23	2.44	7BV2	PRO 620, ARG 624	
Carbenoxolone	164.15	−9.1	−0.22	0.2153	7BV2	PRO 620, ALA 554, PHE 441, PHE 442, PHE 440, ASN 552, LYS 551, GLN 444	

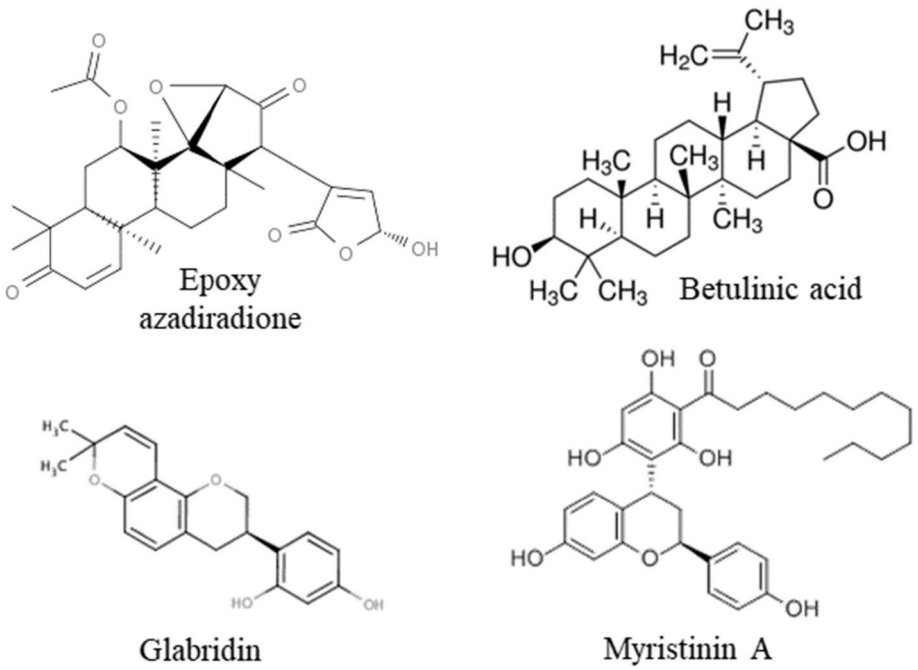

Fig. 6. 3CL^{pro} PDB ID: 6XMK best hits and FDA drugs with similar structures.

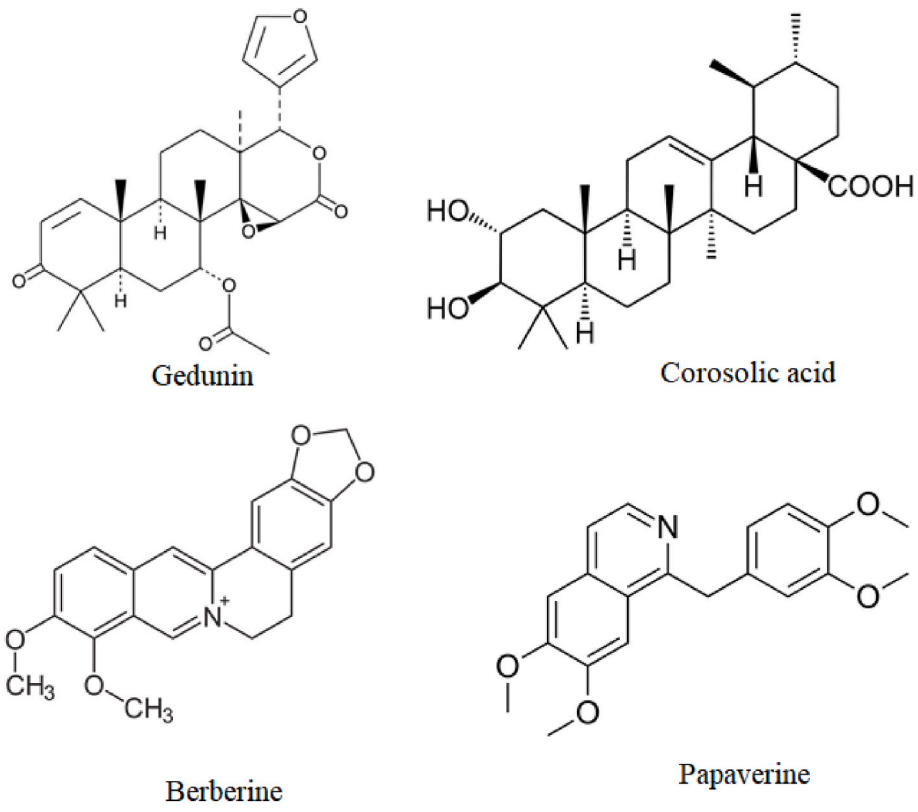

Fig. 7. 3CL^{pro} PDB ID: 6XMK best hits and FDA drugs with similar structures.

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-deoxoglycyrrhtinic 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

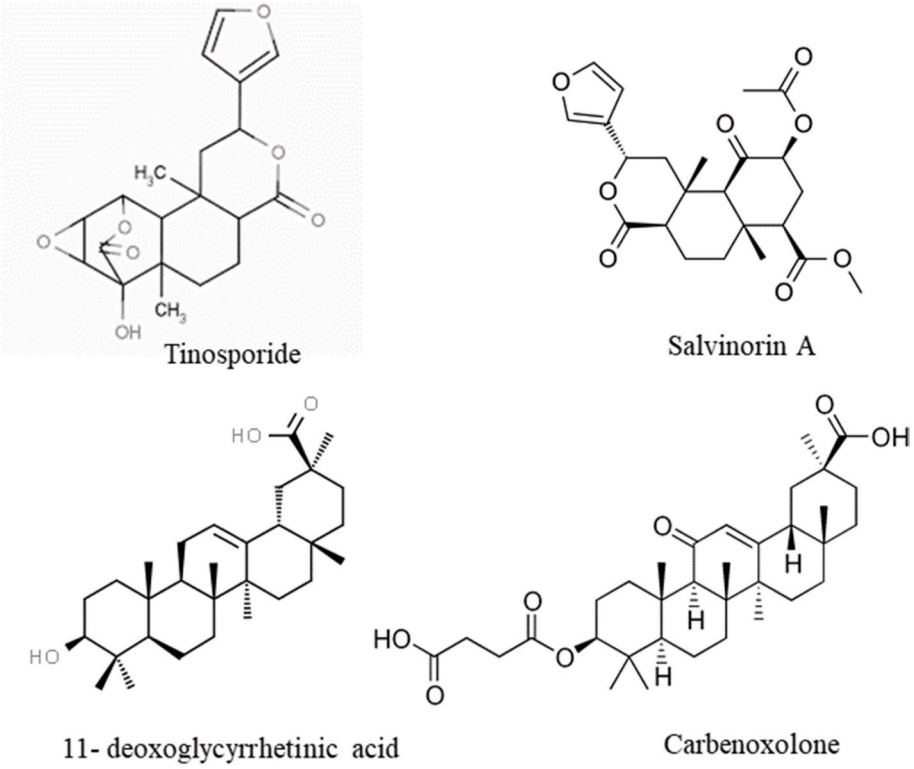

Fig. 8. NSP (12-7-8) trimer complex PDB ID: 6XMK best hits and FDA drugs with similar structures.

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, geduinin 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.

Acknowledgement

Authors are grateful to RV College of Engineering for providing facility to carry out the research work.

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