IN-SILLO STUDY OF INDIAN PLANTS AS THE POTENTIAL INHIBITOR'S AGAINST SARS-COV-2 MAIN PROTEASE (MPRO) AND SPIKE (S) RECEPITORS

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***Abstract*: Coronavirus disease 2019 (COVID-19) is an infectious disease caused by Severe acute Respiratory syndrome virus-2 (SARS CoV-2) temporarily named this pathogen as 2019 novel coronavirus (2019-nCoV) is a single positive strand RNA betacoronavirus (CoV).The coronavirus polyprotein encodes two proteases, which share in its processing and release of the translated nonstructural proteins (NSPs), the main protease is called 3-chymotrypsin-like protease (3CL ^{pro}) and a papainlike protease (PL ^{pro}).[12] Both main protease (3CL^{pro} and PL^{pro}) an attractive drug target for drug discovery studies due to its essential role in processing the polyprotein that are translated from the viral RNA. Lack of an effective vaccine and/or antiviral drugs against SARSCoV-2, the causative agent, has severely hampered the response to this novel coronavirus. Natural products have long been used in traditional medicines to treat various diseases, and purified phytochemicals from Indian medicinal plants provide a valuable scaffold for the discovery of new drug leads. In the present study, we performed a computational screening of a database composed of 50 potent phytochemicals derived from Indian medicinal plants with bronchodilator activity. Structure-based virtual screening was carried out against SARS COV-2 main protease. to identify reputed inhibitors that could facilitate the development of potential antiCOVID-19 drug candidates. Considering the ebselen as a reference, we observed that the inhibition potentials of carotene, glychrhizinic acid are very effective. The inhibitory efficiency of other phytochemical constituents also possesses specific inhibition properties against Covid-19 main protease. These potential phytochemical will be subjected to further in vitro and in vivo studies and may assist the development of effective anti-COVID-19 drugs.**

***Keywords*: Coronavirus 19, Spike protein, Natural products, auto-dock vina, carotene**

INTRODUCTION

Coronavirus disease 2019 (COVID-19) is an infectious disease caused by Severe acute Respiratory syndrome virus-2 (SARS CoV-2) temporarily named this pathogen as 2019 novel coronavirus (2019-nCoV) is a single positive strand RNA beta coronavirus (CoV).[1,2] The novel SARS-CoV-2 was first reported to have emerged in the live wildlife market in the Wuhan region of Hubei province, where it has caused mystic pneumonia-like respiratory illnesses in the human population of the area. [3,4] Before the emergence of S SARS CoV-2, there are 6 known human coronaviruses, including the Middle East respiratory syndrome coronavirus (MERS-CoV) and severe acute respiratory syndrome coronavirus (SARS CoV-1).[5] The SARS-CoV-2 is spherical and has mushroom-shaped proteins termed as spikes that give this virus the shape of a crown. The SARSCoV-2 virus spreads primarily through droplets, saliva, or discharges from the nose of an infected person after sneezing or coughing.[6] The symptoms caused by Sars-CoV-2 infection include acute respiratory distress syndrome (~29%), acute cardiac injury (~12%) or acute kidney injury (~7%) [8], implying that Sars-CoV-2 may infect various human tissues. COVID-19 is a highly infectious disease associated with high mortality. [8, 09,10]The coronavirus polyprotein encodes two proteases, which share in its processing and release of the translated non-structural proteins (NSPs), the main protease is called 3-chymotrypsin-like protease (3CL ^{pro}) and a papain-like protease (PL ^{pro}).[11] Both main protease (3CL^{pro} and PL^{pro}) an attractive drug target for drug discovery studies due to its essential role in processing the polyprotein that are translated from the viral RNA. As of May 16, 2020, there have been more than 4,425,485 confirmed cases reported including 302,059 deaths in more than 216 Countries, areas or territories.[12] In addition; millions of people's lives have been affected as a result of mandatory isolations/quarantines. Thus, there is an unmet requirement for the specific anti-COVID-19 therapeutics to limit the severity of the deadly disease. The present study summarized the various clinicians and researcher's investigation and development antivirals using different strategies combining experimental and in-silico approaches with the goal of identifying novel, selective and potent therapeutic agents to target main proteases for COVID-19 treatment.

1.1 Role of Main protease and SARS-CoV-2 infection cycle

Angiotensin-converting enzyme 2 (ACE2) is a cellular receptor expressed in the lungs, arteries, heart, kidneys, and the intestine. ACE2 is a functional receptor, acting as the entry point into human lung cells for SARS-CoV-2 binds via its spike (S) protein.[13] This S protein is cleaved into two subunits, S1 and S2, by an extracellular protease. Receptor-binding domain (RBD) of S1 bind to ACE2, while S2 is further cleaved and activated by the host surfaceassociated type 2 transmembrane cellular serine protease enzyme (TMPRSS2).[14] Together these act result in hostviral membrane fusion and the release of the RNA genome into the cytoplasm of cell host. Once the virus enters the host cell, the viral RNA is exposed. Firstly, the host translational machinery is hijacked to generate large polyproteins upon genome translation of open reading frame (ORF) 1a and ORF1ab by the host cell machinery. The 5′ end of the SARS-CoV-2 genome encodes two polyproteins, pp1a and pp1ab, collectively termed the replicase (replicase polyproteins). The polyproteins (pp1a and pp1ab) are cleaved into 16 non-structural effector proteins (nsps) by 3- chymotrypsin-

like protease ($3CL^{pro}$) and papain-like proteases (PL^{pro}) allowing them to form the replication complex together with the RNA-dependent RNA polymerase (RdRp or sometimes referred to as nsp12), which is responsible for the replication and transcription of full-length negative viral RNA.[15,16,17] The proteolysis of PP1a and PP1ab by 3CLpro occurs at 11 distinct sites and generates various nsps that are important for the viral replication. PLpro is another crucial cysteine protease, an enzyme that cleaves N-terminus of the replicase polyprotein to release several nsps, among them the nsp3, in which PLpro is encoded and is implicated not only in the viral replication but also in suppressing the host innate immune response. In addition, PLpro possesses a nucleic acid-binding domain (NAB) with a nucleic acid chaperon function and is essential in the virus replication correction.[18] like other β-coronavirus, the 3′ end of SARS-CoV-2 genome encodes four essential structural proteins(Spike (S) glycoproteins, Small envelope (E) proteins, Membrane/Matrix (M) proteins and Nucleocapsid (N) proteins) and five to eight set of accessory proteins, which can all interfere with the host innate immune response.[19,20] The newly synthesized structural and accessory proteins are then trafficked from the ER through the Golgi apparatus, after which new virions assemble in budding Golgi vesicles.[16] Finally, the mature SARS-CoV-2 virions are exocytosed and released from the host cell into the surrounding environment to repeat the infection cycle.[21] Once the cell is infected with COVID-19, the existing molecular machinery of the host cell is taken over by the virus to translate its RNA into long chains of proteins, producing more copies. These long viral proteins are activated when cut into smaller pieces by proteases. Hence, viral proteases have a critical role in the propagation of the virus. Viral replication is summarized in **Figure 1**.As one of the bestcharacterized drug targets among coronaviruses, in the absence of closely related human homologues, the main proteases represents one of the most attractive SARS-CoV-2 drug targets.

Since there is no human protease with similar cleavage specificity, the inhibitors are expected to be nontoxic.

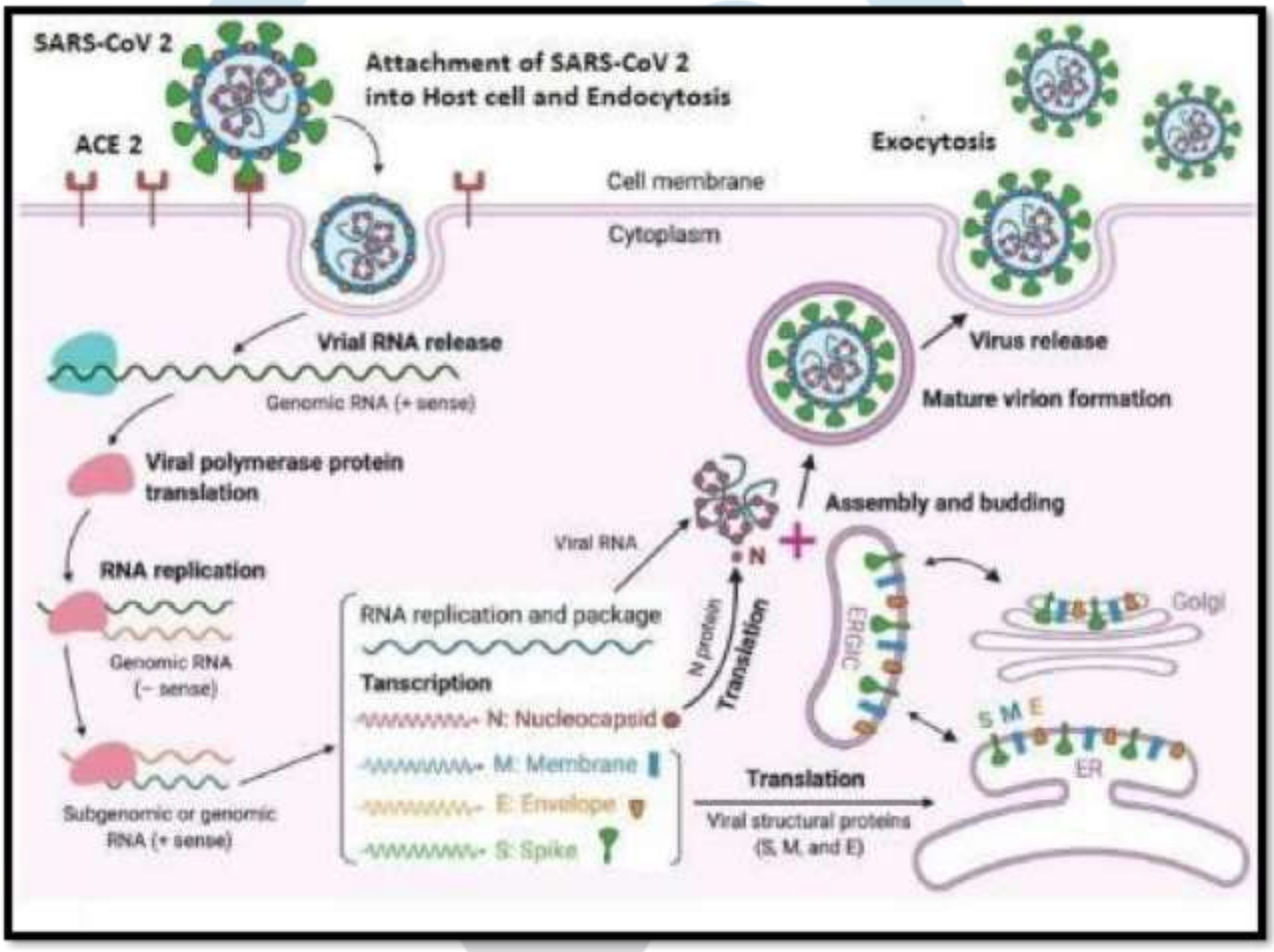


Figure 1: Replication life cycle of SARS -CoV -2

LITRATURE SURVEY

2. Literature review:

2.1 **Tachoua Wafa** and workmates reported Molecular Docking study of COVID-19 Main Protease with Clinically Approved Drugs. CoV M^{pro} (Residues 1-306 aa) is composed of three domains: Domain I (residues 8-101), domain II (residues 102-184) and domain III (residues 201303). The CoV M^{pro} enzymes share a highly conserved substrate-binding pocket, located in the cleft between domains I and II. This pocket serves as a drug target of their selected compounds. In order to identify a novel potent inhibitor they performed docking studies with SwissDock server on the main virus protease (PDB ID: 6LU7) with eight drugs belonging to four pharmacological classes: anti-malarial, anti-bacterial, antiinfective and anti-histamine. The eight approved drugs (Chloroquine, Quinine, Nitazoxanide, Doxycycline, Lymecycline, Cetirizine, Mizolastine, and Indinavir) were able to bind CoV Mpro, with a binding energies of -9.71, -8.09,7.71,-7.52,-8.87,7.99,-8.71,-9.81 Kcal/mol. Among the eight studied compounds, full fitness score revealed that, Lymecycline and Mizolastine had the more favorable binding mode, which is indicated by a more negative full fitness score -1332.56 and -1300.12 Kcal/mol, along with 168 and 256 binding modes detected in the binding substrate pocket, respectivelyIn addition, docking results produced 33 clusters of ligand Lymecycline around the complete CoV Mpro protein. Lymecycline and Mizolastine interact with specific residues in substrate and form three hydrogen bonding to binding cavity. Thus, S and Mizolastione may serve as a tool to fight COVID-19 disease.[22]

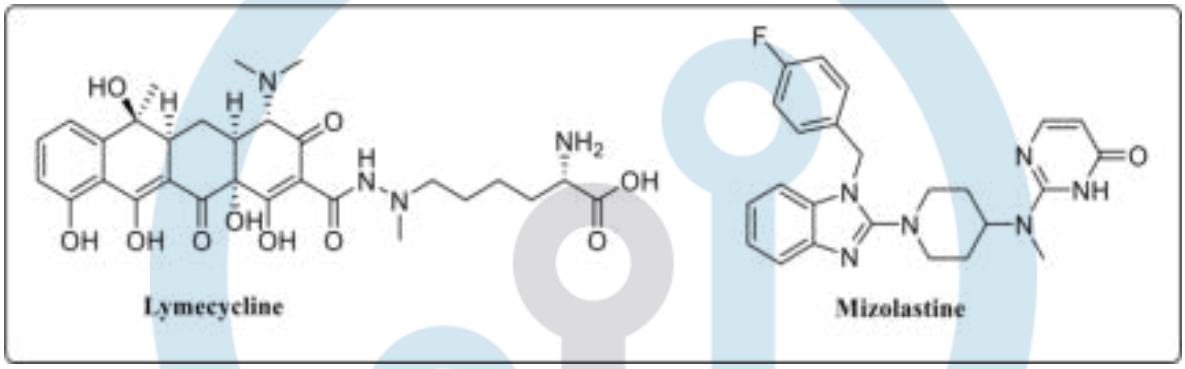


Figure 2: Cheical structure of Lymecycline and Mizolastine

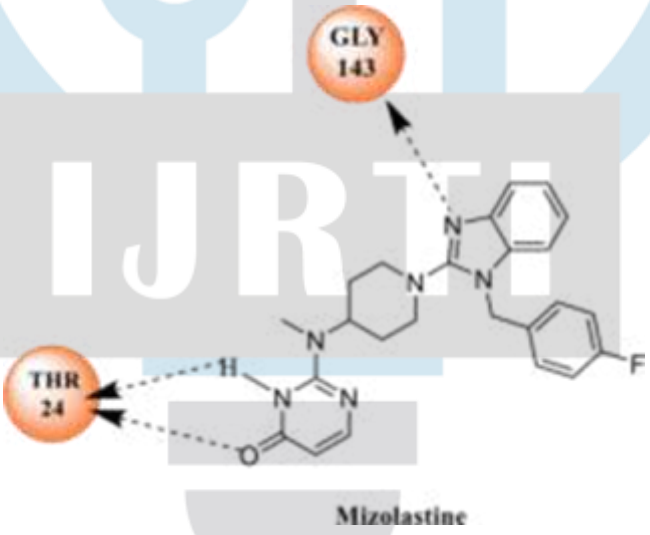


Figure 3: 2D interaction of Mizolastine with SARS-CoV 2main protease

2.2 **Bui Thi Phuong Thuy** carried out analysis of bioactive compounds in Garlic Essential Oil commonly used used to cure chronic colds, pnemonia, and other form of disease by molecular docking studies. A Total of 17 organosulfur bioactive compounds have been identified and studied against SARS-CoV 2main protease **(PDB ID:6lu7)**. Top lead were chosen based on the number of hydrogen bonds, interaction energies, and other criteria defining their efficacy in inhibiting SARS-CoV 2main protease. They determined the qualitative and quantitative eighteen active substances, including 17 organosulfur compounds in garlic essential oil by GC−MS analysis. The main constituents in the garlic essential oil were allyl disulfide (28.4%), allyl trisulfide (22.8%), allyl(E)-1-propenyl disulfide (8.2%), allyl methyl trisulfide (6.7%), and diallyl tetrasulfide (6.5%). They docked this 18 constituents into the crystal structure of main protease (PDB ID:

6LU7) using MOE software.

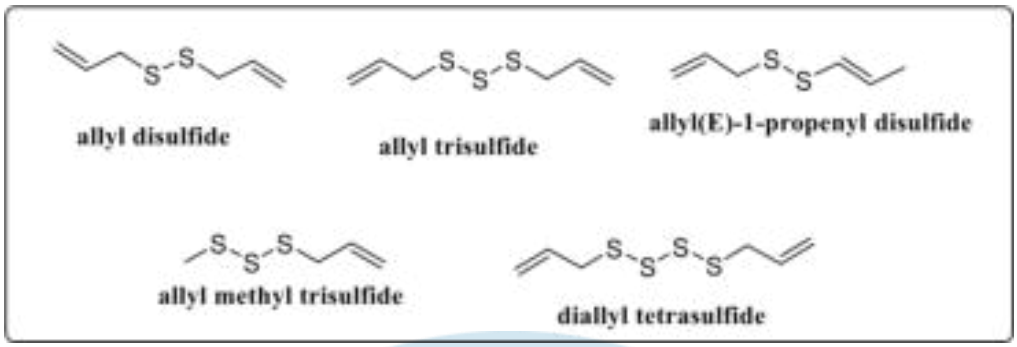


Figure 4: Chemical structure of active constituent of garlic essential oil, allyl disulfide, allyl trisulfide, allyl(E)-**1**-propenyl disulfide, allyl methyl trisulfide, and diallyl tetrasulfide

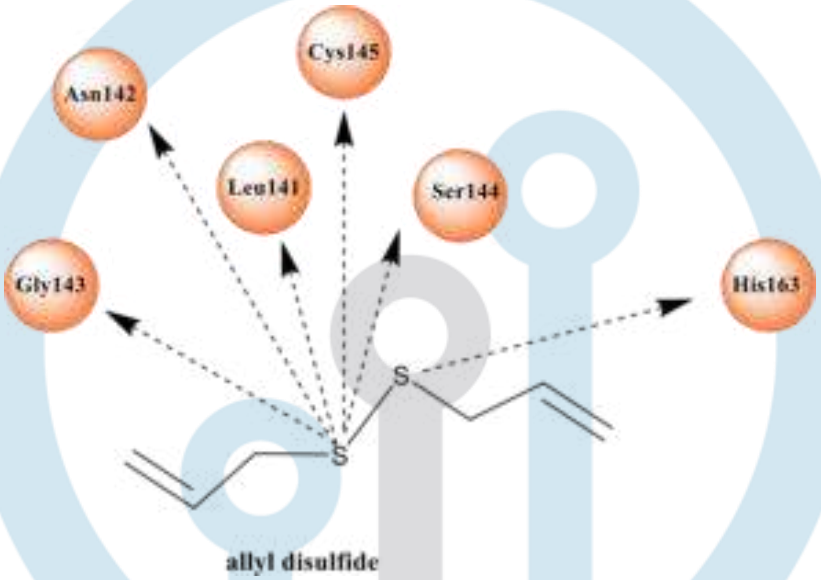


Figure 5: 2D interaction ofallyl disulfide with SARS-CoV 2main protease

The Docking result showed significant interaction between allyl disulfide (-15.32 kcal/mol) and allyl trisulfide (-15.02 kcal/mol) and the human SARS-CoV-2 main protease. Among them the allyl disulfide has high binding score & the stable interaction with SARS-CoV-2 main protease through six amino acid residue i.e. Gly143, Asn142, Leu141, Cys145, Ser144 and His163 respectively.[23]

2.3 **Muhammad Tahir ul Qamar** analysed the 3CLpro sequence, 3D homology model, and screened it against a medicinal plant library containing 32,297 potential antiviral phytochemicals/traditional Chinese medicinal compounds. They performed comparative homology modelling of molecular architecture of SARS-CoV-2 3CLpro using Modeller. They docked ML188 (a potential noncovalent inhibitor of SARS-CoV 3CLpro) with the prepared SARS-CoV-2 3CLpro homology model. They also docked ML188 with the SARS-CoV 3CLpro protein (PDB ID: 3M3V) as a reference, which bind strongly to the Cys-His catalytic dyad (Cys145 and His-41) along with the other residue of SARS-CoV 3CLpro, while this interaction not observed against SARS-CoV-2. In order to screen against the predicted SARS-CoV-2 3CLpro homology model, they generated medicinal plant library containing 32,297 potential anti-viral phytochemicals and traditional Chinese medicinal compounds from their previously collected data and studies. Recently proposed repurposing drug Nelfinavir, Prulifloxacin and Colistin docked taking as a control. Their screening identified nine novel non-toxic, druggable natural compounds

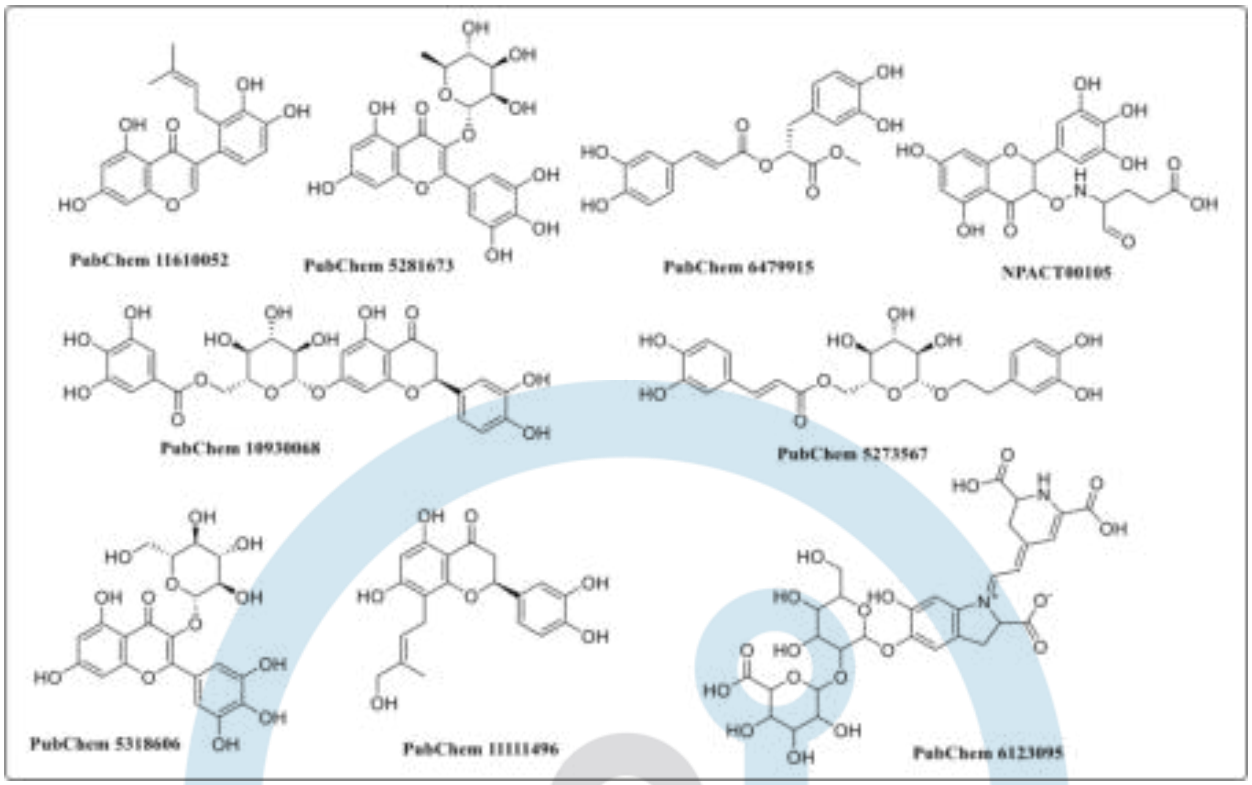


Figure 6: Chemical structure of *insilico* potent screened compound

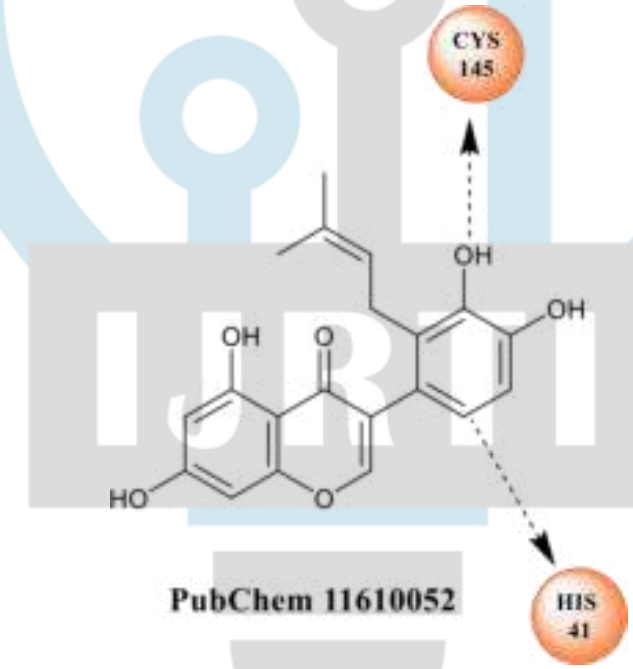


Figure 7: 2D interaction of PubChem11610052 against SARS-CoV 2.

that displayed higher docking scores, stronger binding energies, and closer interactions with the conserved catalytic dyad residues (Cys-145 and His-41) than Nelfinavir, Prulifloxacin and Colistin. Among these screened phytochemicals, 5,7,3´,4´-tetrahydroxy-2'-(3,3-dimethylallyl) isoflavone extracted from Psorothamnus arborescens as an antileishmanial agent, found in traditional Chinese medicine records, displayed the top binding affinity (-29.57 kcal/mol) and docking score (-16.35 kcal/mol), and strong hydrogen bonds interaction with the catalytic dyad residues (Cys-145 and His-41). These results suggested that natural products identified in their study may prove more useful candidates for COVID-19 drug therapy.[24]

2.4 **Ran Yu** & group reported investigation of Lianhuaqingwen (LH) traditional Chinese medicine preparation, which has been shown to have broad-spectrum antiviral effects on a variety of influenza viruses. Honeysuckle and forsythia have been the main component of Lianhuaqingwen, which is widely used as antiviral purpose. Luteolin, the primary flavonoid, is the principal chemical constituent of honeysuckle. Molecular docking of Luteolin flavonoid was performed using Auto Dock Vina software package with crystal structure of the COVID-19 main protease (PDB ID: 6LU7). The Docking study showed that Luteolin's binding energy to the main protease was -5.37 kcal/mol and that of the crystallized ligand (N3) was -3.63 kcal/mol. Leutolin demonstrated

crucial binding interactions with THR26,ASG142 & GLU182 amino acid residues of SARS Cov2 main protease. The binding energy of Luteolin to the active site is also lower than that of the control molecule, meaning that Luteolin has a higher binding activity, which suggests a good antiviral activity. This study demonstrates the potential of traditional Chinese medicine in the treatment of the current coronavirus.[25]

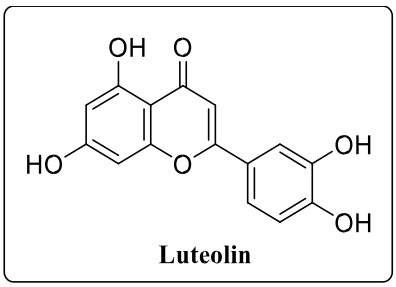


Figure 8: Chemical structure of Luteolin

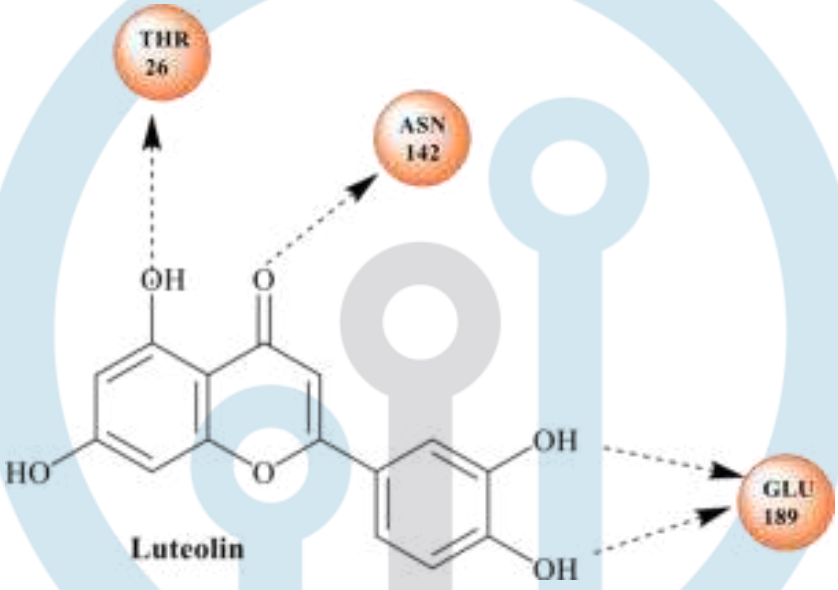


Figure 9: 2D interaction of Luteolin against SARS-CoV 2

RATIONALE and PLAN OF WORK

3. Rationale:

Indian Medicinal plants, especially those employed in traditional Ayurveda medicine, have attracted significant attention because they include bioactive compounds that could be used to develop formal drugs against several diseases with no or minimal side effects.[26] Therefore, the present study was conducted to gain structural insights into the SARS-CoV-2 3CLpro and to discover potent anti COVID-19 natural compounds. Identification of specific inhibitors from natural products against the COVID-19 Mpro might be of great importance in terms of proposing the treatment regimen. Here in the current study, we searched some phytochemical constituents and docked into the Mpro, shows a good binding interactions that might be useful against COVID19 Figure 10.

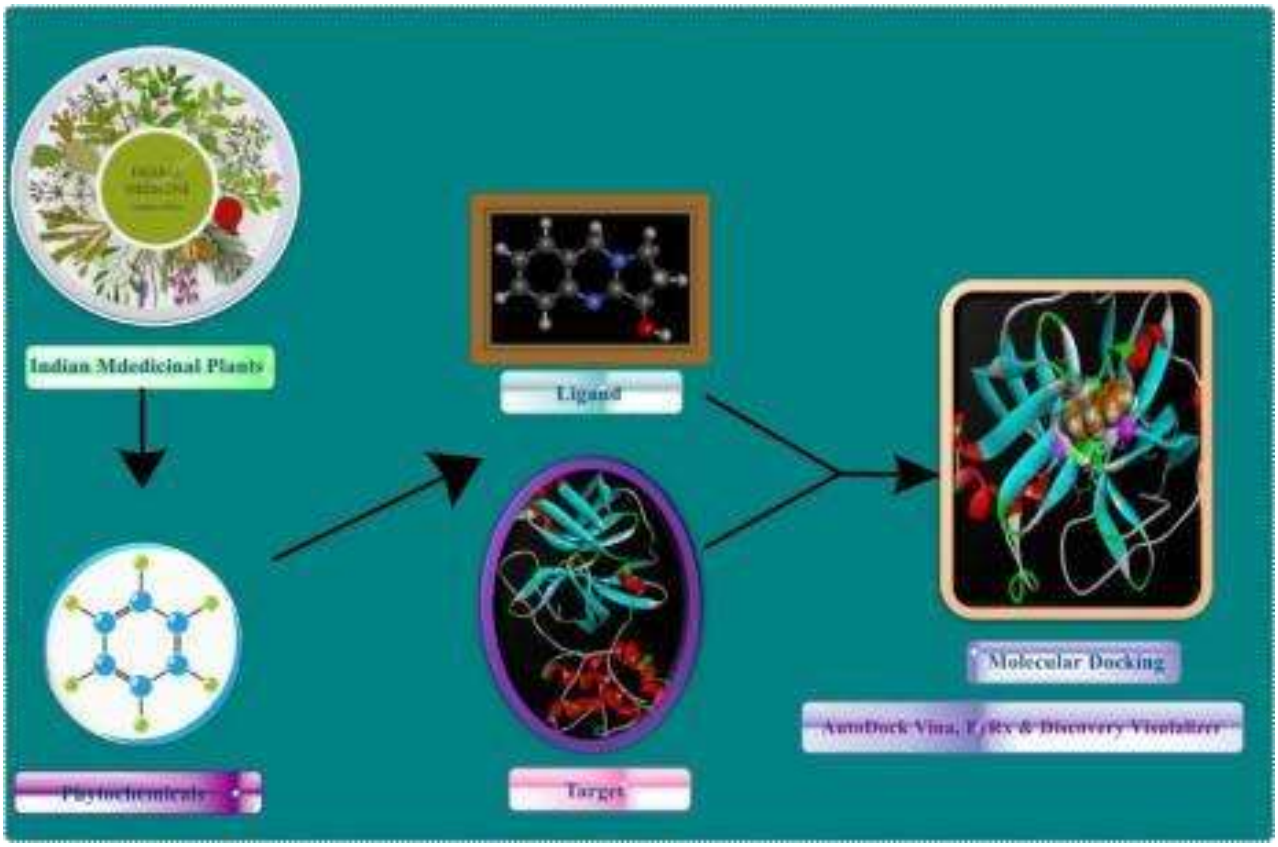


Figure 10: *In-silico* screening of Phytochemicals constituent as a potent inhbitors agains SARS CoV2 main protease.

RATIONALE and PLAN OF WORK

3.1 Plan of Work

1) Based on the literature survey we have prepared the library of potent phytochemicals, extracted from the Indian medicinal plants.

2) It was generated from Pubchem and Chembiodraw software.

3) Molecular Docking study of phytochemical against SARS CoV-2 Main protease (PDB ID:6lu7) by using AutoDock vina and PyRx software.

4) Visualization of Docking results by using Discovery Visualizer software.

5) Comparing of docked molecule with reference molecule

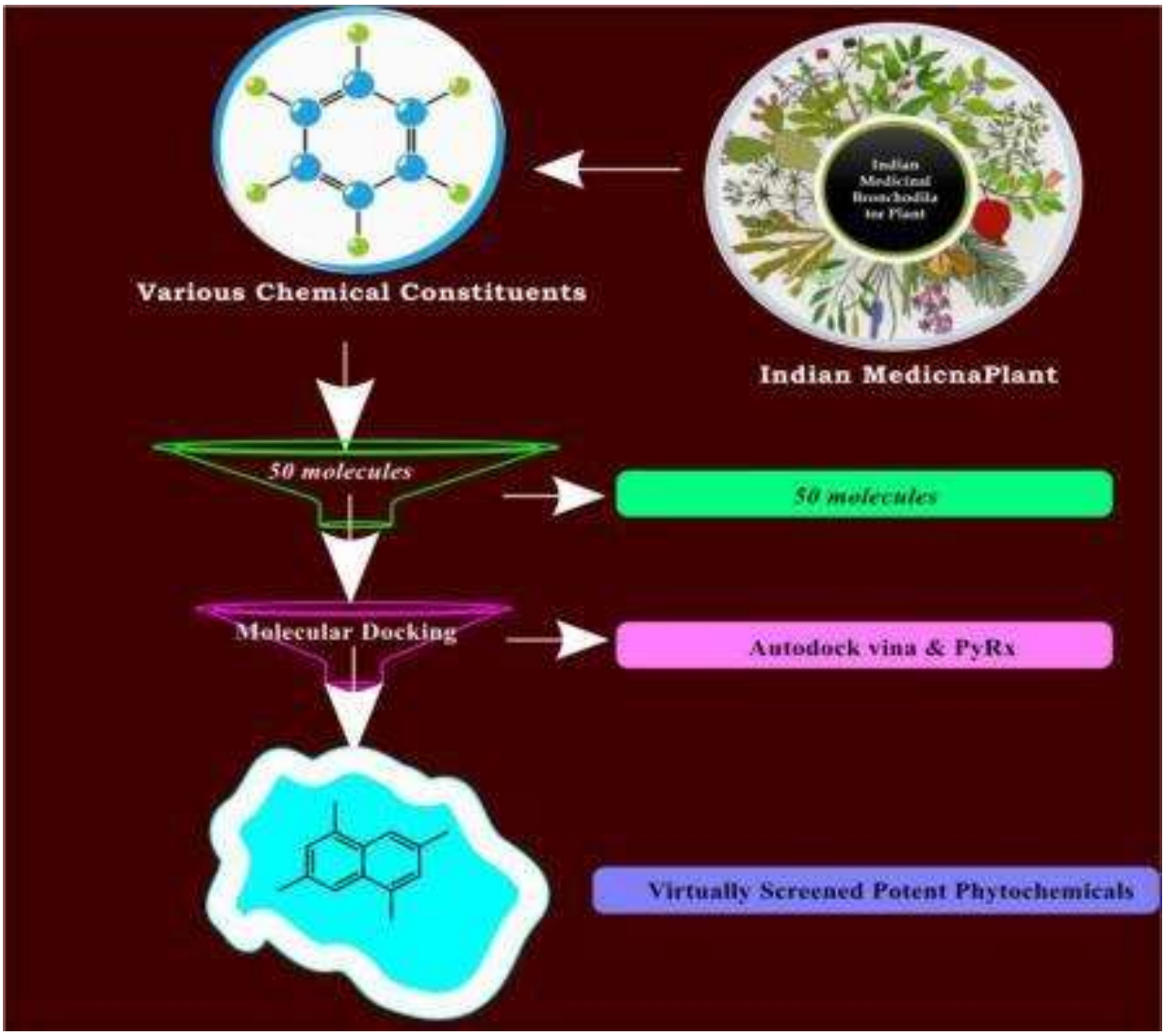


Figure 11: Virtual screening flow chart

CHAPTER 4

EXPERIMENTAL METHODOLOGY

4. Experimental Methodology:

4.1 Protein & Ligand Preparation:

The crystal structure of SARS-CoV-2 Mpro PDB_ID: 6lu7,[27] with the highest resolution of 1.95 Å was retrieved from RCSB (Protein Data Bank) was uses as a target. The structure was subjected to preparation by discovery visualizer. The missing hydrogens were added, and partial charges were assigned. A library containing 50 plant-derived compounds obtained from 08 traditional Indian medicinal plants with Bronchodilator activity were designed. The phytochemicals were collected by comprehensive literature study. It was generated from pubchem[28], and screened against the predicted SARS-CoV-2 3CLpro structure and converted in to PDB with 3 D coordinates using Open Babel[29], an open source chemical toolbox for the interconversion of chemical structures.

4.2 Molecular Docking:

The virtual screening of phytochemical database against the structure of target proteins was performed individually using Autodock vina in PyRx 8.0 virtual screening tool.[30] At first, compounds were imported into OpenBabel program[29], implemented in PyRx for energy minimization using MMFF94 force field and for converting compounds into Autodock PDBQT format. The 3D grid box parameters were set to cover the active site cavity within each protein. The best phytochemical with highest binding energy score against each viral protein were selected as hit compounds for further assessment shown in figure 11.

4.3 Molecular docking protocol:

The molecular docking of candidate phytochemicals against their viral protein's targets were carried out using Autodock vina 1.1.2 program.[31] Autodock Tools 4.2.1 program[32] was employed to prepare the PDB structures of proteins for docking by adding polar hydrogen and to convert proteins and ligands PDB files into Autodock PDBQT format. The PLpro the grid box parameters were set to size 33Å x 36Å x 24Å (x, y and z) and center -10.8366Å x 15.36Å y 59.06Å z (x, y and z). The 2D ligand-protein interaction diagrams were generated by Discovery visualizer tool visualize and analyse the docking results.[33]

5. Results & Discussion

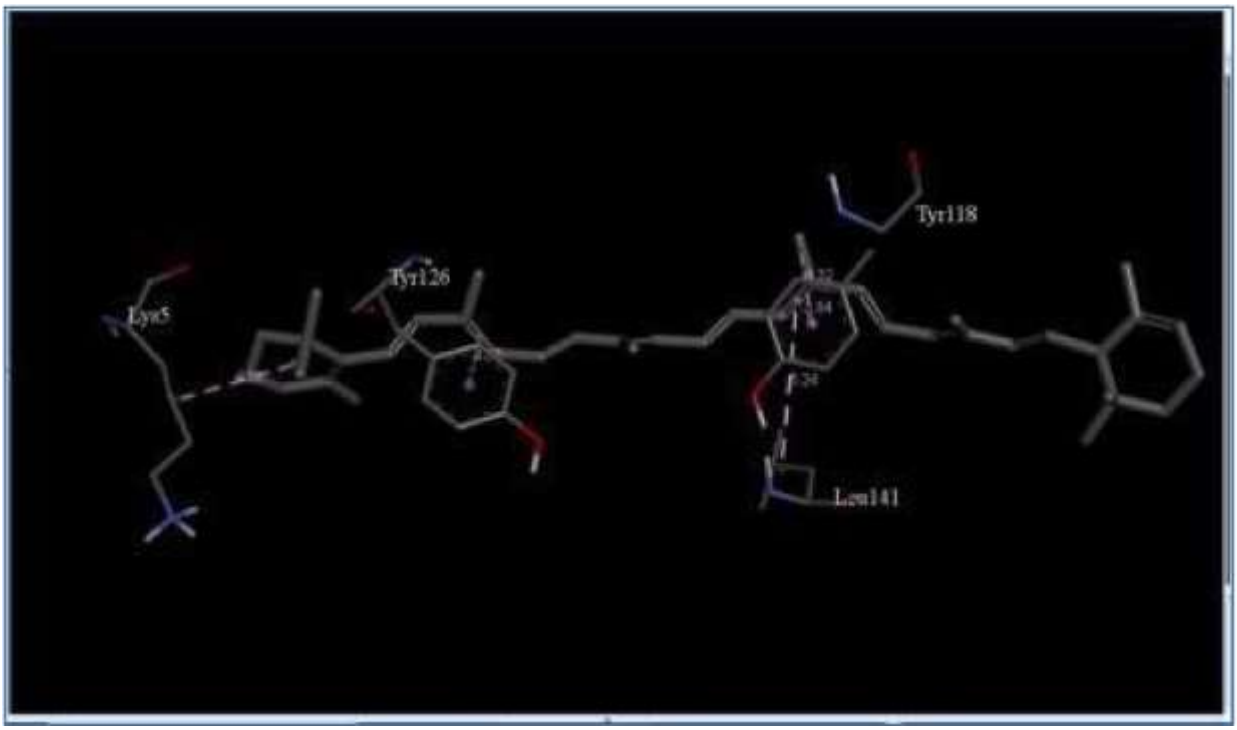
We examined at 11 different types (species) of Indian medicinal plants. The molecular structures of a few (main) chemicals isolated from these plants are shown. We concentrated mostly on chemicals that have been shown to have bronchodilator action. Vasicine, beta sitosterol, inulin, vasicinol, and vasicicnolone are all derived from the leaves of the vasa plant (Malbar Nuts). Alantolactone and isoalantolactone are chemical components of Puskarmula leaves. Glycyrrhizin, glycirhzinic acid, yashimadhu root licorice extract, and licorice. Tvak extracts cinnamomum and zylanicum; Ardraka extracts alpha-carotene, citral, leucine citreniline, and zhingiberol; and Curcumin (C21H20O6) extracted from the dried ground rhizome of turmeric. Linolic acid is a kind of acid. Chebulinic acid is derived from Amalaki. Picrosides, kutkiol sterol, and oleanolic acid were extracted from Kiratika, and kutkiol sterol was taken from Katuki.

Molecular docking studies investigate the mechanism of interaction between ligands and receptors. In the field of drug discovery, the interactions between a ligand and a receptor are critical. The binding affinity (G) of (drug) compounds is determined by the sort of bonding (H-bond) that happens with the protein's active region. Docking findings demonstrate that the chemical ingredient Glycyrrhizinic acid forms H-bonds with Gln-19 and Asn-119(Figure 14,15). Carotene binds to the receptor via a pi contact with Lys-5, Leu-141, and Tyr-118 and 126(Figure 12,13). Picroside has six hydrogen bonds: Glu-166, Phe-140, His-172, Gly-138, Gly-170, Asp-289, and Arg-131. Oleanolic acid, a phytochemical, forms an H-bond with Gln-127. Liquiritin's molecular ingredients produce four hydrogen connections with amino acids such as Gln-69, Gly-1, Glu-19, and Asn-142 (Figure16,17). Vascinolone, Vasicinol, and Vasiccine are phytochemical components of vaska that create hydrogen interactions with Gly-120.Kutkin forms hydrogen binding with three amino acids i.e. Tyr-118,Gly 71 and 120(Figure 18 and 19).

As a result of our docking analysis, we believe that extracts of Indian medicinal plants can inhibit the COVID-19 protease (6LU7). Using ebselen[34] as a reference, we discovered that the inhibitory potentials of carotene and glychrhizinic acid (tABLE-1) are quite effective. Other phytochemical components have particular inhibitory effects against Covid-19 major protease.

Table 1: Docking Score

Sr. NO	Ligand	Binding Affinity
1	6lu7_Ebselen_mmff94_E=166.27	-6.8
2	6lu7_Carotene_mmff94_E=188.01	-7.6
3	6lu7_Glycyrrhizinic_acid_mmff94_E=389.20	-7.4
4	6lu7_Oleonolic_acid__mmff94_E=122.41	-6.7
5	6lu7_Stigmasterol__mmff94_E=104.90	-6.7
6	6lu7_liquiritin_mmff94_E=159.31	-6.6
7	6lu7_Kutkin_)_mmff94_E=168.63	-6.5
8	6lu7_Clerosterol_)_mmff94_E=106.88	-6.3



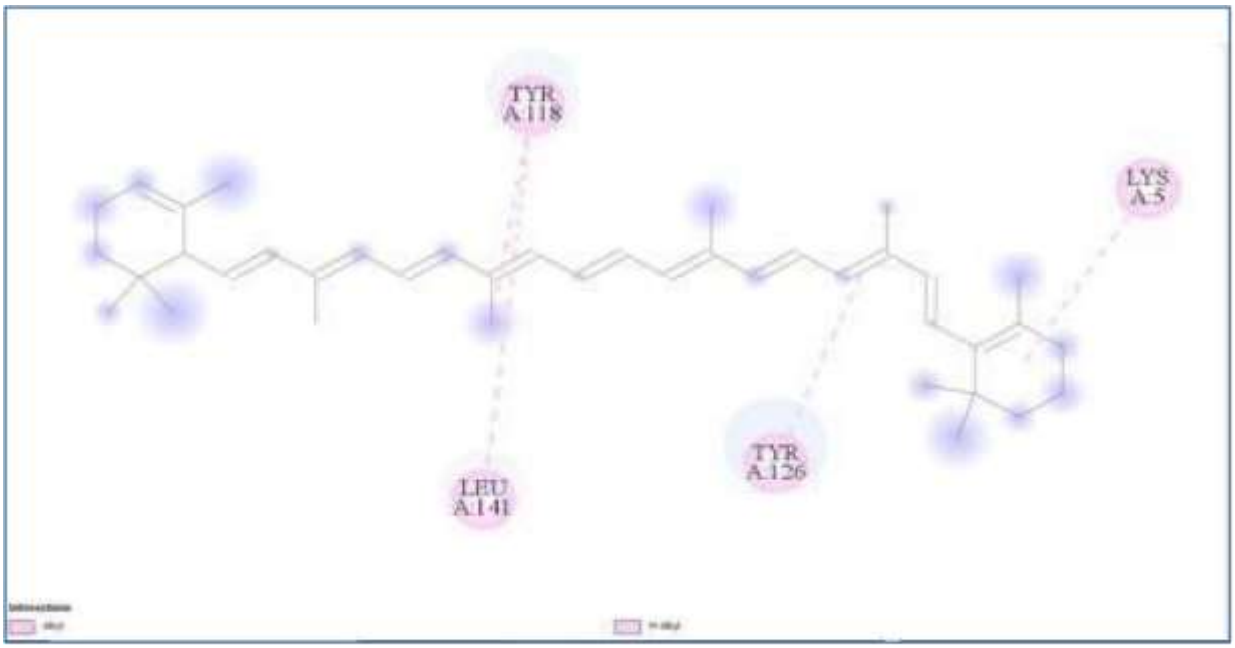


Figure 13: 2D interaction of carotene with SARS-COV-2 main protease of covid19 (PDB:6lu7)

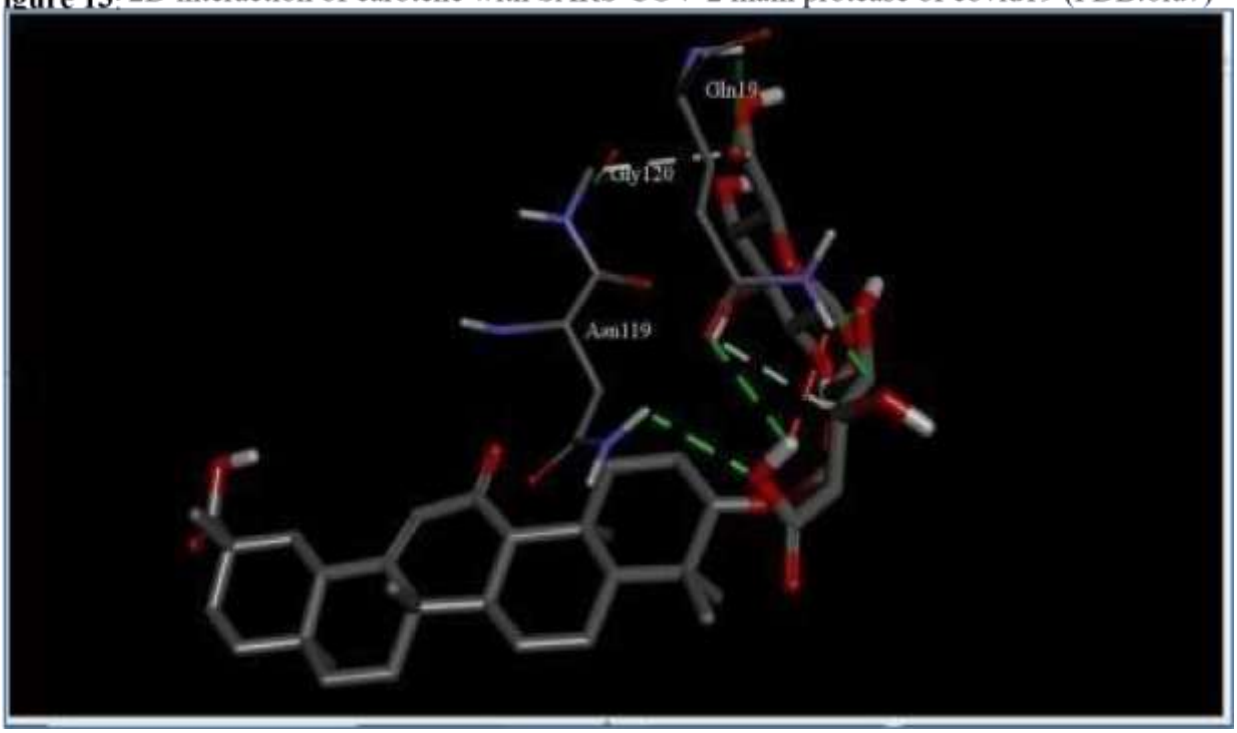


Figure 14: 3D interaction of Glychirhhizic acidwith SARS-COV-2 main protease of covid19 (PDB:6lu7)

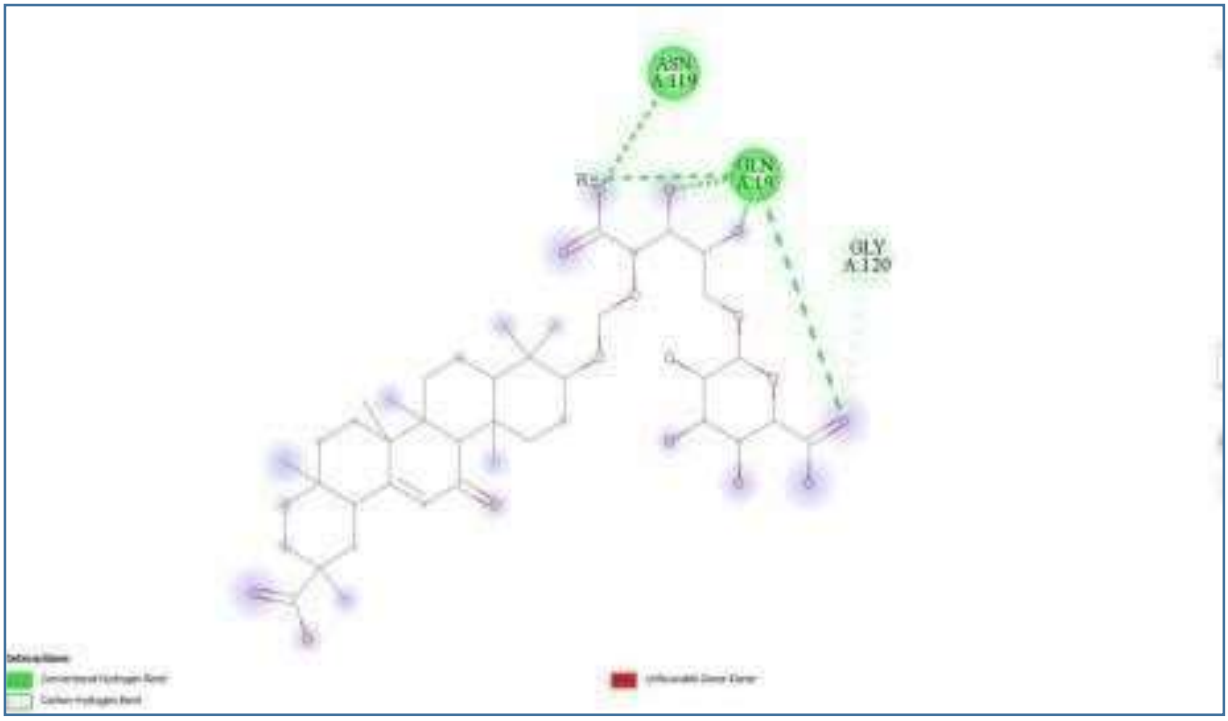


Figure 15: 2D interaction of Glychirhhizic acidwith SARS-COV-2 main protease of covid19 (PDB:6lu7)

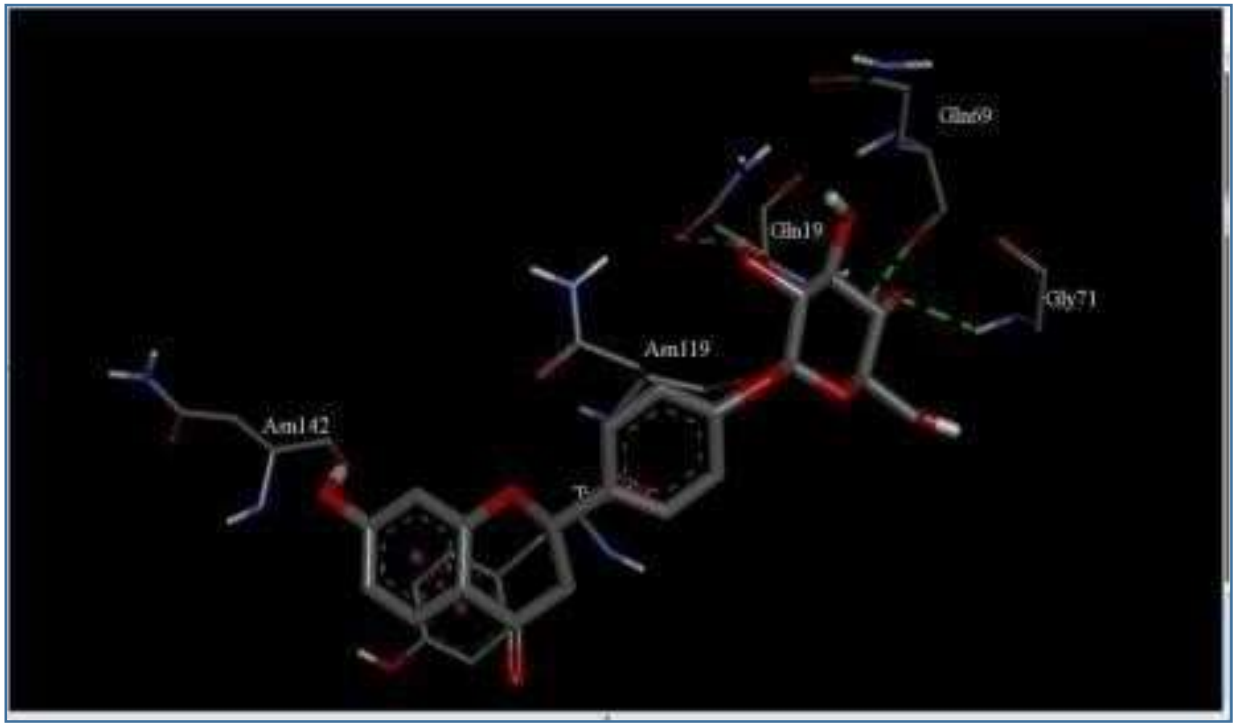


Figure 16: 3D interaction of liquitrin with SARS-COV-2 main protease of covid19(PDB:6lu7)

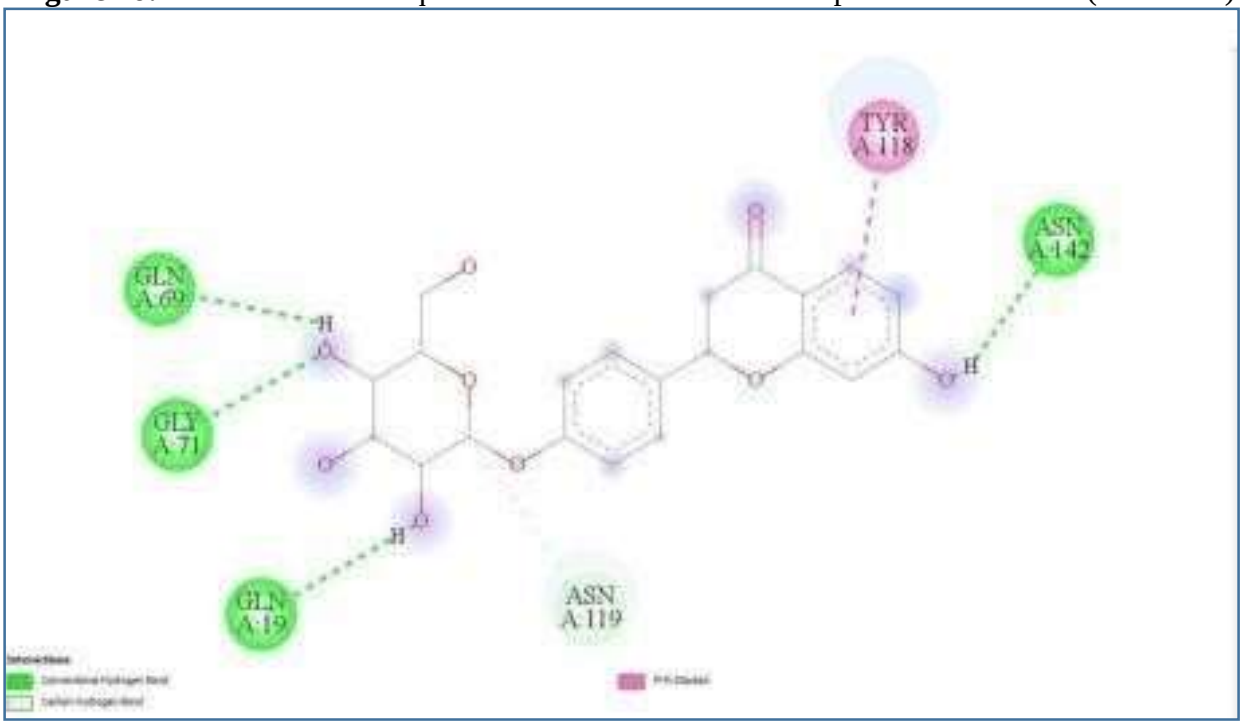


Figure17: 2D interaction of liquitrin with SARS-COV-2 main protease of covid19(PDB:6lu7)

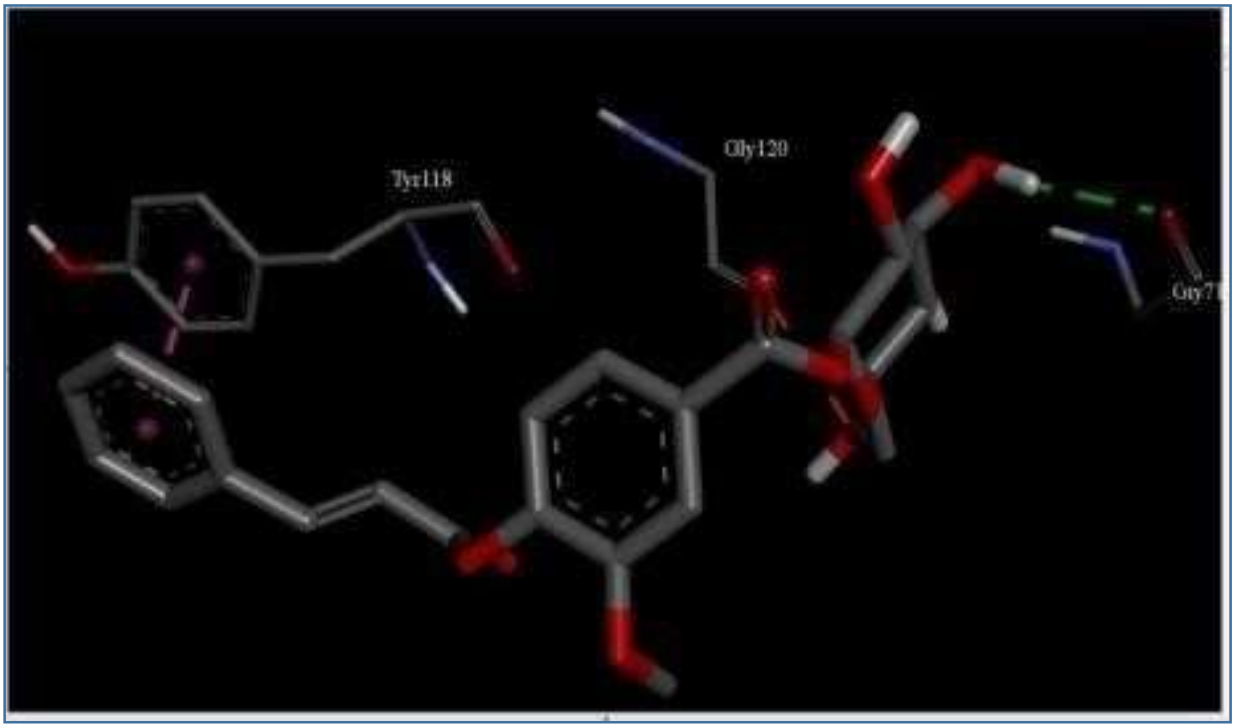


Figure 18: 3D interaction of Kutkin with SARS-COV-2 main protease of covid19 (PDB:6lu7)

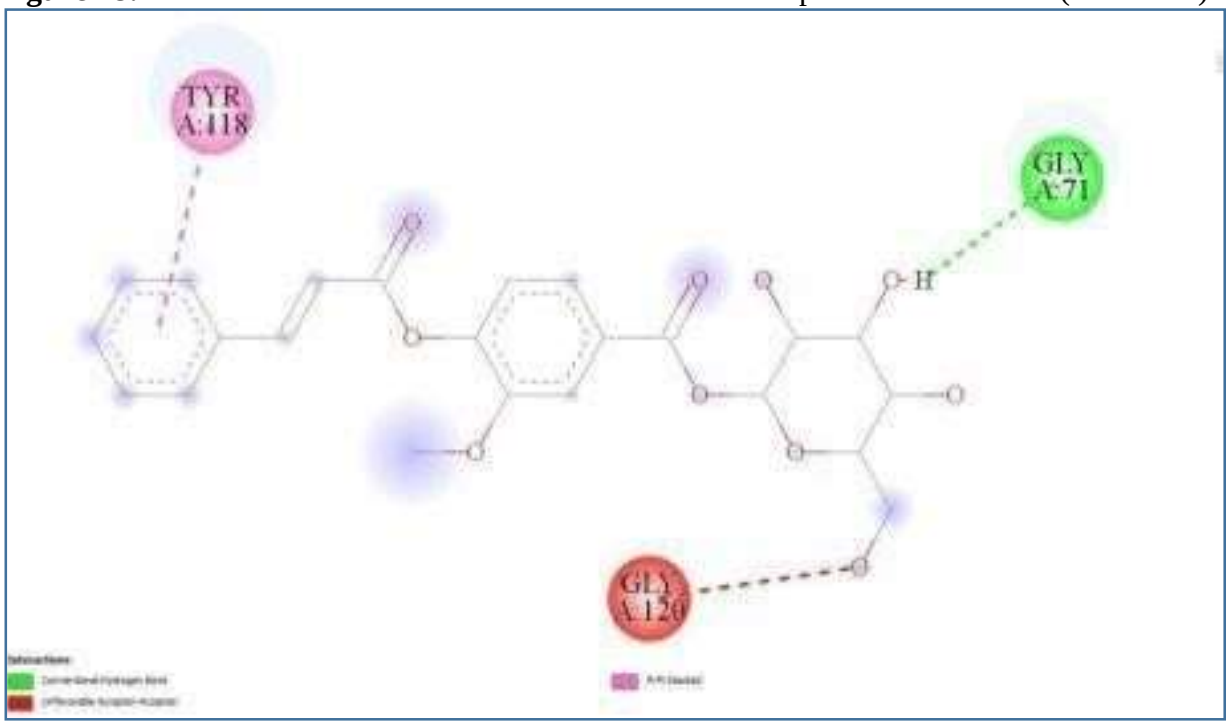


Figure 19: 2D interaction of Kutkin with SARS-COV-2 main protease of covid19 (PDB:6lu7)

SUMMARY AND Conclusion

6. Summary & Conclusion

We investigated the inhibition of COVID-19 protease by extracts of Indian medicinal plants in silico. We discovered that all of these plants have some level of inhibition. Based on binding affinity, the phytochemicals carotene and Glychirhizic acid appear to be the most powerful inhibitors among the eleven plants. Carotene and Glychirhizic acid have more inhibitory potentials than ebselen's reference compounds. The synthetic chemical is active, but it also has some side effects and toxicity. As a result, our findings have piqued our interest in producing alternative (Indian medicinal plant) treatments with no discernible negative effects. We anticipate swift action in this direction to address COVID-19.

6.1 Future Perspective

Future in vitro and in vivo studies of these phytochemicals (carotene and glychirhizic acid) may confirm their antiviral activity and, as a result, their potential to serve as therapeutic candidates for the development of target-specific anti-COVID-19 therapies.

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