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IN SILICO ANALYSIS OF COVID-19 PROTEASE TARGET PROTEIN AGAINST SELECTED PLANT BASED ANTIVIRAL COMPOUNDS

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Abstract : The recent pandemic Covid-19 infection started from China in the later of 2019 and has spread all over the world which killed many people. Due to the lack of immediate medicines and treatment, biologist from many places of the world started finding a new medicines. Many antiviral drugs have been used for treatment but no specific drugs have supported completely to control Covid -19. Plant based compounds are highly potential for treating all types of microbial infection. Hence, screening plant compounds using Molecular docking technique is one of the fast and more reliable smethods for finding potential lead against present pandemic condition. We have selected fifteen plant based antiviral compounds from Literature search. Among those compounds Vincaleucoblastine , Solasonine , Hesperetin and Acrimarine have shown good inhibitory profiles against crystal structure of COVID-19 main protease and 2019-nCoV spike glycoprotein and were also compared with standards Remdesivir and Favipiravir.

Keywords: SARS – CoV -2; Protease and spikeprotein; Multi –targeting drugs; Molecular docking analysis

I.INTRODUCTION

The virus strain causing severe acute respiratory syndrome corona virus 2 (SARS-CoV-2) is a single-stranded RNA virus. COVID-19 is a respiratory problem with the common symptoms of shortness of breath, cough and fever. This disease also reported to show other symptoms like muscle aches, loss of smell, fatigue and abdominal pain (Wang *et al*., 2020). The first infected patient was detected in December, 2019 at Wuhan, China (Guo *et al*., 2020). Afterwards, a total of 12768307 confirmed COVID-19 cases were reported globally with the death of 566654 by 13th July 2020. The outbreak of SARS-CoV-2 was declared as a public health emergency of international concern (PHEIC) and a pandemic respectively on 30th Jan 2020 and 11thMarch 2020 by World Health Organization (WHO).

Covid 19 in INDIA

As of May 8th, 2020 in India, 56,342 positive cases have been reported. India, with a population of more than 1.34 billion the second largest population in the world has difficulty in controlling the transmission of severe acute respiratory syndrome coronavirus 2 among its population. Multiple strategies would be highly necessary to handle the current outbreak; these include computational modelling, statistical tools and quantitative analyses to control the spread as well as the rapid development of a new treatment. The Ministry of Health and Family Welfare of India has raised awareness about the recent outbreak and has taken necessary actions to control the spread of COVID-19. The central and state governments are taking several measures and formulating several wartime protocols to achieve this goal. Moreover, the Indian government implemented a 55-days lockdown throughout the country that started on March 25th, 2020 to reduce the transmission of the virus.

Current Scenario in India

As of May 8th, 2020 the World Health Organization (WHO) had documented 3,759,967 positive COVID-19 cases and the death toll attributed to COVID-19 had reached 259,474 worldwide.

So far, more than 212 countries and territories have confirmed cases of SARS-CoV2 infection. On January 30th, 2020 the WHO declared COVID-19 as a Public Health Emergency of International Concern. The first SARS-CoV-2 positive case in India was reported in the state of Kerala on January 30th, 2020. Subsequently, the number of cases drastically rose. According to the press release by the Indian Council of Medical Research (ICMR) on May 8th, 2020 a total of 14,37,788 suspected samples had been sent to the National Institute of Virology (NIV), Pune and a related testing laboratory (Lee *et al*., 2019). Among them, 56,342 cases tested positive for SARS-CoV-2 (Zhu *et al*., 2020). As of May 8th, 2020. Maharashtra, Delhi, and Gujarat states were reported to be the hotspots for COVID-19 with 17,974, 5,980 and 7,012 confirmed cases respectively. To date, 16,540 patients have recovered and 1,886 deaths have been reported in India. To impose social distancing, the “Janata curfew” (14- hr lockdown) was ordered on March 22nd, 2020. A further lockdown was initiated for 21 days, starting on March 25th, 2020 and the same was extended until May 3rd, 2020 but, owing to an increasing number of positive cases, the lockdown was extended for the

third time until May 17th, 2020 (Guo *et al*., 2019). Currently, out of 32 states and eight union territories in India, 26 states and six union territories have reported COVID19 cases. Additionally, the health ministry has identified 130 districts as hotspot zones or red zones, 284 as orange zones (with few SARS-CoV-2 infections) and 319 as green zones (no SARS-CoV-2 infection) as of May 4th, 2020. These hotspot districts have been identified to report more than 80% of the cases across the nation. Nineteen districts in Uttar Pradesh are identified as hotspot districts and this was followed by 14 and 12 districts in Maharashtra and Tamil Nadu, respectively (Chan *et al*., 2020). The complete lockdown was implemented in these containment zones to stop/limit community transmission (Wang *et al*., 2020). As of May 8th 2020, 310 government laboratories and 111 private laboratories across the country were involved in SARS-CoV-2 testing. As per ICMR report, 14,37,788 samples were tested till date, which is 1.04 per thousand people (Udhaya Kumar *et al*., 2020).

Bioinformatics

Bioinformatics is a branch of Biology which is a multi disciplinary approach to predict biological problems and methods that involves computational, mathematical and statistical methods to decipher the biological information. It is an emerging field having a lot of scope for research and development. Currently students and researchers are showing more interest in getting deep into this new arena of biology, where mathematics, statistics and computer science knowledge is being implemented for deciphering biological concepts. And since the completion of the Human Genome Project (HGP), the importance and demand has become more imperative in this field.

The virus generally spread from the infected person through close contact along with the droplets spilled during talking, coughing and sneezing (Chan *et al*., 2020). After infection with the virus, the symptoms likely to appear within two to fourteen days that depends on the age of person and weak immunity due to other illness like diabetes, asthma, heart ailment etc. (Velavan *et al*., 2020). The lack of recommended drugs or vaccines to deal with the COVID-19 along with the human to human transmission nature is the main concern of this pandemic. Therefore, at present scenario, efforts have been made to identify the infected persons through rapid diagnosis followed by quarantine to stop the further spread of this disease. Also, other recommended steps such as using masks, washing hands with soap and maintaining social distancing are suggested to control the spread of this virus. Simultaneously, the approved drugs, drugs under clinical trial and molecules from medicinal plants extracts are investigated randomly to deal with the COVID-19 infection (Vellingiri *et al*., 2020). In searching drugs / molecules from a library that contained in lakhs, the computational approaches like molecular docking, drug-likeness screening, simulations etc. can expedite the research on drug discovery for COVID-19 (Meng *et al*., 2011).

Potential Therapeutic Target against SARS-CoV and SARS-CoV-2:

Several similarities between SARS-CoV-2 and SARS-CoV have been reported. Since the sequencing of SARS-CoV-2 has been done (Zhu *et al*., 2020). It has given rise to various molecular modelling experiments in order to find the potential drug against novel coronavirus SARS-CoV-2. The SARS-CoV-2 is originated from bat has been revealed by the phylogenetic analysis. (Zhu *et al*., 2020 ; Wan *et al*., 2016) have performed some molecular modelling experiments which revealed similarlity in the 3-D structures of SARS-CoV-2 and SARS-CoV-2 in the RBD (receptor binding domain). This leads to designing of various approaches to find out the potential target for development of potential drug candidate against SARS-CoV-2. The analysis of crystal structure and several biochemical studies revealed that the S protein (spike protein) of SARS-CoV possess strong affinity for binding to human ACE-2 receptors (Li *et al*., 2005). Some of the potential targets for drug design spike protein, envelop protein, membrane protein, protease, nucleocapsid protein, hem-agglutinin esterase and helicase have been identified (Prajapat *et al*., 2020).

S PROTEIN

The S protein (spike protein) consisting of ectodomain region (ED), intracellular domain and TM region has been identified. The S protein is type I-transmembrane (TM) proteins which appear as clove shaped. The ED region consists of two receptor binding domains (RBD), S1 and trimeric stalk containing S2 subunit associated on C-terminal. By association of S proteins, the virion appear as trimeric form which give rise to crown like structure, thus it is called as corona virus (Belouzard *et al*., 2012). The S protein is found to have potential role in viral entry inside the host (Li *et al*., 2016). The activation of host immune response against the virus by S protein has been reported. This protein is considered as a potential target for drug discovery because S1 domain and host ACE2 for SARS-CoV and di-peptidyl peptidase-4 (DPP4) for MERS-CoV associated with host and viral membrane fusion mediated by S2 segment potentiate the CoV to release its RNA in host cell Proteases (Li *et al*., 2016). There are 16 non-structural polyproteins (NSPs) containing PP1a and PP1b present in genome of corona virus encoded by replicase (Lindner *et al*., 2005). The release of NSPs is mediated by the action of protease on polyproteins. The chymotrypsin-like cysteine protease also known as main protease (Mpro) or 3-C like protease (3CLPro) carries out cleavage at C-terminal of polyproteins. The papain like protease (PLpro) facilitate cleavage of polyproteins at N-terminal (Lindner *et al*., 2005). The release of 16 NSPs takes place as PLpro facilitate cleavage at first three sites of polyproteins whereas CLpro facilitates the cleavage at 11 sites (Jo *et al*., 2020). The Cys-His dyad present on the active sites of 3CLPro is reported to show protease activity (Shimamota *et al*., 2015). This protease has ability to carry out cleavage at 11 sites in the p1 region of PP1a and PP1ab. It can generate mature protein which facilitates replication (Hilgenfeld *et al*., 2014 ; Hsu *et al*., 2005) and also helps in releasing NSPs (Barretto *et al*., 2005). Some of the HIV protease inhibitors including Lopinavir and Ritonavir are also found to inhibit Mpro (Liu *et al*., 2020).The papain-like protease (PLpro) potentiate the cleavage polyprotein (PP) at N-terminal to form NSP 1, 2 and 3 (Hilgenfeld., 2014 ; Hsu *et al*., 2005). The catalytic domain of PLpro consists of 316 amino acids which are known to facilitate cleavage of substrates for replicase mediated by a consensus sequence (LXGG) (Barretto *et al*., 2005). The inhibition of CLpro and

PLpro at higher doses of Zinc and its conjugates have been reported. There are several protease inhibitors including combinations of Ritonavir and Lopinavir are being used for treatment of COVID-19(Han *et al*., 2005).

N Protein

The N protein (nucleocapsid protein) consists of N-arm, central linker (CL) and the C-tail which are also known as characteristic intrinsically disordered regions (IDRs), (Chang *et al*., 2016). The major structural and functional domains of the N protein are N-terminal domain (NTD) and C-terminal domain (CTD). The NTD is known to facilitate the RNA binding and CTD plays an important role in dimerization (Chang *et al*., 2016 ; McBride *et al*., 2014). Along with the arginine and serine, the CL region comprises of several phosphorylation sites (Lin *et al*., 2014). The C-tail plays a vital role in interactions of N-M proteins and oligomerization of N protein (Chang *et al*., 2014).N protein is reported to cause inhibition of cell growth in human via inhibition of cytokinesis process (Zhou *et al*., 2008). The N protein peptide (N220) has found to have promising activity towards destruction of N protein expressing cells in transgenic animals. Thus, it can be recognized as potential target for developing DNA vaccine (Cheung *et al*., 2007).

E Protein

E protein (envelop protein) the smallest transmembrane structural protein of coronavirus consists of hydrophobic domain and cytoplasmic tail. It is of 8.4-12 kDa size. The process of ion channels generation is mediated through oligomerization of E protein (Kuo *et al*., 2007 ; Venkatagopalan *et al*., 2015). During viral assembly and release, E protein is known to facilitate viral morphogenesis. In the mammalian cells expressed with SARS-CoV envelop protein, hexamethyleneamiloride has found to inhibit E protein-mediated ion channel activity (Pervushin *et al*., 2009). Along with assembly and release, E protein is also found to be responsible for virulence of virus. M Protein by, virtue of interaction with proteins, introducing Golgi complex in virion and stabilizing the nucleocapsid protein, M proteins modulates the shape of envelope of virus (Venkatagopalan *et al*., 2015 ; Schoeman and Fielding 2019). M protein is known to help intracellular homeostasis in virus via various protein interactions (Schoeman and Fielding, 2019). It consists of a short N-terminal and a long C-terminal (Venkatagopalan *et al*., 2015). The entry of virus takes place in association with interaction of M–M, M–S and M–N proteins. The introduction of spike protein in new virus takes place via M-S interactions (Schoeman and Fielding, 2019). The stabilization of nucleocapsid-RNA complex (RNP complex) is associated with M-N interactions. Despite of regulating the shape of the virus, M and N proteins also facilitate the generation and release of virus like particles. M protein is known to potentiate sensitization of host by virus (Schoeman and Fielding, 2019). The SARS-CoV Helicase (NTPase) enzyme is a member of the superfamily 1. It facilitates hydrolysis of all NTPs (Karpe *et al*., 2010). Helicase can be utilized as potential target for designing numerous drug candidates against various disorders (Anand *et al*., 2020). In order to design the helicase inhibitors, toxicity is a major concern as non-specificity leads to precipitation of toxic effects (Karpe *et al*., 2010).

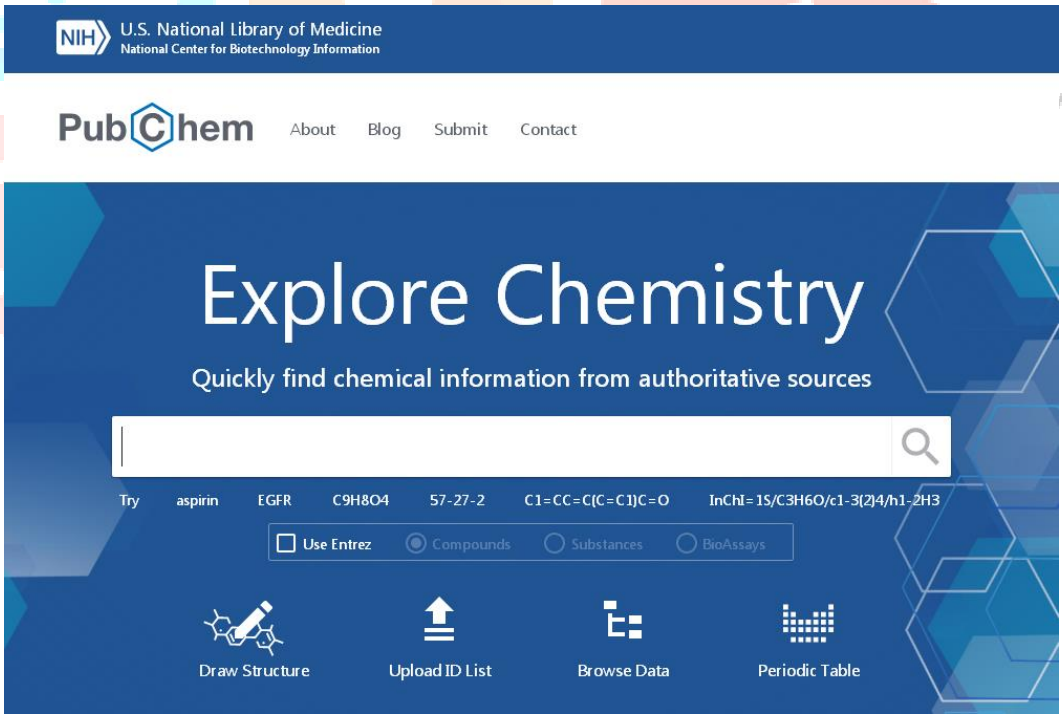
In the present study fifteen plant based antiviral compounds such as Acrimarine, Atropine, Caffeine, Canavanin, Carbine, Coriandrin, Cryptopleurine, Favipiravir, Gingerol, Hesperetin, Ochropamine, Papaverine, Psychotrine, Remdesivir, Solasonine and Vincaleucoblastine have been selected based on literature search for finding potential inhibitors towards the virus SARS- CoV-2.

II. MATERIALS AND METHODS

PubChem

PubChem is an open chemistry database at the National Institutes of Health (NIH). "Open" means that we can put our scientific data in PubChem and that others may use it. (Fig. 1) Since the launch in 2004, PubChem has become a key chemical information resource for scientists, students and the general public. PubChem mostly contains small molecules but also larger molecules such as nucleotides, carbohydrates, lipids, peptides and chemically-modified macromolecules. We collect information on chemical structures, identifiers, chemical and physical properties, biological activities, patents, health, safety, toxicity data and many others. PubChem records are contributed by hundreds of data sources such as government agencies, chemical vendors, journal publishers and more. The amount of data in PubChem is ever-growing.

Fig. 1: PubChem



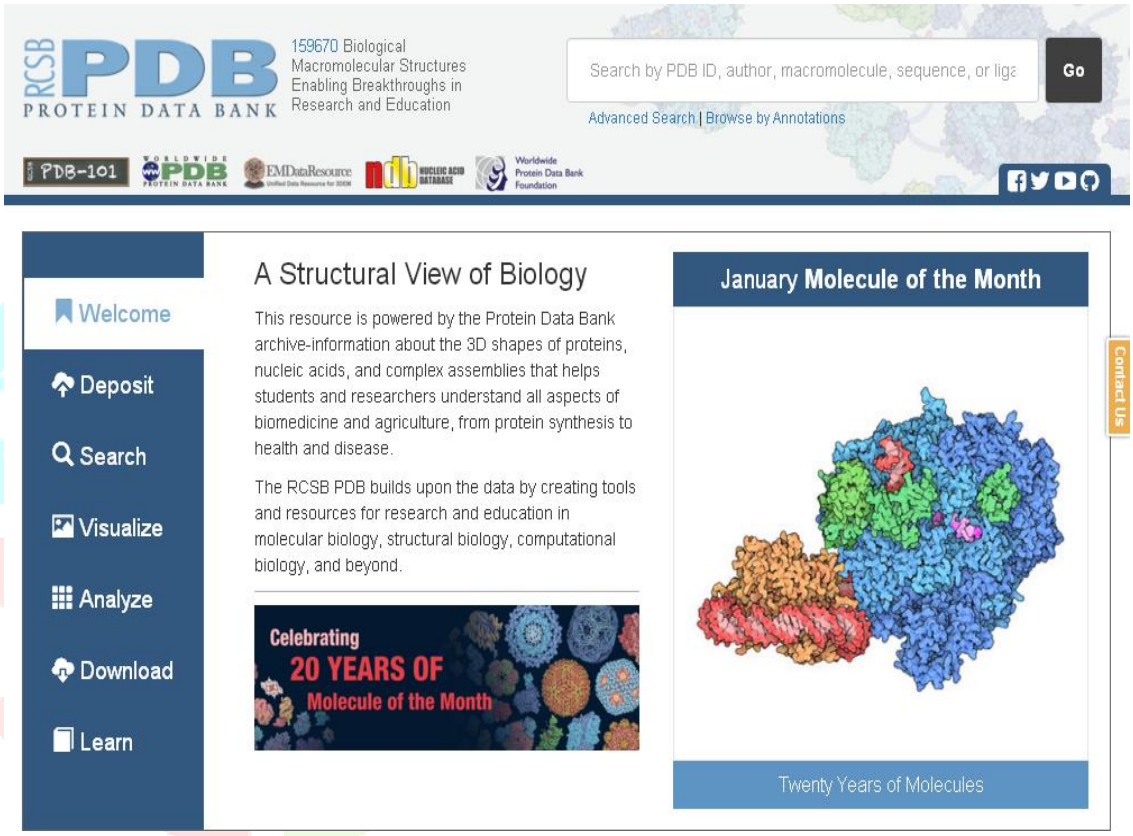
Protein Data Bank (PDB)

The Protein Data Bank (PDB) is a database for the three-dimensional structural data of large biological molecules, such as proteins and nucleic acids (Fig.2). The data typically obtained by X-ray crystallography, NMR spectroscopy or cryo-electron microscopy and submitted by biologists and biochemists from around the world are freely accessible on the Internet via the websites of its

member organizations (PDB, PDBj, RCSB and BMRB). The PDB is overseen by an organization called theWorldwide Protein Data Bank, wwPDB.

The PDB is a key in areas of structural biology such as structural genomics. Most major scientific journals and some funding agencies, now require scientists to submit their structure data to the PDB. Many other databases use protein structures deposited in the PDB. For example, SCOP and CATH classify protein structures, while PDBsum provides a graphic overview of PDB entries using information from other sources, such as Gene ontology.

Fig. 2: Protein Data Bank (PDB)

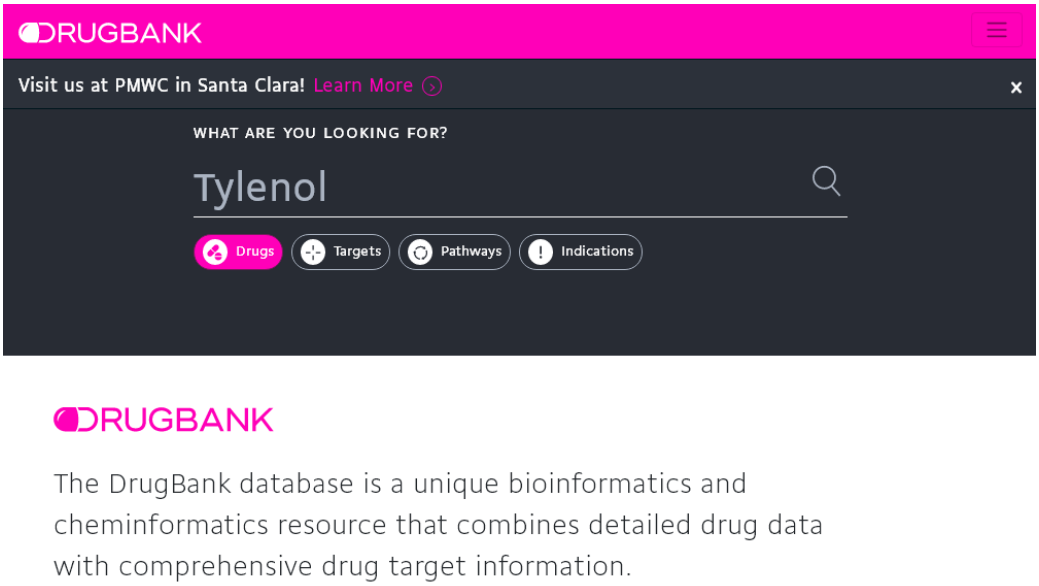


Drug Bank

The DrugBank database is a comprehensive, freely accessible, online database containing information on drugs and drug targets. As both a bioinformatics and a cheminformatics resource, DrugBank combines detailed drug (i.e. chemical, pharmacological and pharmaceutical) data with comprehensive drug target (i.e. sequence, structure, and pathway) information (Fig. 3). Because of its broad scope, comprehensive referencing and unusually detailed data descriptions, DrugBank is more akin to a drug encyclopedia than a drug database. As a result, links to DrugBank are maintained for nearly all drugs listed in Wikipedia. DrugBank is widely used by the drug industry, medicinal chemists, pharmacists, physicians, students and the general public. Its extensive drug and drug-target data has enabled the discovery and repurposing of a number of existing drugs to treat rare and newly identified illnesses.

The latest release of DrugBank (version 5.1.5, released 2020-01-03) contains 13,491 drug entries including 2,638 approved small molecule drugs, 1,364 approved biologics (proteins, peptides, vaccines, and allergenics), 130 nutraceuticals and over 6,356 experimental (discovery-phase) drugs. Additionally, 5,178 non-redundant protein (i.e. drug target / enzyme / transporter / carrier) sequences are linked to these drug entries. Each entry contains more than 200 data fields with half of the information being devoted to drug / chemical data and the other half devoted to drug target or protein data.

Fig. 3: Drug bank



KEGG (Kyoto Encyclopedia of Genes and Genomes)

KEGG (Kyoto Encyclopedia of Genes and Genomes) is a collection of databases dealing with genomes, biological pathways, diseases, drugs and chemical substances. KEGG is utilized for bioinformatics research and education, including data analysis in genomics, metagenomics and the studies, modeling and simulation in systems biology and translational research in drug development (Fig. 4).

- **Systems information**
  - ❖ PATHWAY - pathway maps for cellular and organismal functions
  - ❖ MODULE - modules or functional units of genes
  - ❖ BRITE - hierarchical classifications of biological entities
- **Genomic information**
  - ❖ GENOME - complete genomes
  - ❖ GENES - genes and proteins in the complete genomes
  - ❖ ORTHOLOGY - ortholog groups of genes in the complete genomes

- **Chemical information**
  - ❖ COMPOUND, GLYCAN - chemical compounds and glycans
  - ❖ REACTION, RPAIR, RCLASS - chemical reactions
  - ❖ ENZYME - enzyme nomenclature
- **Health information**
  - ❖ DISEASE - human diseases
  - ❖ DRUG - approved drugs
  - ❖ ENVIRON - crude drugs and health-related substances

Systems information

The KEGG PATHWAY database, the wiring diagram database, is the core of the KEGG resource. It is a collection of pathway maps integrating many entities including genes, proteins, RNAs, chemical compounds, glycans, and chemical reactions, as well as disease genes and drug targets, which are stored as individual entries in the other databases of KEGG. The pathway maps are classified into the following sections:

- Metabolism
- Genetic information processing (transcription, translation, replication and repair, etc.)
- Environmental information processing (membrane transport, signal transduction, etc.)
- Cellular processes (cell growth, cell death, cell membrane functions, etc.)
- Organismal systems (immune system, endocrine system, nervous system, etc.)
- Human diseases
- Drug development

The metabolism section contains aesthetically drawn global maps showing an overall picture of metabolism, in addition to regular metabolic pathway maps. The low resolution global maps can be used, for example, to compare metabolic capacities of different organisms in genomics studies and different environmental samples in metagenomics studies. In contrast, KEGG modules in the KEGG MODULE database are higher-resolution, localized wiring diagrams, representing tighter functional units within a pathway map, such as subpathways conserved among specific organism groups and molecular complexes. KEGG modules are defined as characteristic gene sets that can be linked to specific metabolic capacities and otherphenotypic features, so that they can be used for automatic interpretation of genome and metagenome data.

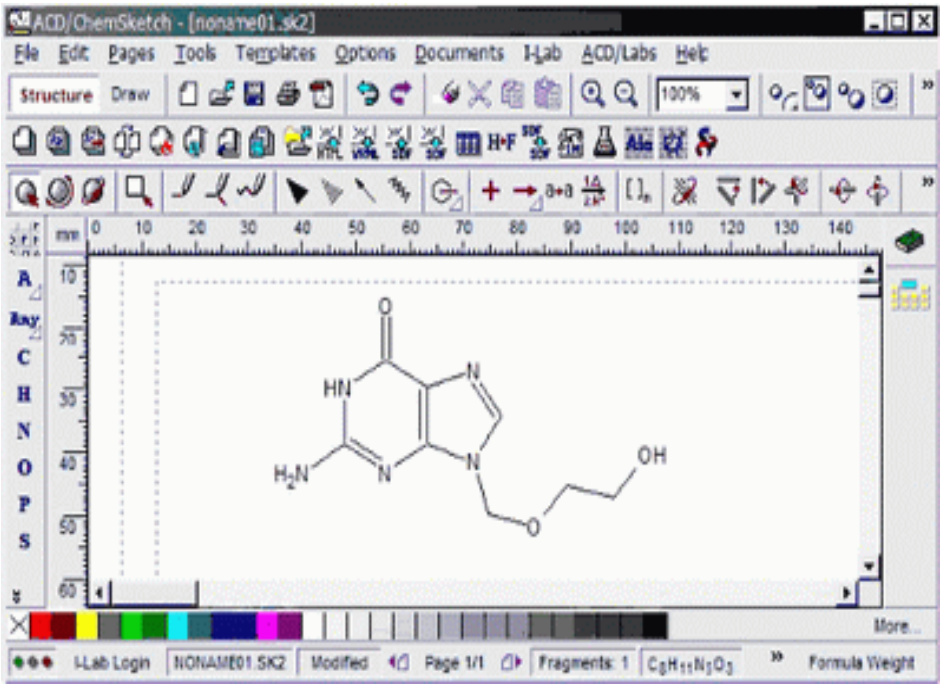
Fig. 4: Kyoto Encyclopedia of Genes and Genomes (KEGG)

KEGG BRITE

Another database that supplements KEGG PATHWAY is the KEGG BRITE database. It is an ontology database containing hierarchical classifications of various entities including genes, proteins, organisms, diseases, drugs and chemical compounds. While KEGG PATHWAY is limited to molecular interactions and reactions of these entities, KEGG BRITE incorporates many different types of relationships.

ACD/ChemSketch is an advanced chemical drawing tool and is the accepted interface into the industry's best NMR and molecular property predictions, nomenclature and analytical data handling software (Fig. 5).

Fig. 5: KEGG BRITE



ACD/ChemSketch is also available as freeware, with functionalities that are highly competitive with other popular commercial software packages. The freeware contains tools for tautomer prediction, 2D structure cleaning, 3D optimization and viewing, drawing of polymers, organometallics, and Markush structures - capabilties which are not even included in some of the commercial packages from other software producers. Also included is an IUPAC systematic naming capability for molecules with fewer than 50 atoms and 3 rings. The capabilities of ACD/ChemSketch can be further extended and customized by programming.

Receptors selected for this study

The three dimensional structure of the target proteins, the crystal structure of COVID-19 main protease in complex with an inhibitor N3.(PDB ID 6LU7) and Prefusion 2019-nCoV spike glycoprotein with a single receptor-binding domain (PDB ID 6VSB) were downloaded from protein Data Bank. The downloaded structures were prepared for docking measures.

Ligands selected for this study

The three dimensional conformers of plant based compounds and commercially available drugs were downloaded from Pubchem compound database. The downloaded inhibitor molecules were energy minimized and prepared for docking study.

Open Babel

Open Babel is a free, open-source version of the Babel chemistry file translation program. Open Babel is a project designed to pick up where Babel left off, as a cross platform program and library designed to interconvert between many file formats used in molecular modeling, computational chemistry and many related areas.

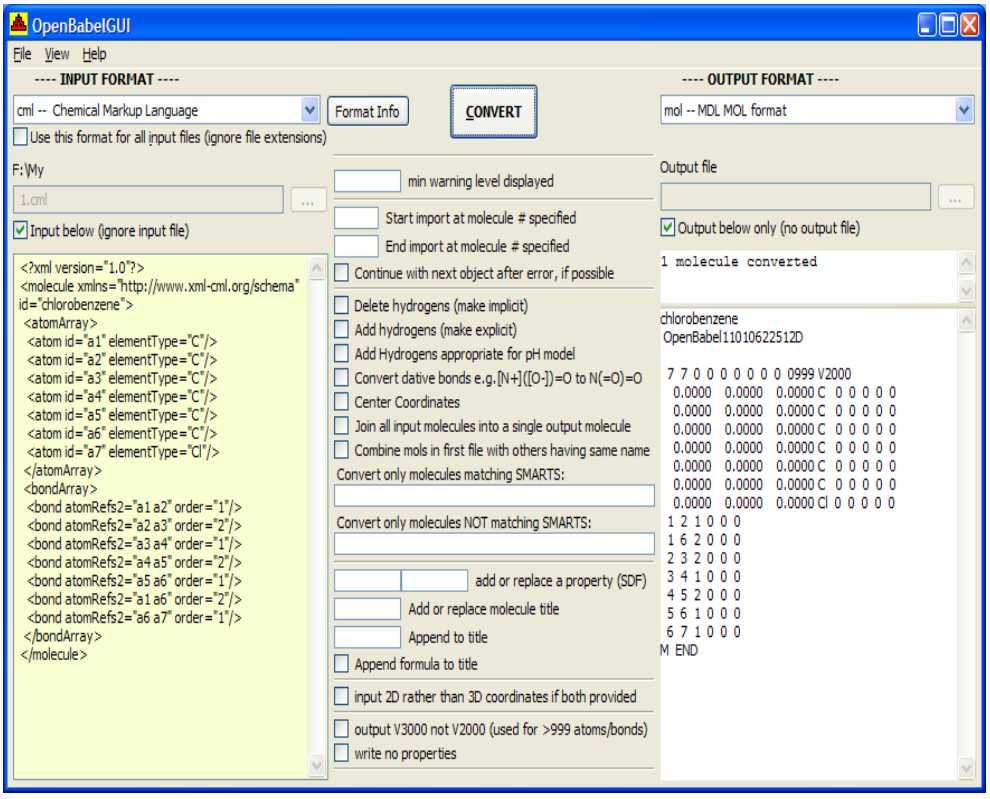
Open Babel includes two components, a command-line utility and a C++ library. The command-line utility is intended to be used as a replacement for the original Babel program, to translate between various chemical file formats (Fig. 6). The C++ library includes all of the file-translation code as well as a wide variety of utilities to foster development of other open source scientific software.

The file formats currently supported are some of the more common file formats such as:

- documentation on the file format
- working code to read the file format or translate it
- example files in the new file format and in some other format

The SMILES format contains 2D information on the molecule. That is, it says which atoms are connected to which other atoms and what type of bonds are present. MOL2, PDB and several other formats contain 3D coordinate information not present in the SMILES format. Since Open Babel does not attempt to generate 3D structure, by default, all of the coordinates are set to zero. However, it is possible to generate 3D structure with the release of Open Babel 2.2.0 using the `--gen3d` option.

Fig. 6: Open Babel

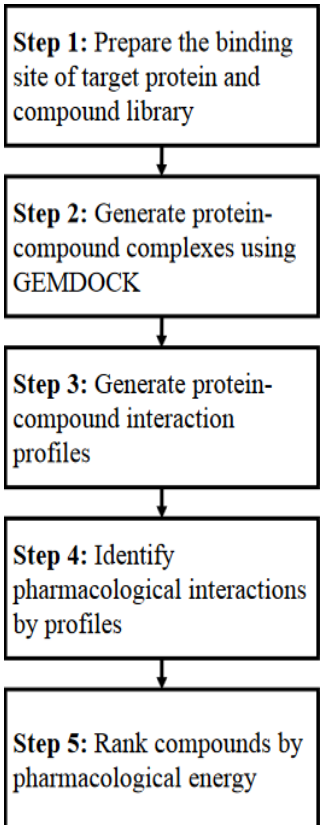


Molecular docking study

iGEMDOCK

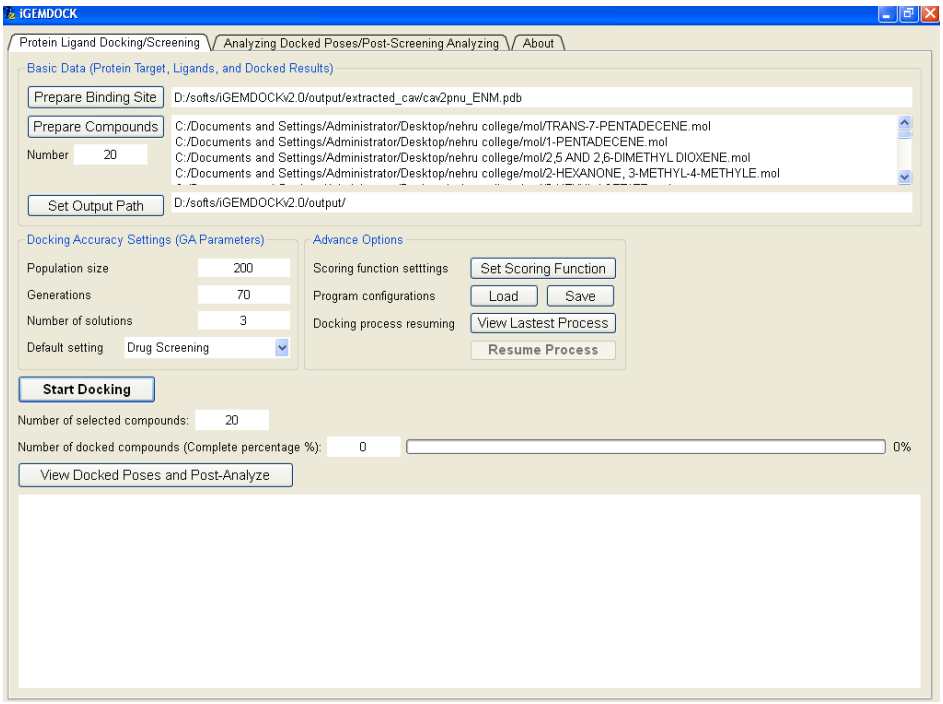
Structure-based virtual screening and post-screening analysis are emergent tasks in computer-based drug discovery. Combining these two methods to effectively reduce the false positives from a large compound database is considered as a key step to find the lead compounds (Fig. 7). In this study, we have developed a graphical-automatic drug discovery system called iGEMDOCK, for integrating docking, screening, post analysis and visualization. To our best knowledge, iGEMDOCK is the first system for this requirement. The core of iGEMDOCK is the GEMDOCK, which is a robust and well-developed tool. Using iGEMDOCK, the predicted poses generated from the GEMDOCK are able to be directly visualized by a molecular visualization tool and analyzed by post-analysis tools. iGEMDOCK provides the post-analysis tools by using k-means and hierarchical clustering methods based on the docked poses (i.e. protein-ligand interactions) and compound properties (i.e. atomic compositions). Atomic composition (AC), which is similar to the amino acid composition of a protein sequence, is a new concept for measuring compound similarity. The protein-ligand docking accuracy and screening accuracies of iGEMDOCK is validated by using a test set with 100 protein-ligand complexes and four targets, respectively, which are thymindine kinase, estrogen receptor for antagonists and agonists and human DHFR. Experimental results show that iGEMDOCK keeps the advantages of iGEMDOCK and provided graphical-integrated environment for virtual screening and docking. The AC on a test set with 76 compounds is evaluated. The results indicate that the AC method performs better than the comparative methods in this set. The iGEMDOCK which

integrates the structure-based virtual screening and post-screening analysis, is a useful system for drug discovery. The core of iGEMDOCK is the GEMDOCK.



In the present study iGEMDOCK is used as a molecular docking tool in order to carry out the docking simulation iGEMDOCKv is an integrated Virtual Screening environment from pre-preparation through post-screening analysis with pharmacological interactions. The docking protocol consisted of 70 generations per ligand andthe population size of 200 random individuals. All the docking conformations were performed twice using genetic evolutionary algorithm and the fitness of the docked structures were calculated. The hydrophobic preference and electrostatic preference were set to 1.00. The binding site of the target was identified at a distance 8Å. The empirical scoring function of iGEMDOCK was estimated as: Fitness = vdW + Hbond + Elec. Here, the vdW term is van der Waal energy. Hbond and Elect terms are hydrogen bonding energy and electro statistic energy, respectively. The pharmacological scoring function of iGEMDOCK was estimated as Epharma=EGEMDOCK+E (E) pharma+2E (H) pharma+0.5E (V) pharma Where EGEMDOCK is the docked energy of GEMDOCK and E (E) pharma, E (H) pharmaand E (V)pharmaare the pharmacological scores of electrostatic, hydrogen bonding and vdW interactions, respectively. Based on these profiles and compound structure, iGEMDOCK infers the pharmacological interactions and clusters the screening compounds for the post screening analysis. Finally iGEMDOCK ranks and visualizes the screening compounds by combining the pharmacological interactions and energy-based scoring functions of iGEMDOCK.

Fig. 7: iGEMDOCK



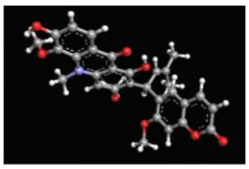
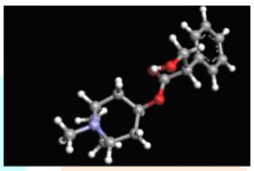
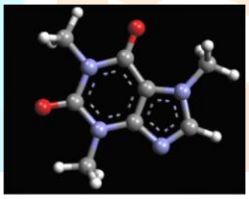
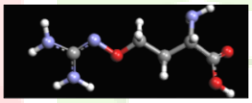
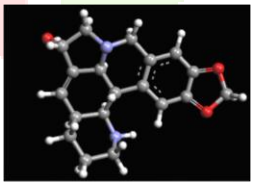
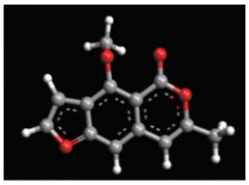
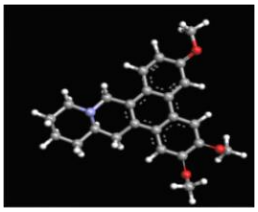
III. RESULTS AND DISCUSSION

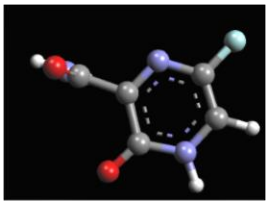
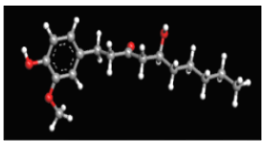
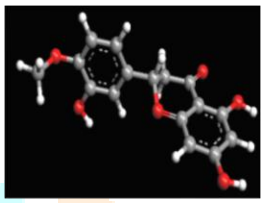
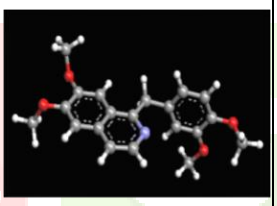
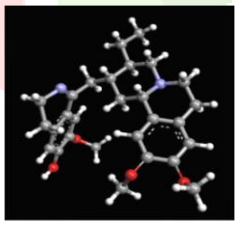
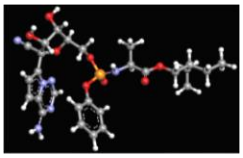
The crystal structure of COVID-19 main protease in complex with an inhibitor N3 was downloaded from Protein Data Bank (PDB Code6LU7). which was predicted by the Method of X-RAY DIFFRACTION with Resolution of 2.16 Å (Fig. 8). Perfusion 2019-nCoV spike glycoprotein with a single receptor-binding domain 3D structure was downloaded from Protein Data bank (PDB Code 6VSB) which was predicted by the Method of ELECTRON MICROSCOPY with Resolution: 3.46 Å (Fig. 9). The plant compounds and currently used drug molecules were downloaded in 2D and 3D coordinates from Pubchem compound database and Drug bank for interaction studies. The downloaded molecules were converted into .mol file format using open Babel software which is acceptable chemical file formats for most of molecular docking softwares. Compounds downloaded from Pubchem is not readily available for docking inputs since it provides molecules in .SDF and other non-acceptable chemical formats.

Information like compounds name, structure and Lipinki's parameter has been provided in Table 1. Drug-likeness of our inhibitors including absorption, distribution, metabolism and excretion of these inhibitors within the body, was predicted using SCF Bio, an online server. The Egan BOILED-Egg method available in SwissADME tool was used for the determination of the absorption of the inhibitors in the gastrointestinal tract and brain. BOILED-Egg (Brain Or IntestinaL EstimateD permeation predictive model), also called Egan egg, provides a threshold (WLOGP $\leq$ 5.88 and TPSA $\leq$ 131.6) and a clear graphical representation of how far a molecular structure is from the ideal one for good absorption (Daina *et al*., 2016). In this 2D graphical representation, the yolk area represents the molecules that can passively permeate through the blood–brain barrier (BBB).whereas the molecules located in the white region are predicted to be passively absorbed by the gastrointestinal (GI) tract. Binding scores, VDW, hydrogen bond score and Electrostatic score of molecules were studied against crystal structure of COVID-19 main

protease obtained from iGEMDOCK molecular docking software Table 2. Binding scores, VDW, hydrogen bond score and Electrostatic score of molecules were studied against nCoV spike glycoprotein with a single receptor-binding domain obtained from iGEMDOCK molecular docking software. Table 3.

Table 1: Results of Molecular Docking studies on SARS -CoV-2

S.No	Compounds	Structure	Lipinki's parameter	Lipinki's Status
1	Acrimarine		mass: 543.000000 hydrogen bond donor: 2 hydrogen bond acceptors: 9 LOGP: 5.466202 MR: 149.750198	Negative
2	Atropine		mass: 289.000000 hydrogen bond donor: 1 hydrogen bond acceptors: 4 LOGP: 1.930900 MR: 79.955772	Positive
3	Caffeine		mass: 194.000000 hydrogen bond donor: 0 hydrogen bond acceptors: 5 LOGP: 0.061900 MR: 49.100494	Positive
4	Canavanin		mass: 176.000000 hydrogen bond donor: 7 hydrogen bond acceptors: 7 LOGP: -2.006600 MR: 41.989994	Negative
5	Caribine		mass: 326.000000 hydrogen bond donor: 2 hydrogen bond acceptors: 5 LOGP: 1.133100 MR: 87.175476	Positive
6	Coriandrin		mass: 230.000000 hydrogen bond donor: 0 hydrogen bond acceptors: 4 LOGP: 2.661370 MR: 60.234486	Positive
7	Cryptopleurine		mass: 377.000000 hydrogen bond donor: 0 hydrogen bond acceptors: 4 LOGP: 4.696699 MR: 111.929962	Positive

8	Favipiravir		mass: 157.000000 hydrogen bond donor: 3 hydrogen bond acceptors: 5 LOGP: -1.189100 MR: 33.963097	Positive
8	Gingerol		mass: 294.000000 hydrogen bond donor: 2 hydrogen bond acceptors: 4 LOGP: 3.233799 MR: 82.752571	Positive
9	Hesperetin		mass: 302.000000 hydrogen bond donor: 3 hydrogen bond acceptors: 6 LOGP: 2.518499 MR: 76.746880	Positive
10	Ochropamine		mass: 366.000000 hydrogen bond donor: 0 hydrogen bond acceptors: 4 LOGP: 2.972900 MR: 104.741470	Positive
11	Papaverine		mass: 339.000000 hydrogen bond donor: 0 hydrogen bond acceptors: 5 LOGP: 3.859998 MR: 97.198975	Positive
12	Psychotrine		mass: 464.000000 hydrogen bond donor: 1 hydrogen bond acceptors: 6 LOGP: 4.184439 MR: 131.839767	Positive
13	Remdesivir		mass: 602.000000 hydrogen bond donor: 5 hydrogen bond acceptors: 13 LOGP: 2.312181 MR: 149.834305	Negative

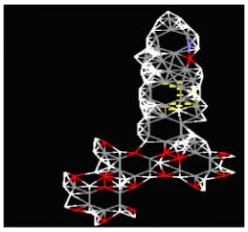
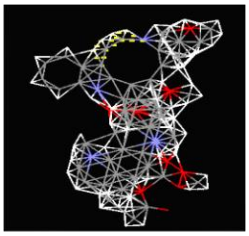
14	Solasonine		mass: 883.000000 hydrogen bond donor: 8 hydrogen bond acceptors: 16 LOGP: 8.156656 MR: 241.162292	Negative
15	Vincalcucoblastine		mass: 810.000000 hydrogen bond donor: 1 hydrogen bond acceptors: 9 LOGP: 7.202292 MR: 224.554749	Negative

Table 2: The docking results by using iGEMDOCK

Ligand	Total Energy	VDW	H Bond	Elec
cav6lu7_02J-Acrimarine-0.pdb	-66.2106	-54.6088	-11.6018	0
cav6lu7_02J-Atropine-1.pdb	-64.5896	-55.0896	-9.5	0
cav6lu7_02J-Caffeine-0.pdb	-47.6063	-38.3852	-9.22113	0
cav6lu7_02J-Canavanin-1.pdb	-61.0868	-40.4569	-20.6299	0
cav6lu7_02J-Caribine-1.pdb	-65.4598	-58.4598	-7	0
cav6lu7_02J-Coriandrin-1.pdb	-56.9267	-43.8718	-13.055	0
cav6lu7_02J-Cryptopleurine-0.pdb	-62.7697	-60.2697	-2.5	0
cav6lu7_02J-Favipiravir-0.pdb	-50.9369	-38.398	-12.5389	0
cav6lu7_02J-gingerol-1.pdb	-64.401	-56.6288	-7.77221	0
cav6lu7_02J-Hesperetin-0.pdb	-77.6105	-61.7976	-15.8129	0
cav6lu7_02J-Ochropamine-1.pdb	-61.6517	-49.9578	-11.6939	0
cav6lu7_02J-Papaverine-0.pdb	-65.05	-61.55	-3.5	0
cav6lu7_02J-Psychotrine-1.pdb	-61.8285	-58.3285	-3.5	0
cav6lu7_02J-REMDESIVIR-0.pdb	-63.9859	-51.4202	-12.5657	0
cav6lu7_02J-Solasonine-0.pdb	-133.989	-116.272	-17.7169	0
cav6lu7_02J-Vincaleucoblastine-0.pdb	-130.437	-119.88	-10.5569	0

Table 3: The docking results by using the docking server

Ligand	Total Energy	VDW	HBond	Elec
cav6vsb_NAG-Acrimarine-0.pdb	-74.75	-41.0912	-33.6588	0
cav6vsb_NAG-Atropine-1.pdb	-59.8241	-44.54	-15.2841	0
cav6vsb_NAG-Caffeine-0.pdb	-47.8974	-30.193	-17.7044	0
cav6vsb_NAG-Canavanin-0.pdb	-55.9319	-31.0809	-24.851	0
cav6vsb_NAG-Caribine-1.pdb	-66.9197	-50.3865	-16.5332	0
cav6vsb_NAG-Coriandrin-1.pdb	-58.0021	-35.248	-22.7541	0
cav6vsb_NAG-Cryptopleurine-1.pdb	-63.2409	-60.5013	-2.73966	0
cav6vsb_NAG-Favipiravir-1.pdb	-53.3197	-30.9619	-22.3577	0
cav6vsb_NAG-gingerol-0.pdb	-60.8169	-39.4793	-21.3376	0
cav6vsb_NAG-Hesperetin-0.pdb	-76.1759	-41.7262	-34.4496	0
cav6vsb_NAG-Ochropamine-1.pdb	-63.2672	-52.5563	-10.7109	0
cav6vsb_NAG-Papaverine-0.pdb	-68.0934	-56.3728	-11.7206	0
cav6vsb_NAG-Psychotrine-1.pdb	-76.6558	-59.2104	-17.4454	0
cav6vsb_NAG-REMDESIVIR-1.pdb	-84.5622	-63.1416	-21.4206	0
cav6vsb_NAG-Solasonine-1.pdb	-136.316	-96.6787	-39.6375	0
cav6vsb_NAG-Vincaleucoblastine-0.pdb	-150.877	-129.736	-21.1408	0

The Lipinski's rule of five was published in 1997 by Christopher A. Lipinski and is also known as the Pfizer's rule of five or Rule of five (Ro5). It is a rule of thumb to evaluate the drug-likeness and to determine if a chemical compound with a certain pharmacological or biological activity has properties that would make it a likely orally active drug in humans. Ro5 depends on four simple physiochemical parameter ranges: the molecular weight (MW), which should be less than 500 g/mol, lipophilicity (Log P) less than 5 and number of hydrogen bond donors and acceptors less than 5 and 10, respectively, as seen for 90% of orally functional drugs that have obtained phase II clinical status. These parameters are connected with intestinal permeability and aqueous solubility and determine the first step of oral bioavailability. These rules explain molecular properties valuable for a drug's pharmacokinetics in the human body including their absorption, distribution, metabolism and excretion (ADME). If a ligand fails to fulfill the parameters of Ro5, then it is highly probable that it will cause trouble if ingested (Mendis *et al*., 2011). ADME predictions of our inhibitors are shown in Table1. BOILED-egg results, showing the possibility of absorption and penetration of inhibitors in the GI tract and brain using WLOGP and TPSA parameters. All of the inhibitors

or ligands discussed herein satisfy the Lipinski's rule, except for teprotide, which significantly violates three parameters (MW > 500, number of hydrogen bond donors > 5 and number of hydrogen bond acceptors > 10); furthermore, it also violates the BOILED-egg method. Although teprotide has the highest binding affinity for human ACE among all the inhibitors, it is not proposed as an orally active drug due to violation of the Lipinski's rule. An Egan's egg graph for the inhibitors was generated using SwissADME. The graph showed that only Allicina herbal compound, is absorbed by the brain, though in the acceptable range. The remaining inhibitors showed gastrointestinal absorption within an acceptable range, except for teprotide and lisinopril (WLOGP >5.88 and TPSA >131 (Fig. 10-19).

DOCKING RESULTS OF 6LU7

Fig. 10: Two hydrogen bonds were formed between crystal structure of COVID-19 main protease and Favipiravir.

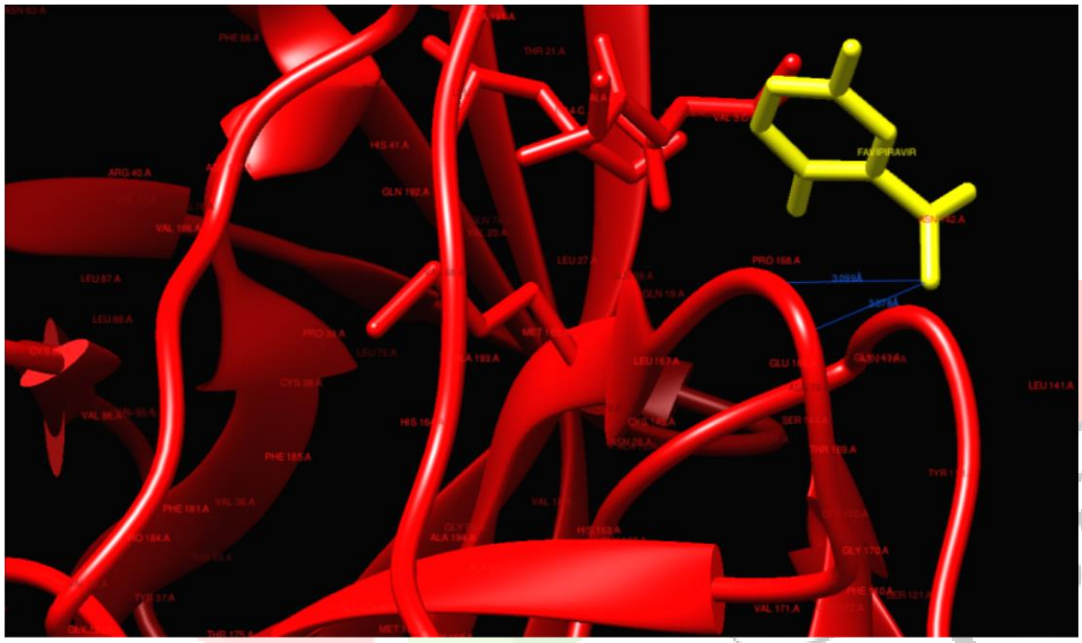


Fig. 11: Two hydrogen bonds were formed between crystal structure of COVID-19 main Protease and Hesperetin

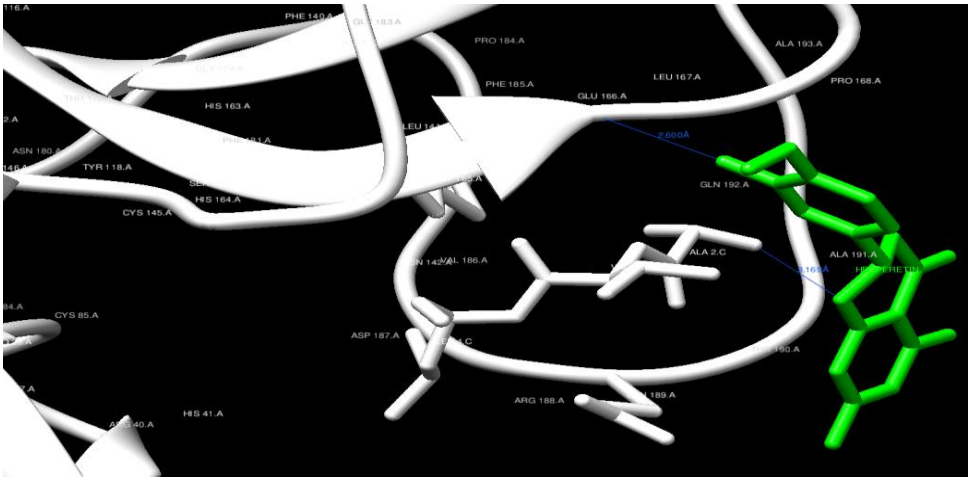


Fig. 12: Two hydrogen bonds were formed between crystal structure of COVID-19 main protease and Caribine

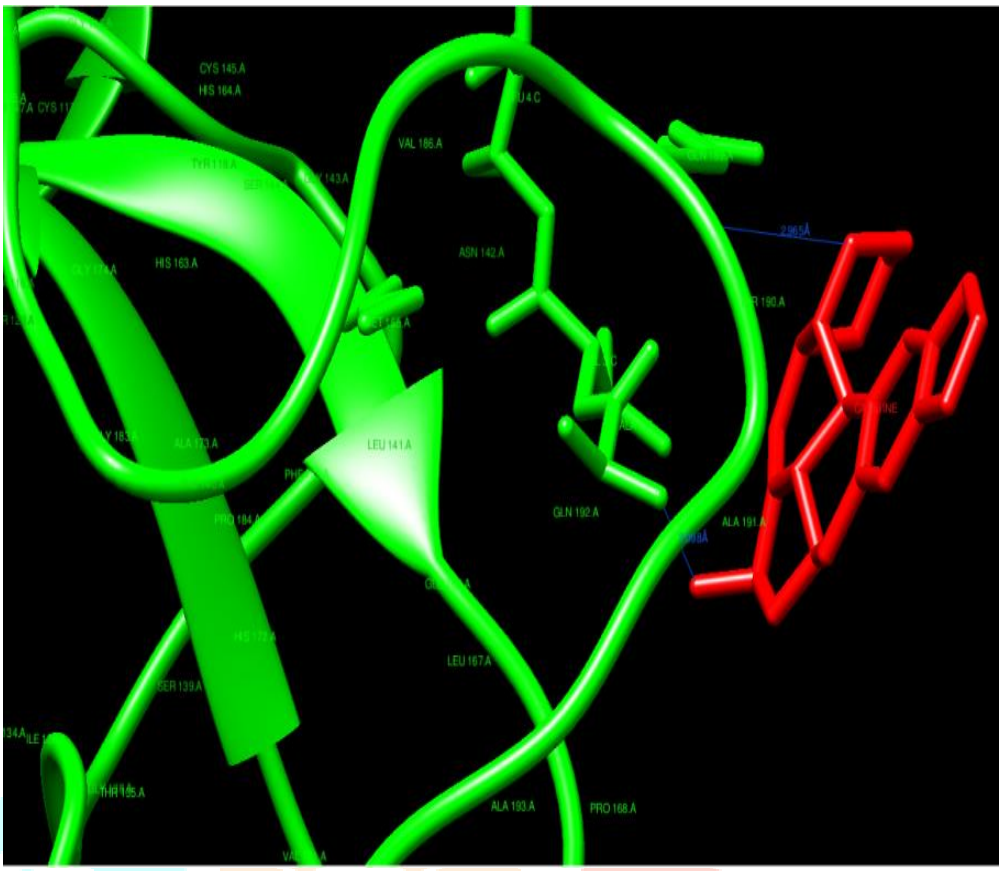


Fig. 13 : Two hydrogen bonds were formed between crystal structure of COVID-19 main protease and Atropine

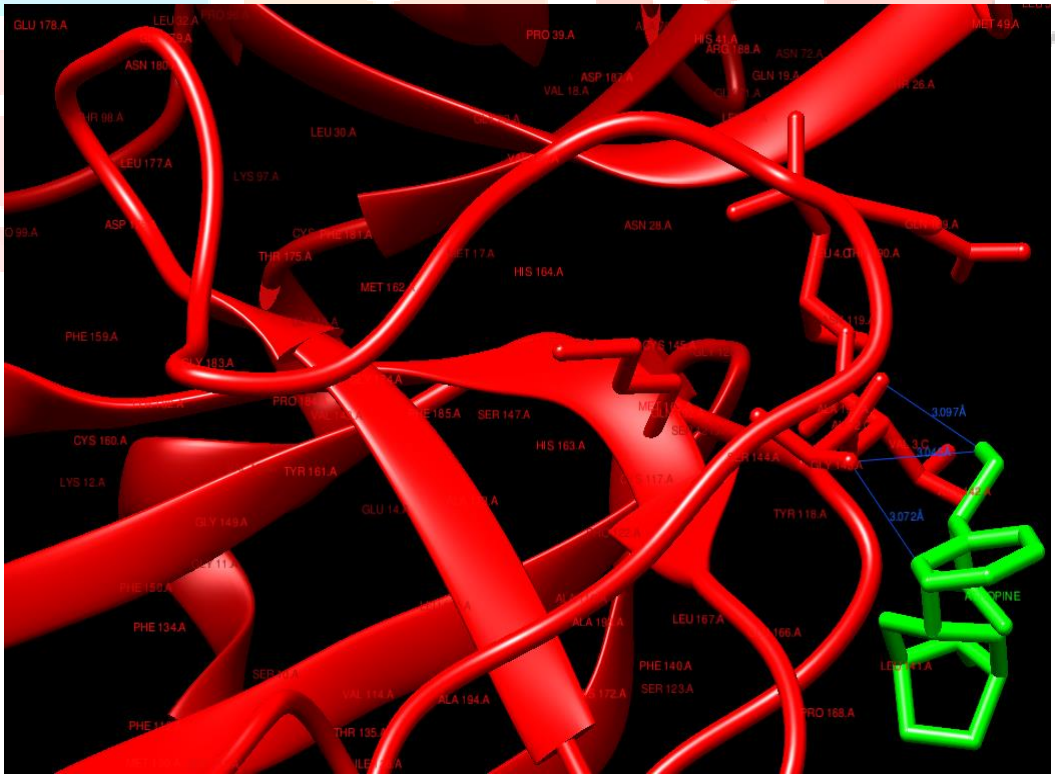


Fig. 14 : Two hydrogen bonds were formed between crystal structure of COVID-19 main protease and Gingerol

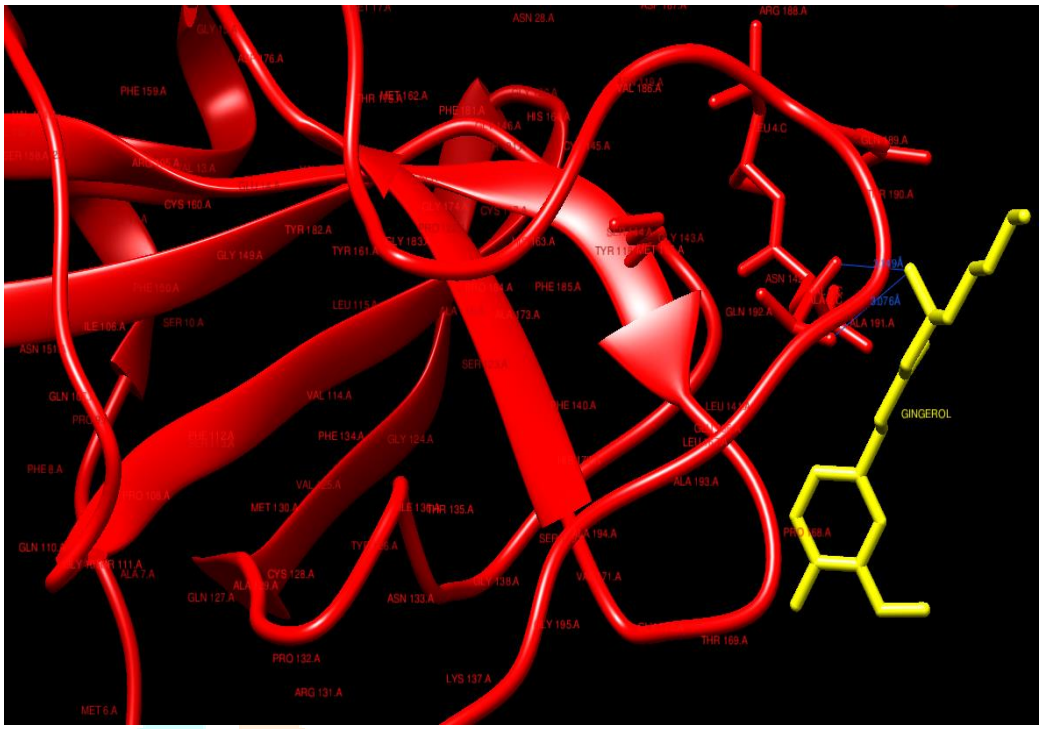


Fig. 15 : One hydrogen bond was formed between crystal structure of COVID-19 main protease and papaverin

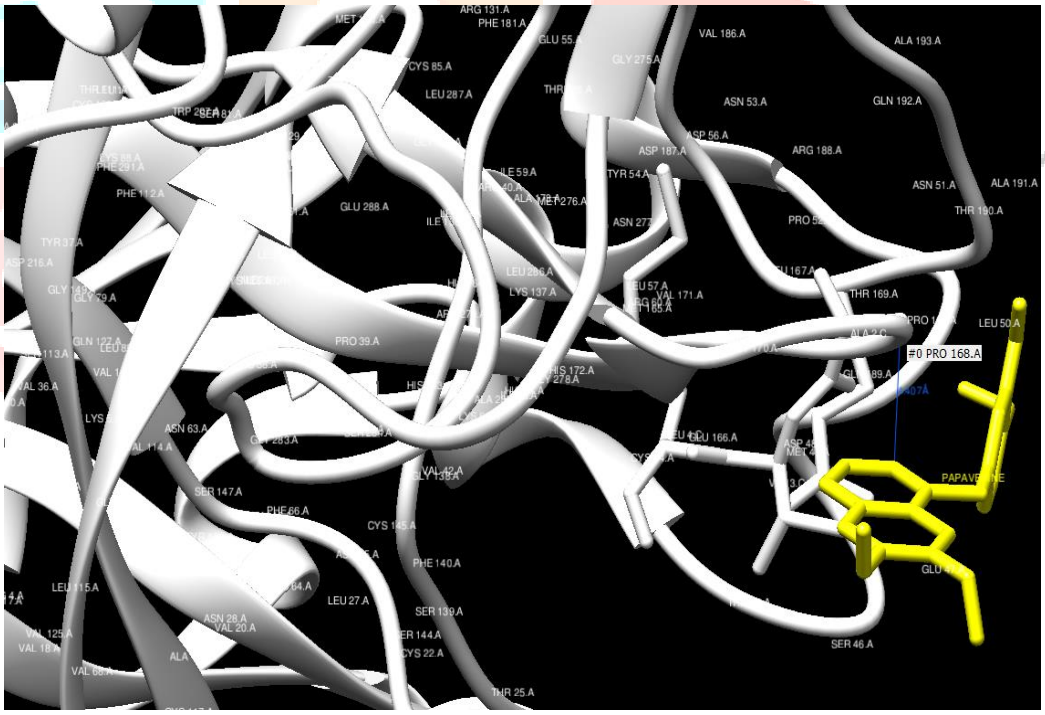
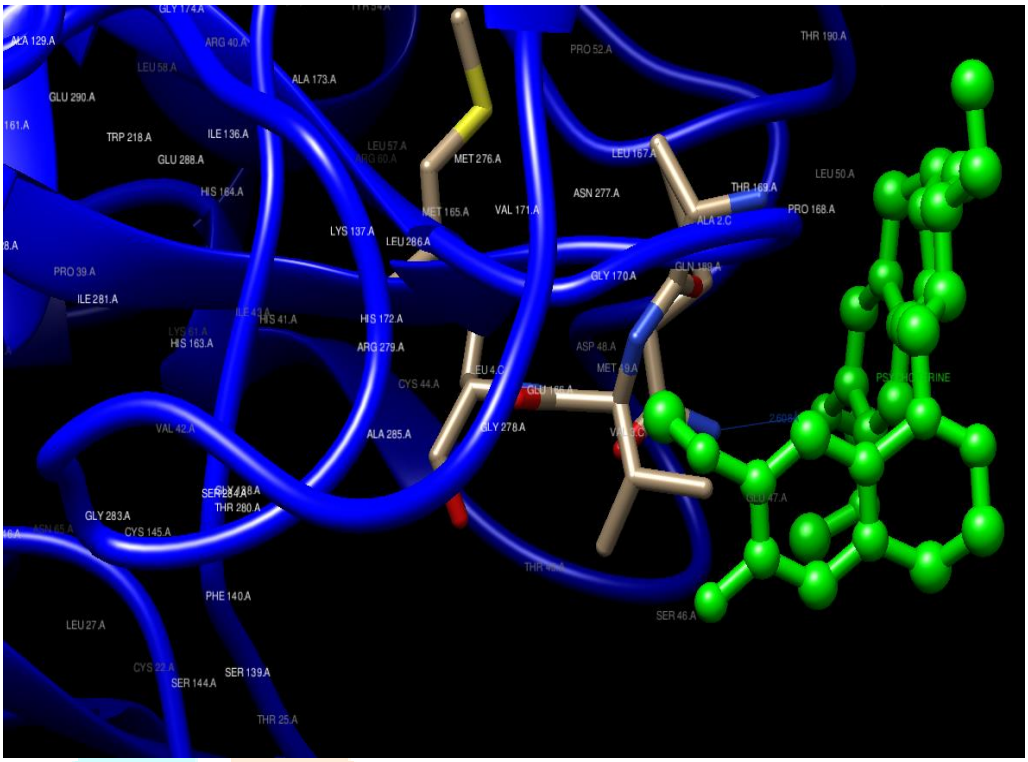


Fig. 16: One hydrogen bond was formed between crystal structure of COVID-19 main protease and Psychotorine



DOCKING RESULTS OF 6VSB

Fig. 17: One hydrogen bond was formed between nCoV spike glycoprotein with a single receptor-binding domainand Acrimarine

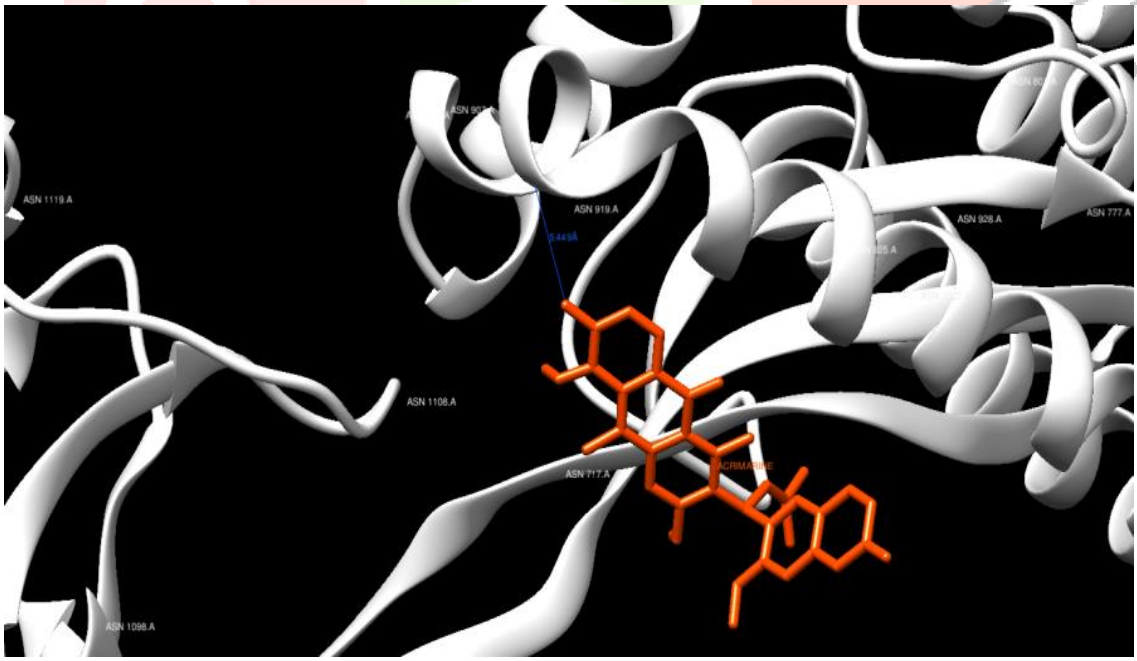


Fig. 18: One hydrogen bond was formed between nCoV spike glycoprotein with a single receptor-binding domain and Caffiene

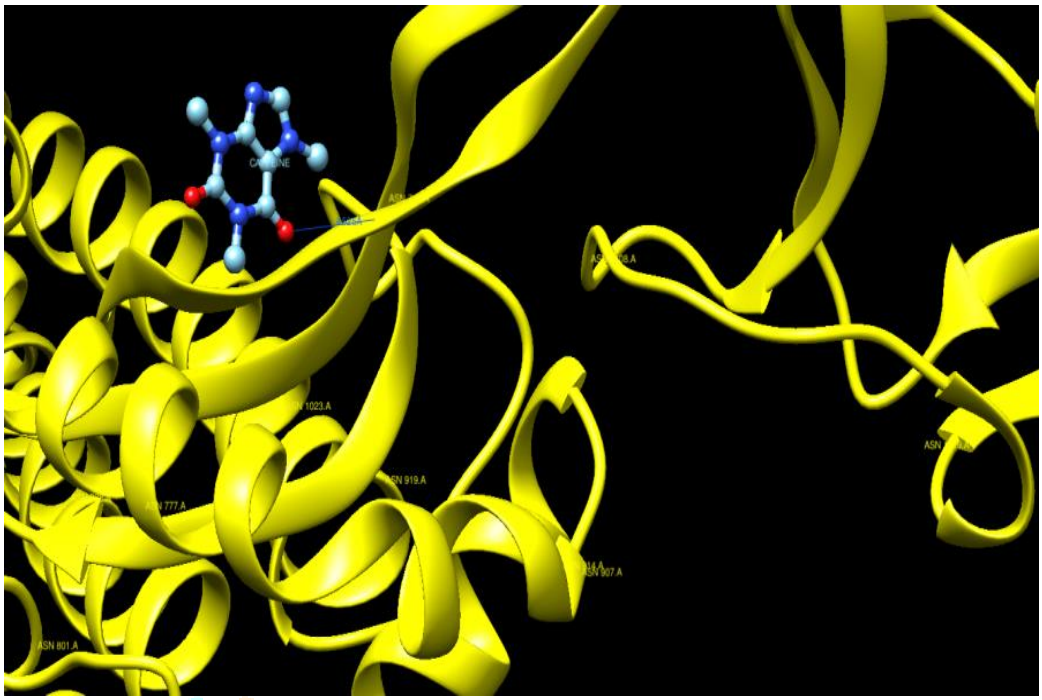
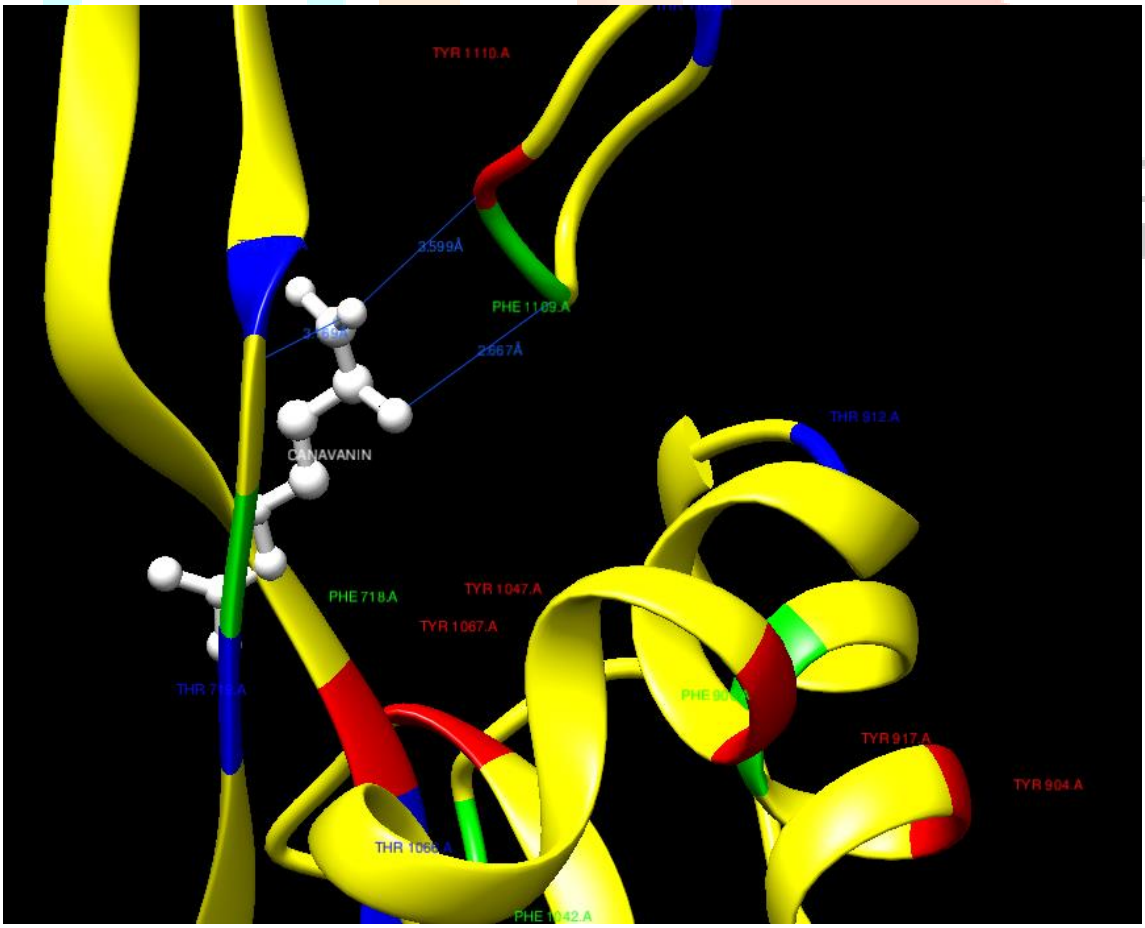


Fig. 19: Three hydrogen bonds were formed between nCoV spike glycoprotein with a single receptor-binding domain and Canavanin



Currently, Coronavirus has become a big challenge for every country. The outbreak of this virus is spreading worldwide and causing several deaths. In the present study, 15 plant compound namely Acrimarine, Atropine, Caffeine, Canavanin, Caribine, Coriandrin, Cryptopleurine, Favipiravir, Gingerol, Hesperetin, Ochropamine, Papaverine, Psychotrine, Remdesivir, Solasonine, Vincalcucoblastine were tested for the anti-viral activity against SARS-CoV-2. Similar studies were performed by (Joshi *et al*. 2020). Prepared a phytochemicals library from 11 medicinal plants. These phytochemicals were subjected to molecular docking against two enzymes Mpro and ACE2. Based on molecular docking study, seven plants namely *Piper Longum, Phaseolus Vulgaris, Curcuma Longa, OcimumGratissimum, SyzygiumAromaticum, Artemisia Absinthium, InulaHelenium* were found which have such compounds showing better and significant binding energy against these receptors. As a suggestion since there are no effective drugs against Corona virus, the infected people should keep the immune system healthy because a healthy immune system reduces the chance of viral infection and help the body to clear the virus rapidly Many such medicinal plants are available which have antiviral, antibacterial and antifungal activity as well as these plants can enhance the immune system. The phytochemicals of these medicinal plants can be used against Corona virus.

In the current study, molecular docking analysis reveals that although all natural ligands interacted in the active site of target enzyme COVID -19 main protease but some of them could not interact through hydrogen bonds with target which are required for enzyme inhibition. Similar results have been obtained by (Agrawal *et al*., 2020).

The mechanism of action of most of these drugs is to target the viral replication process or block viral entry into the host cell. Among these drugs, sixteen approved and investigational drugs were originally developed to treat influenza, HIV, HCV, and other respiratory infections. Besides, two approved anti-malarial drugs namely Remdesivir and Hesperetin. Were proposed for clinical investigation as prospective anti-COVID-19 drugs (Cavasotto *et al*., 2020).

The study was unlined to find novel plant based inhibitors against covid – 19 using molecular docking methods. The plant compounds selected based on their biological activity against virus, bacterial and other microbes were tabulated and downloaded from chemical database Molecules interacted with PDB file with estimated fitness ranges from -150.87 Kcal/mol to -47.89 Kcal/mol against n-Cov spike glycoprotein and the estimated fitness ranges from -133.98 Kcal/mol to -47.60 Kcal/mol against covid -19 main protease enzyme. Molecules which have occupied top rank with reference to highest binding energy have also proved good drug properties. All the compounds showed H- bond length below 1.A which was assigned durinSg H-bond analysis parameter of chimera. From this study binding affinity towards the two target proteins Solasomine(-133.98 Kcal/mol) against covid 19 main protease and Vincaleucoblastine (-150.87 Kcal/mol) against n- Cov spike glycoprotein are selected as potential inhibitors but these two compound failed during Lipinski's parameter evolution. It has been further enlarged that molecules with good binding energy and possessing positive status during Lipinski's parameter evolution was Hesperetin (-77 Kcal/mol) and Psychotrine (-76 Kcal/mol). It has been concluded that hesperetin, potential inhibitors against both drug targets of covid -19 and considering psychotrine, it was potential against n-Cov spike

glycoprotein. The post screening analysis molecule of the iGEMDOCK is useful for clustering and selecting compounds based on interaction profile compounds were compared with standard drugs (given during hospitalization of covid patients) like Favipiravir and Remdesivir, Remdesivir have shown binding energy higher than hesperetin against spike glycoprotein only, in the mean timeFavipiravir had shown good binding protein against both spike glycoprotein and nCoV main protease . From this study it has been concluded that Hesperetin serves as a potential inhibitor against Covid -19 virus.

IV.CONCLUSION

From this study it has been concluded that plant compounds are potent inhibitors against protease and spike proteins of COVID pandemic. Protease and spike proteins were two important drug targets used for many research studies for finding potential inhibitor towards the virus. Our study has provided some valuable comparison result for evaluating many specific antiviral compounds obtained from literature search which were compared with currently using drugs. This study needs more molecular level analysis and future experiments to map the plant based compounds which are used against covid treatment.

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