

Targeting multiple SARS-CoV-2 domains by Indian medicinal plants – A Drug repurposing study using molecular docking, ADME-Tox analysis

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Abstract

The rapid transmission of SARS-CoV-2 and its capability to spread in humans has brought about the development of new approaches for treatment against COVID-19. Drugs and vaccines available currently either target the virus ectodomain or endodomain. Thus, repurposing the use of natural products that target more than one part of the virus is the fastest option available for treatment. Plants are a repository of important constituents with proven significant efficacy against many human viruses. The present study focused on employing computational approaches for screening phytochemicals from 4 Indian medicinal plants, by targeting more than one part of SARS-CoV-2 for the identification of natural antiviral therapeutics to determine their feasibility as potential inhibitors of target viral proteins. Here, we used a multi-target, ligand virtual screening study on 9 target proteins important in SARS-CoV-2 lifecycle, namely Spike glycoprotein, Nucleocapsid phosphatase, Spike protein ACE-2, Non-structural protein 10 and 12, RdRp, Envelope protein, Main protease/3CL protease, and Papain like protease. Out of the 58 plant phytochemicals screened, Z-5-methyl-6-heneicosen-11-one from *Piper nigrum*, Arjunetin from *Terminalia arjuna*, Rutin from *Azadirachta indica* and Makisterone A from *Tinospora cordifolia* exhibited highest binding affinity with 9 viral targets. In addition, ADMET analysis indicated Ursodeoxycholic acid, Ellagic Acid, Epicatechin and Isocolumbin, Ecdysterone, Columbin from *Piper nigrum*, *Terminalia arjuna*, *Azadirachta indica*, and *Tinospora cordifolia* have good binding energetics with the target viral proteins. The research thus enlightens the suitable pharmacological properties and the anti-viral activity of potential medicinal plant molecules for human administration using extensive in-silico techniques.

1. INTRODUCTION

In recent years, many viruses have emerged worldwide and many more are on the verge of rising (Mourya *et al.*, 2019). These diseases include respiratory diseases, due to the rapid propagation nature of respiratory viruses. These kinds of respiratory diseases include the severe acute respiratory syndrome coronavirus (SARS-CoV), Middle East respiratory syndrome coronavirus (MERS-CoV), Avian influenza, etc. In December 2019, cases of atypical pneumonia began to arise in the Wuhan province of China (Lu *et al.*, 2020). By January 2020, the etiological pathogenic agent was classified as a new member of the Coronaviridae family and β -coronavirus genus (2019-nCoV) that differ from SARS-CoV and MERS-CoV. As per Rothan and Byrareddy *et al.*, 2020, the genome of 2019-nCoV shares an 82% sequence identity with SARS-CoV-2.

To enter host cells, SARS-CoV-2 uses the angiotensin-converting enzyme 2 (ACE-2) cell receptor (Hoffmann *et al.*, 2020). The SARS-CoV-2 genome consists of ~ 29.8 kb nucleotides; 14 open reading frames (ORFs) that encode 27 proteins. The ORF has 2 parts: ORF1a and ORF1b. ORF1a/b encodes two polyproteins by employing polymerase frameshifting. The polyproteins are then post-translationally cleaved into 15 non-structural proteins (NSPs): NSP1-10 and NSP 12–16. The rest of the genome encodes 4 structural proteins namely: spike protein (S protein), an envelope protein (E protein), membrane protein (M protein), and nucleocapsid protein (N protein). (Wu A. *et al.*, 2020). These 4 structural proteins of the virus altogether form the virion and mediate cell entry; whereas the 16 non-structural proteins (Nsps) form the viral replication-transcription complex, thereby inhibiting host immune responses. The spike protein is a homotrimeric protein, composed of three identical polypeptide units; it is the spike protein that mediates viral entry through recognition by its receptor-binding domain (RBD) of the host angiotensin-converting enzyme 2 (ACE2) receptor (Smith *et al.*, 2020). On entering the human body, the host ribosome synthesizes a precursor polyprotein, which is activated by Nsp3 (or PLPro) and Nsp5 (or MPro) proteases via auto-cleavage into the 16 different Nsps (Hartenian *et al.* 2020). It was observed that most recent drug repurposing studies focus only on screening the same classical antiviral targets, which target either one protein of the virus or a maximum of 2 proteins of the virus. Potential drugs for repurposing in therapeutics could target numerous additional viral protein sites. The current research thus explores molecular docking for the identification of the most important active sites and analysis of the interactions that occur between the docked molecules and the identified active site.

Indian medicinal plants and their multipurpose therapy date back to ancient times. Although Indian flora is widely known as an abundant medicinal plant species, only a few ethnobotanical studies have been identified, for their use to treat

recently occurring respiratory diseases including SARS-CoV-2. As per World Health Organization (WHO) 2021, statistics demonstrate that developing countries are not preserved and are swarmed by COVID-19 cases, which now occupy an important place in the global incidence of transmissible diseases (WHO World Health Organization (2021)). Traditional medicine has consequently gained interest in health care services. In addition, as per Biyiti *et al.*, 2004, it is estimated that 25% of all modern medicines are directly or indirectly derived from medicinal plants. And considering the increasing issues that arise as side effects due to chemical-based drugs, the focus on studying traditional medicine needs to be more. In this study, *Piper nigrum*, *Terminalia arjuna*, *Azadirachta indica*, and *Tinospora cordifolia* were used. As per the literature review, all of these plants possess antiviral activity among other biological activities. *Piper nigrum* (Black pepper), a commonly found spice in Indian households, is known for its antioxidant, antiviral, anticancer, and antimicrobial activity (Patil *et al.*, 2017). *Terminalia arjuna* is known for its antimicrobial as well as antiviral activity. Casuarinin is a hydrolyzable tannin present in the bark of *Terminalia arjuna*, that is capable of antiviral activity against Herpes simplex virus type – 2 (HSV 2) (Cheng *et al.*, 2002) Structural re-orientation of phytoconstituents (PCs) from *Azadirachta indica* was observed as the mechanism for SARS-CoV-2 viral cell entry inhibition (Daniel *et al.*, 2021). *Terminalia arjuna* has also been found to possess an antiviral effect against HIV, and HTLV by immunomodulation and selective inhibition of the virus to target T helper cells. (Singh *et al.*, 2017). A study by Patil *et al.*, 2023, found out the anti-viral activity of *Piper nigrum*, *Terminalia arjuna*, *Azadirachta indica*, and *Tinospora cordifolia* in-vitro, using qRT-PCR.

As a part of our ongoing biodiversity prospection for natural anti-SARS-CoV-2 agents, we observed significant antiviral activity of the above-mentioned common medicinal plants that have been used largely against various diseases including respiratory diseases. This observation prompted us to conduct an in-silico study on the 4 Indian medicinal plants. The study aims to promote the use of natural phytoconstituents-derived traditional medicine as anti-viral agents against SARS-CoV-2. The phytochemical ligands were used for docking studies against multiple target proteins of SARS-CoV-2. Indeed, the interaction modes of 58 potential antiviral phytoconstituent candidates were studied against the SARS-CoV-2 target proteins using virtual screening, molecular docking, and ADME-Toxicological techniques.

2. RESULTS

Phytochemical shortlisted as per Lipinski's rule

On conducting LCMS analysis of the extracts obtained from *Piper nigrum* peppercorns, *Terminalia arjuna* stem, *Azadirachta indica* leaves and *Tinospora cordifolia* stem, by Patil *et al.*, 2023, 20, 7, 16, and 15 phytochemicals were identified from the plants respectively. The ones selected after molecular docking and ADME-Toxicological analysis as per Lipinski's rules are mentioned in table no. 2.

Molecular docking

Comprehensive docking virtual screening studies of the 58 phytoconstituent ligands from the 4 medicinal plants *Piper nigrum*, *Terminalia arjuna*, *Azadirachta indica*, and *Tinospora cordifolia* is commonly known for their antiviral properties were carried out against 9 viral target proteins using AutoDock Vina. The virtual screening of 58 phytochemical ligands from the above-mentioned medicinal plants was conducted at different precision levels was conducted with 6VYB (Spike glycoproteins), 6VYO (Nucleocapsid phosphatase), 6M17 (Spike protein ACE-2), 6W4H (NSP-10), 6M71 (RdRp), 5X29 (EPro), 6LU7 (Mpro/3CLpro), 7D47 (PL pro), 6NUS (NSP-12) SARS-CoV-2 targets. The docking results for the medicinal plant phytochemical ligands and viral target proteins are presented in table no. 3. Total of 58 PCs were docked against the receptors mentioned in table no. 1, which resulted in 542 docking scores. The range of dock scores taken between – 8 to -11. On considering the criteria of percentage BA more than 50%, the PCs shortlisted further are as mentioned in table no. 3.

As per Wan *et al.*, 2020, SARS-CoV-2 enters the host using the S-Protein in it, which interacts with the host receptor called ACE2 (Angiotensin converting enzyme-2). The two subunits of S-protein are S1 and S2. S1 is the receptor-binding domain (RBD), whereas the S2 subunit is the fusion protein. As per their study, SARS-CoV-2 recognizes human ACE2 by its amino

acid residues Gln and Leu, thereby enhancing viral binding to human ACE2. While, Leu enhances ACE-2 binding (Bharadwaj *et al.*, 2021 Satarker *et al.*, 2020). Gln supports the virus with energy and biosynthetic requirements during the infection cycle (Bharadwaj *et al.*, 2021), Leu enhances ACE-2 binding (Satarker *et al.*, 2020), Arg is an essential amino acid in the life cycle of many viruses, including SARS-CoV-2 (Grimes *et al.*, 2021), Lysine is essential for NSP inhibition of host gene expression (Shen *et al.*, 2021), and Tyr forms strong contacts with hACE2 residues (Chowdhury *et al.*, 2020). Hence, the interaction of phytochemicals with Gln, Leu, Arg, Lys, or Tyr is capable of inhibiting the interaction of viral proteins with the ACE2 receptor and stopping the entry of SARS-CoV-2 into the host cell.

Ursodeoxycholic acid from *Piper nigrum* formed two hydrogen bonds, one hydrophobic bond and one unfavorable bond with the 6VYB receptor. The highest BA of this ligand was shown with 6VYB viral protein. Two CHB were formed from the residues, and one CHB formation from the A: His 1048 donates the hydrogen from the amine group to the ligand's oxygen atom with acts as a hydrogen acceptor. Another CHB formed from the ligand's hydrogen atom donates hydrogen to the A: Arg 1039. A: Lys 1038 alkyl interacts with the ligand's alkyl group to form one alkyl hydrophobic bond. A: Asp 1041 on donating hydrogen made an unfavorable bond with a ligand hydrogen atom.

Ellagic acid from *Terminalia arjuna* on molecular docking has formed six hydrogen bonds and one hydrophobic bond with the receptor 6VYB. Each CHB was formed from the residues A: Leu 977, A: Met 740, and A: Ile 742. Three CHB were formed by A: Asn 978 with the ligand. C: Thr 572 formed a pi-sigma hydrophobic bond with the ligand. The highest BA of this ligand was seen with the 6VYB receptor. The interaction between the ellagic acid ligand and its highest binding viral protein is depicted in Fig. 2. (Left side of the figures represents the 3D interactions of the protein-ligand complex, whereas the Right side of the figures represents the 2D interactions of the protein-ligand).

From *Azadirachta indica*, on molecular docking between the phytoconstituent epicatechin (compound C7) and the viral receptor 6VYB, eight hydrogen bonds and two unfavorable bonds. The highest BA of the ligand was seen with 6VYB, 6VYO, and 6W4H. Seven CHB were formed, and three CHB were formed from the residues A: Thr 549, A: Thr 573, B: Asn 978 with the oxygen atoms of the ligand. The rest of the CHB were formed from the hydrogen atoms of the ligand with oxygen atoms of the residues B: Met 740, B: Tyr 741, A: Thr 549 and B: Asp 745. One pi donor hydrogen bond was formed between the B: Asn 978 with pi orbitals of the ligand. No hydrophobic bonds formed between the complex. The compound ligand formed four hydrogen bonds, three hydrophobic bonds, one electrostatic, and one unfavorable bond with the receptor 6VYO. Compound four hydrogen bonds, two hydrophobic bonds, one electrostatic bond, and one pi-sulfur bond with 6W4H receptor.

3 PCs Isocolumbin, Ecydsterone, and columbin from *Tinospora cordifolia* exerted the highest molecular docking with the viral receptors. Isocolumbin had the highest BA with 7D47 viral protein. The ligand formed four hydrogen bonds with the receptor 7D47. Four CHB were formed, the residues A: Glu 124, A: Tyr 137, A: Arg 140, B: Glu 124 with oxygen atom of the ligand. Ecydsterone on molecular docking demonstrated the highest BA with 6VYB receptor. The ligand formed five hydrogen bonds with the receptor 6VYB. A: Asn 978 formed two CHB with the ligand. Each residue A: Thr 912, A: Lys 1036, and B: Gln 1036 formed CHB with the ligand. On the other hand, phytoconstituent Columbin showed the highest BA with 7D47. The ligand formed six hydrogen bonds with the receptor 7D47. Each residue of A: Glu 124, B: Glu 124, A: Glu 133, A: Lys 126 (hydrogen donor) formed CHB with the oxygen atoms of the ligand. The Ligand hydrogen atom interacts with the hydroxyl group of A: Tyr 137 to form one CHB. Hydroxyl group of the A: Thr 137 interacts with the oxygen atom of the ligand as depicted in Figs. 3 and 4. (Left side of the figures represents the 3D interactions of the protein-ligand complex, whereas the Right side of the figures represents the 2D interactions of the protein-ligand).

Drug likeness prediction and ADME-Toxicological analysis

Based on Lipinski's rule of 5, distinguishing between compounds that are drug-like and non-drug-like molecules is helpful. Drug-likeness prediction for the best-docked compounds was done in this study, with Lipinski's rule of 5, and ADME analysis was performed using ADMETSar and pkCSM web server. Furthermore, with the help of ADMETSAR and pkCSM,

analysis was carried out for the prediction of absorption parameters - Water solubility (log mol/L), intestine absorption (%), skin permeability (log Kp), P-glycoprotein substrate, P-glycoprotein 1 inhibitor, P-glycoprotein 2 inhibitor; for digestion parameters - VDss (log L/kg), BBB permeability (log BB), CNS permeability (log PS), Metabolism parameters - CYP2D6 substrate, CYP3A4 substrate, CYP1A2 inhibitor, CYP2C19 inhibitor, CYP2C9 inhibitor, CYP2D6 inhibitor, CYP3A4 inhibitor; and toxicological analysis using AMES toxicity, Oral rat acute toxicity (LD50), oral rat chronic toxicity (IC50), Hepatotoxicity, Skin sensitization, and Minnow toxicity. Out of 23 PCs identified to possess the highest BA to SARS-CoV-2 viral receptors, 6 PCs followed Lipinski's rule of 5. The results for the ADME-Toxicological analysis for the 6 PCs are mentioned in tables no. 4, 5, 6, 7, and 8.

3. MATERIALS AND METHODS

Construction of phytochemical database and data retrieval: The dataset of this study was engineered by shortlisting medicinal plants *Piper nigrum*, *Terminalia arjuna*, *Azadirachta indica*, and *Tinospora cordifolia* that are commonly used against respiratory infections and using LC-MS analysis to identify their PCs. This set constructed was as follows: 20 PCs from *Piper nigrum*, 7 from *Terminalia arjuna*, 16 from *Azadirachta indica*, and 15 from *Tinospora cordifolia*. 58 phytochemicals were developed and converted into a single database format (SDF) using Biovia Drug Discovery studio 2020 software for performing molecular docking. Molecular docking was performed for each ligand against viral targets with Spike glycoproteins, Nucleocapsid phosphatase, Nsp10, RdRp, Mpro/3CLpro, and Nsp12. (Table 1). ADME-T (Absorption, Distribution, Metabolism, Excretion, Toxicity), pharmacological and pharmacokinetic properties of the PCs were assessed using AMDETSar 2.0 (Yang *et al.*, 2019). For determining the ADMET characteristics of the compounds and involvement of the assessment of drug-like characteristics, their possible toxicity was assessed using the pkCSM server. Out of the 4 medicinal plants, a total of 6 PCs were shortlisted using Lipinski's rule (Table 2). The 3D structures of these phytochemicals were obtained from the PubChem database.

Ligand–protein targets virtual screening and molecular docking: Molecular docking is considered to be one of the primary computational modeling methods to determine the BA of molecules (ligands) with the targets (proteins) because it predicts the binding conformation mode of the ligand against the receptor's binding site. To all the compounds, hydrogen atoms were added, and 3D conformers of the compounds were generated and converted from sdf to mol2 to pdbqt formats after energy minimization in MMFF94 force field through OPENBABEL (O'Boyle *et al.*, 2011). The 3D structure of the proteins was retrieved from the PDB. Protein preparation was carried out to get the Raw protein form by deleting the hetatm, water molecules, bound ligand compounds, and metal ions in the Drug Discovery Studio. For purifying the pure protein structures, polar hydrogen atoms and Kollman charges were added and saved in PDBQT format for docking. The binding site was defined as the volume occupied by the co-crystallized ligands in each of the receptor's active sites. The protein, ligands, as well as the ligand entry site into the protein pockets, were prepared in the present work by using Discovery Studio software. Extensive studies on the stability of binding and highest BA of the individual PCs of the medicinal plants against SARS-CoV-2 targets were performed and ligand-protein interactions using AutoDock Vina by constructing grid dimensions by UCSF Chimera (Trott *et al.*, 2020, Pettersen *et al.*, 2004). According to the results of recent molecular studies, Remdesivir antiviral drug has been chosen to serve as a reference ligand with SARS-CoV-2 spike and membrane proteins, RdRp, nucleoproteins, viral proteases in the study by Eweas *et al.*, 2021. The results obtained in the current study were evaluated according to the binding energy value and the molecule with the lowest binding energy (i.e Best/Highest affinity score) was considered the best one that interacts with the target. Of the 58 compounds, 23 compounds that showed the highest BA (Above 50%) were selected for further studies using molecular docking studies. To calculate the BA percentage, the BA range from – 8 to -11 was considered and the formula applied was: $\text{Virus targets bound with highest affinity (i.e- 8 to -11) / Total viral target (i.e. 23)} \times 100$. The adenosine analog Remdesivir is known to inhibit RNA strand elongation, thereby inhibiting viral replication as per Warren *et al.*, 2016. In addition, in a study by Eweas *et al.*, 2021, a high BA of remdesivir to RdRp was confirmed. As per this study, the high BA of remdesivir to both Mpro and PLpro of SARS-CoV-2 indicates its potential as a protease inhibitor. In this study, Remdesivir showed considerable BA with a molecular docking score of –

111.007 and protein–ligand interactions molecular docking score of – 122.699. It formed one H-bond with Leu303, Glu309, and Asn953. Remdesivir was also found to have a high binding affinity to the main protease of SARS-CoV-2. (Hall and Ji, 2020).

ADME-Toxicological analysis

Drug candidates need to match the criteria for being considered as orally consumed drugs. Physicochemical properties (molecular weight, H-bonding, heavy atoms, etc.), drug-likeness properties (Lipinski *et al.*, 2001), bioavailability, water solubility, pharmacokinetics, stability, and low cost of production are assessed to finalize on adoption of the drugs or their rejection. In the current research, the pkCSM web server was used for the assessment of ADMET properties of top-selected ligands, and Lipinski's descriptors were determined from the ADMETsar (Yang *et al.*, 2019, Pires *et al.*, 2015). The toxicological properties of the selected biological molecules were also determined; where the values of oral toxicity, cytotoxicity, and carcinogenicity, were predicted using computational approaches. A few toxicity-related evaluations such as minnow toxicity (MT), hepatotoxicity (HP), and skin sensitization (SS) have also been added to this research.

4. DISCUSSION

In modern medicine, COVID-19 is treated with synthetically-derived antiviral agents and also using hormone-based corticosteroid therapies individually, as well as in combination (WHO 2020). Natural PCs from medicinal plants such as *Piper nigrum*, *Terminalia arjuna*, *Azadirachta indica*, and *Tinospora cordifolia* can be used, as they are less toxic than synthetic drug compounds. In-silico methods such as molecular docking and ADME-toxicological analysis of the PCs from these 4 medicinal plants have shown promising results, considering the BA, interactions, and stability observed in this study. In addition to deteriorating the host's immunity or respiratory system, SARS-CoV-2 also affects other organs of infected patients (Jain *et al.*, 2020). This research study states that some of the PCs from the 4 medicinal plants have been found effective against COVID-19, as per in-silico methodology. As 25% of all modern medicines are derived from medicinal plants directly or indirectly, these 4 medicinal plants can help to prevent interactions of SARS-CoV-2 viral protein into the host cells and also be safe and effective against COVID-19 without toxicity or fatal side effects.

5. CONCLUSION

The present study was accomplished with the aim to find out natural PCs with antiviral activity against SARS-CoV-2. Targeting multiple viral receptors in the ectodomain as well as endodomain of the virus with natural phytochemicals may inhibit viral entry, which can thereby resist further replication and propagation into the host body. From the present study, we conclude that the 6 PCs from medicinal plants *Piper nigrum*, *Terminalia arjuna*, *Azadirachta indica*, and *Tinospora cordifolia* were shown to bind to multiple SARS-CoV-2 receptors on the ectodomain and endodomain, by following Lipinski's rule. These PCs thus have high potential inhibition and the best BA with SARS-CoV-2 receptors. Thus, the 6 best-docked phytochemicals with drug-like properties and safe ADMET profile may help to inhibit SARS-CoV-2 activity and can override synthetic drugs by being natural anti-COVID-19 agents. Furthermore, experimental investigations should be carried out to assess their in vitro and in vivo efficiency against SARS-CoV-2.

Abbreviations

SARS CoV-2 – Severe acute respiratory syndrome Coronavirus -2.

RCSBPDB - Research Collaboratory for Structural Bioinformatics PDB.

MMFF94 - Merck Molecular Force Field.

PDBQT - Protein Data Bank, Partial Charge (Q), & Atom Type (T).

SDF - Structure-data file.

3D – 3 dimensional.

2D – 2 dimensional.

PC - phytochemicals/phytoconstituents

BA - Binding affinity

Declarations

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AUTHOR CONTRIBUTIONS

NNP conceptualized the study. RSJ and NNP designed the study. RSJ and PM identified phytoconstituents from Pubchem and identified SARS-CoV-2 protein targets in PDB. PM conducted in-silico analysis for molecular docking and ADME-Tox analysis. RSJ, NNP, and PM interpreted the results. RSJ, NNP, and PM discussed the results. RSJ wrote the manuscript and NNP edited the manuscript. RSJ and NNP are equal first authors of the research paper. NNP, RSJ, RCP, and BS proofread the manuscript. All authors contributed to the article and approved the submitted version.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Tables

Table no. 1: SARS-CoV-2 receptors retrieved from Protein Data Bank (PDB)

PDB ID	Virus targets	Description	References
6VYB	Spike glycoproteins	SARS-CoV-2 spike ectodomain structure	Walls AC, Park YJ, Tortorici MA, Wall A, McGuire AT, Veesler D. Structure, Function, and Antigenicity of the SARS-CoV-2 Spike Glycoprotein [published correction appears in Cell. 2020 Dec 10;183(6):1735]. Cell. 2020;181(2):281–292.e6.
6VYO	Nucleocapsid phosphatase	Crystal structure of RNA binding domain of nucleocapsid phosphoprotein from SARS-CoV-2	Madej T, Lanczycki CJ, Zhang D, et al. MMDB and VAST+: tracking structural similarities between macromolecular complexes. Nucleic Acids Res. 2014;42.
6M17	Spike protein ACE 2	The 2019-nCoV RBD/ACE2-B0AT1 complex	Yan R, Zhang Y, Li Y, Xia L, Guo Y, Zhou Q. Structural basis for the recognition of SARS-CoV-2 by full-length human ACE2. Science. 2020;367(6485):1444–1448.
6W4H	Nsp10	1.90 Angstrom Resolution crystal structure of Nsp16-Nsp10 complex from SARS-CoV-2	Rosas-Lemus M, Minasov G, Shuvalova L, et al. High-resolution structures of the SARS-CoV-2 2'-O-methyltransferase reveal strategies for structure-based inhibitor design. Sci Signal. 2020;13(651).
6M71	RdRp	SARS-Cov-2 RNA-dependent RNA polymerase in complex with cofactors	Gao Y, Yan L, Huang Y, et al. Structure of the RNA-dependent RNA polymerase from COVID-19 virus. Science. 2020;368(6492):779–782.
5X29	Epro	NMR structure of the SARS Coronavirus E protein pentameric ion channel	Surya W, Li Y, Torres J. Structural model of the SARS coronavirus E channel in LMPG micelles. Biochim Biophys Acta Biomembr. 2018;1860(6):1309–1317.
6LU7	Mpro/3CLpro	The crystal structure of COVID-19 main protease in complex with an inhibitor N3	Jin Z, Du X, Xu Y, et al. Structure of Mpro from SARS-CoV-2 and discovery of its inhibitors. Nature. 2020;582(7811):289–293.
7D47	PLpro	Crystal structure of SARS-CoV-2 Papain-like protease C111S	Gao X, Qin B, Chen P, Zhu K, Hou P, Wojdyla JA, Wang M, Cui S. Crystal structure of SARS-CoV-2 papain-like protease. Acta Pharm Sin B. 2021 Jan;11(1):237–245.
6NUS	Nsp12	SARS-Coronavirus NSP12 bound to NSP8 co-factor	Kirchdoerfer RN, Ward AB. Structure of the SARS-CoV nsp12 polymerase bound to nsp7 and nsp8 co-factors. Nat Commun. 2019;10(1):2342.

Table no. 2. Phytochemicals identified and shortlisted as per Lipinski's rule and their PUBCHEM IDs

Compound code	Phytoconstituents	PUBCHEM ID
A1	Ursodeoxycholic acid	31401
B1	Ellagic acid	5281855
C1	Epicatechin	72276
D1	Isocolumbin	24721165
E1	Ecdysterone	5459840
F1	Columbin	442015

Table no. 3. BA scores of the phytochemicals of the medicinal plants against the SARS-Co-V2 proteins

Phytoconstituents	Targets									Percentage Binding affinity
	6VYB	6VYO	6M17	6W4H	6M71	5X29	6LU7	7D47	6NUS	
Ursodeoxycholic acid	-8.9	-7.7	-8.5	-8.4	-7.8	-8.2	-7.4	-8.6	-7.4	55%
Ellagic acid	-9	-8.6	-8.3	-8.5	-8.1	-7.3	-7.8	-8.9	-8.2	77%
Epicatechin	-8.5	-8.5	-8	-8.5	-7.2	-7.5	-7.1	-7.6	-8.2	55%
Isocolumbin	-8.8	-7.7	-8.6	-8.4	-8.3	-8.3	-7.6	-9.1	-7.7	66%
Ecdysterone	-8.9	-8.3	-7.9	-8.2	-7.9	-8.5	-7.5	-8.6	-7.9	55%
Columbin	-9.5	-7.7	-8.6	-9	-8.4	-8.4	-7.8	-9.7	-8.3	77%

Table no. 4: Absorption studies of 6 PCs using ADMETSar and pkCSM

Phytoconstituents	Water solubility (log mol/L)	Intestine absorption (%)	Skin permeability (log Kp)	P-glycoprotein substrate	P-glycoprotein 1 inhibitor	P-glycoprotein 2 inhibitor	No. of times Lipinki's rule is violated
Ursodeoxycholic acid	-4.19	95.75	-2.733	Yes	No	Yes	1
Ellagic acid	-3.18	86.684	-2.735	Yes	No	No	0
Epicatechin	-3.117	68.829	-2.735	Yes	No	No	0
Isocolumbin	-3.588	97.485	-3.694	No	No	No	1
Ecdysterone	-3.885	64.542	-3.229	Yes	No	No	0
Columbin	-3.588	97.485	-3.694	No	No	No	1

Table no. 5: Distribution studies of 6 phytoconstituents using ADMETSar and pkCSM

Phytoconstituents	VDss (log L/kg)	BBB permeability	CNS permeability	No. of times Lipinki's rule is violated
Ursodeoxycholic acid	-0.86	-0.603	-1.992	1
Ellagic acid	0.375	-1.272	-3.533	0
Epicatechin	1.027	-1.054	-3.298	0
Isocolumbin	0.137	-0.284	-2.433	0
Ecdysterone	-0.547	-1.071	-3.986	0
Columbin	-0.137	-0.284	-2.433	0

Table no. 6: Metabolism studies of 6 PCs using ADMETsar and pkCSM

Phytoconstituents	CYP2D6 substrate	CYP3A4 substrate	CYP1A2 inhibitor	CYP2C19 inhibitor	CYP2C9 inhibitor	CYP2D6 inhibitor	CYP3A4 inhibitor	No. of times Lipinki's rule is violated
Ursodeoxycholic acid	No	Yes	No	No	No	No	No	1
Ellagic acid	No	Yes	No	No	No	No	No	1
Epicatechin	No	No	No	No	No	No	No	2
Isocolumbin	Yes	No	No	No	No	No	No	1
Ecdysterone	No	No	No	No	No	No	No	2
Columbin	Yes	No	No	No	No	No	No	1

Table no. 7: Excretion studies of 6 PCs using ADMETsar and pkCSM

Phytoconstituents	Total clearance (log/ml/min/kg)	Renal OCT2 substrate	No. of times Lipinki's rule is violated
Ursodeoxycholic acid	0.607	No	1
Ellagic acid	0.537	No	1
Epicatechin	0.183	No	2
Isocolumbin	0.79	Yes	0
Ecdysterone	0.576	No	0
Columbin	0.79	Yes	0

Table no. 8: Toxicological studies of 6 PCs using ADMETsar and pkCSM

Phytoconstituents	AMES toxicity	Oral rat acute toxicity (LD50)	Oral rat chronic toxicity (IC50)	Hepatotoxicity	Skin sensitisation	Minnow toxicity	No. of times Lipink's rule is violated
Ursodeoxycholic acid	No	2.659	1.853	No	No	1.166	0
Ellagic acid	No	2.399	2.698	No	No	2.11	0
Epicatechin	No	2.428	2.5	No	No	3.585	0
Isocolumbin	No	2.946	1.036	Yes	No	0.894	1
Ecdysterone	No	2.671	2.174	No	No	3.492	0
Columbin	No	2.946	1.036	Yes	No	0.894	1

Figures

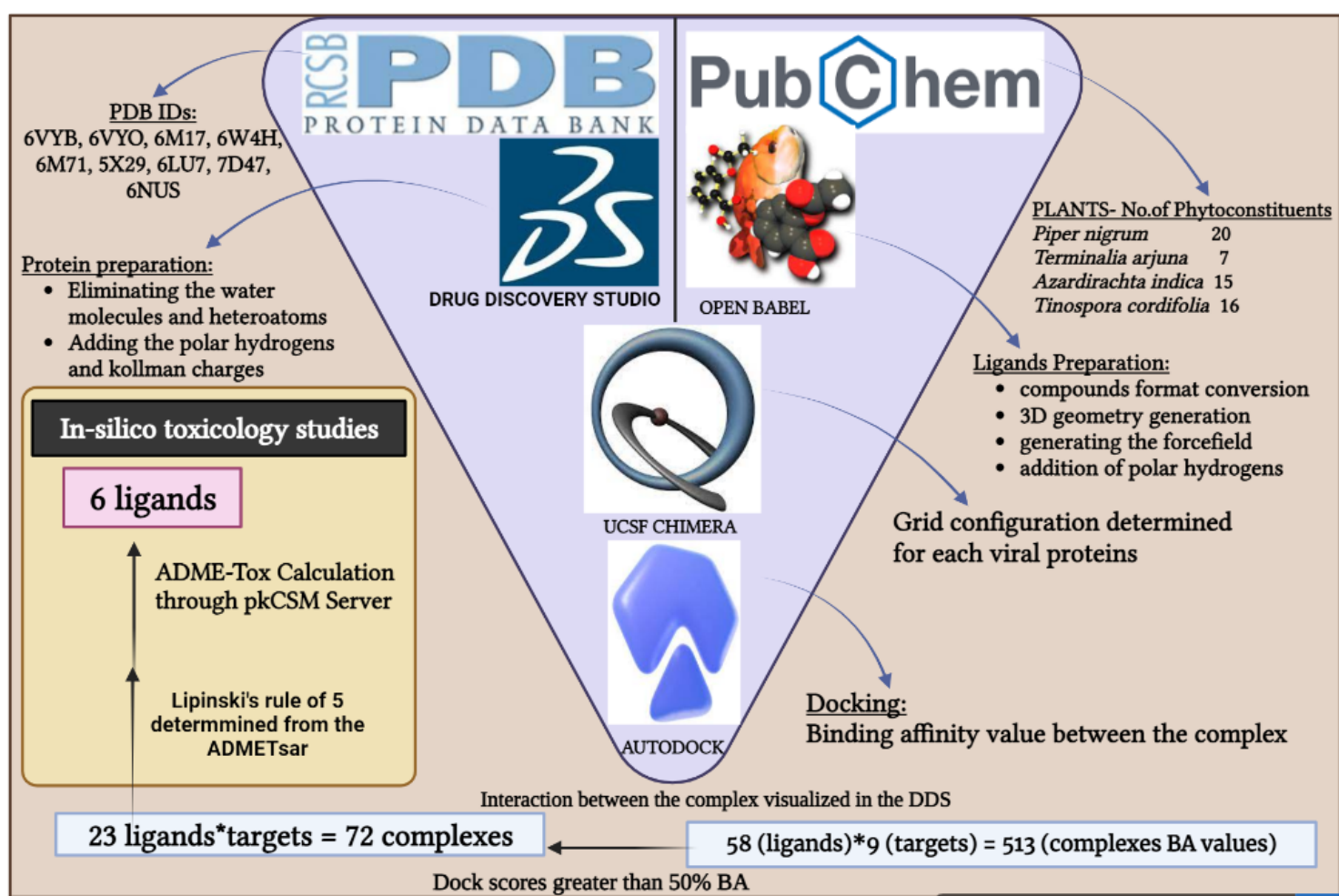


Figure 1

Graphical abstract of in silico studies conducted using Indian medicinal plants against SARS-CoV2

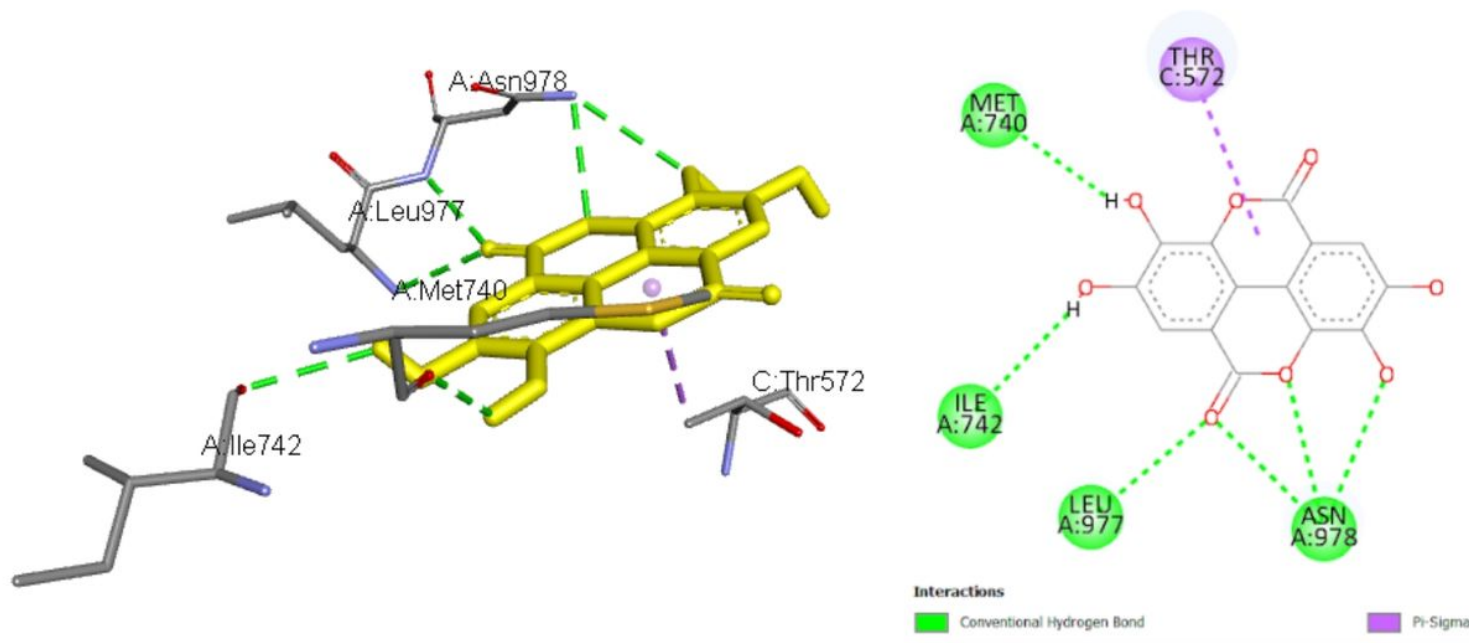


Figure 2

Interaction between Ellagic acid ligand from *Terminalia arjuna* and SARS-CoV-2 viral protein 6VB

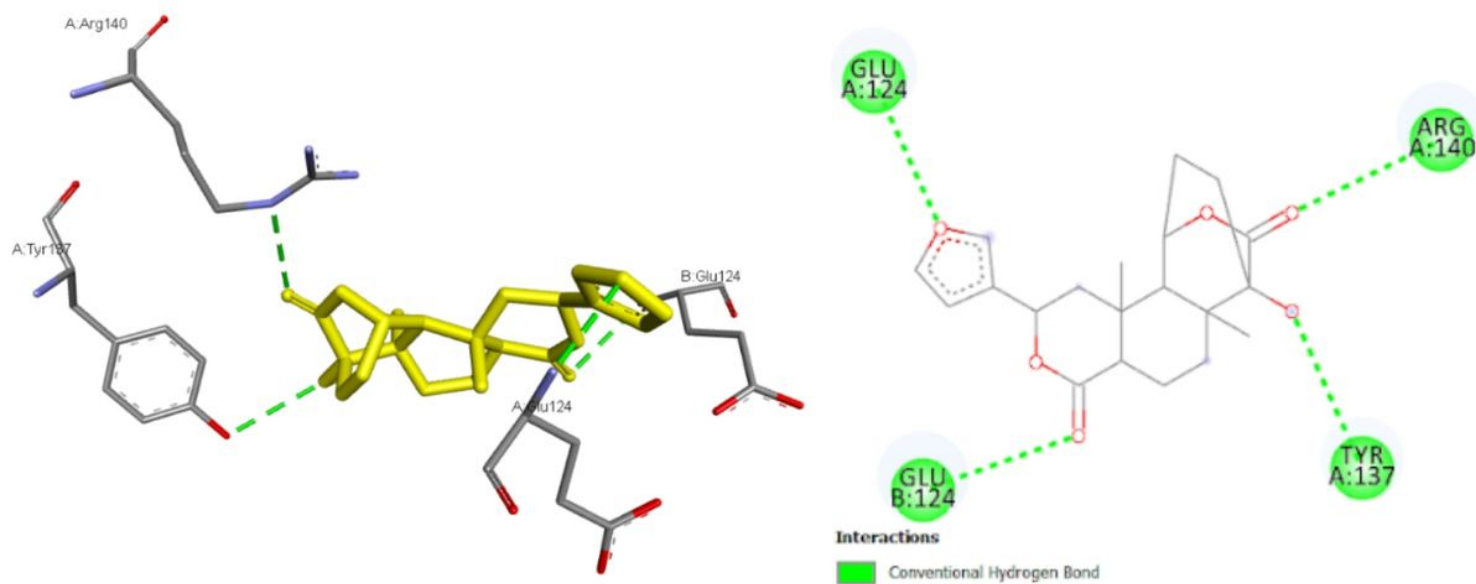


Figure 3

Interaction between Isocolumbin ligand from *Tinospora cordifolia* and SARS-CoV-2 viral protein 7D47

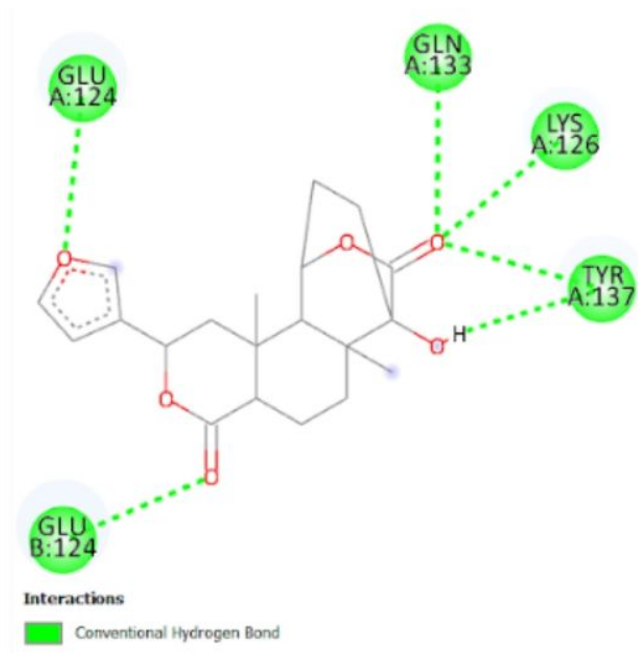
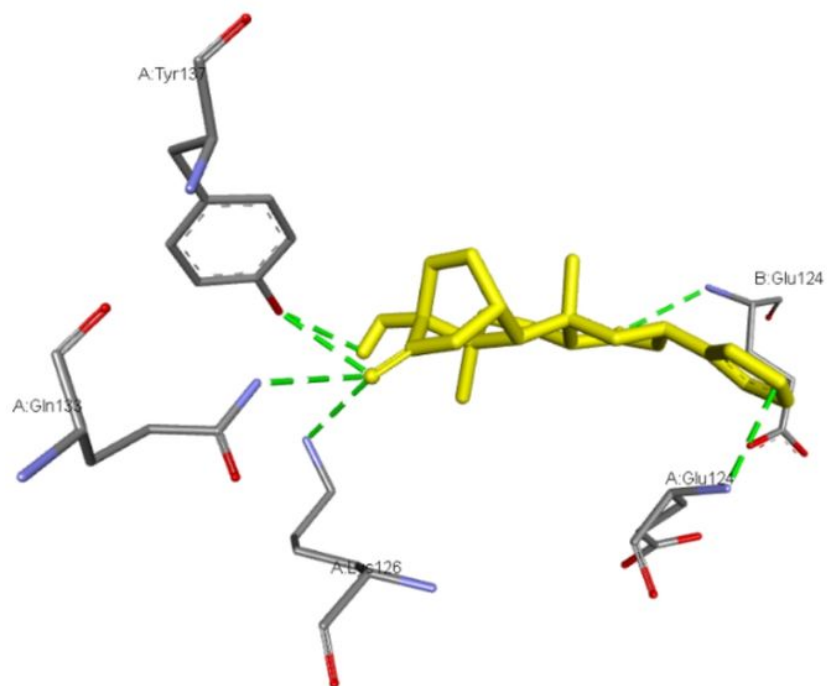


Figure 4

Interaction between Columbin ligand from *Tinospora cordifolia* and SARS-CoV-2 viral protein 7D47