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Evaluation of plant-derived compounds to inhibit COVID-19 through *in silico* studies

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The recent threat which has received worldwide attention is COVID-19, a rapidly spreading new strain of Coronavirus. It has affected more than 176 countries and due to the lack of efficacious drugs or vaccines against SARS-CoV-2, it has further worsened the situation everywhere. After infecting the host, the ssRNA genome of SARS-CoV-2 is translated into a large polyprotein which will be further processed into different nonstructural proteins to form a viral replication complex by the virtue of virus-specific proteases namely main protease (3-CL protease) and papain protease. The crystallized form of SARS-CoV-2 main protease (Mpro) is demonstrated to be a novel therapeutic drug target according to current research. The present study was conducted to evaluate the efficacy of few plant-based bioactive compounds against COVID-19 Mpro (PDB ID: 6LU7: Resolution 2.16 Å) by molecular docking study. Molecular docking investigations were performed by using Auto DockVina to analyze the inhibition capacity of these compounds against COVID-19 as a whole complex and also in the absence of Chain C which is present with protein as a peptide. According to the obtained results, Ritonavir and Curcuminwere found to be more effective on COVID-19 than nelfinavir which is an anti-HIV drug. This is followed by Glycyrrhizin and Piperine, which correlates with COVID-19 as a whole complex and also in the absence of Chain C. So, this study will pave a way for performing more advanced experimental research and to evaluate the natural compounds to cure COVID-19.

Keywords: COVID-19, *In silico* studies, Plant-derived compounds, SARS-CoV-2.

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Introduction

Coronavirus infection was first identified in Wuhan in China in December 2019 which became the centre of a pneumonia outbreak of unknown cause[1]. In early January 2020, Chinese scientist pinpointed a novel Coronavirus strain from people of Wuhan which started at a local seafood/wild animal market[2]. Severe acute respiratory syndrome [SARS] associated with this disease was named 2019-nCOV, which is now named COVID-19[3]. Coronavirus is not a new virus, it was discovered in 1960 causing illness among animals including camels, cattle, cat, and bats. Transmission from animal to human was not reported until 2002. SARS outbreak and later in 2012 as MERS outbreak was due to transmission from animal to human confirmed transmission[4]. Coronavirus which is transmitted by respiratory droplet and contaminated surfaces is known to cause common colds. At present, there are a total of seven strains of human coronavirus which include alpha coronavirus [HCOV-229E; HCOV-NL63] and beta CORONA VIRUS [HCOV-OC43; HCOV-HKU1; SARS-COV-1; MERS-COV and now SARS-COV-2][5]. The first reported International case in Thailand on 13 January 2020 was transmitted by an infected person from Wuhan and slowly started spreading to other parts of the world. On 30 January 2020, the World Health Organization (WHO) emergency committee designated the novel coronavirus outbreak as Public health emergency of International concerns (PHEIC). After 6 weeks (11.03.2020), WHO declared COVID-19 as pandemic[6]. People who have developed pneumonia due to SAR-COVwere characterized with a distinct lung appearance of ground glass opacity in specific shapes and sites as the severity of the disease progresses[7]. It is suggested that SAR-COV may be

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infectious for up to 4 weeks, twice as long as most of the current quarantine measures. Health care professionals and others with close contact with these infected individuals should use standard, contact and airborne precaution and well as wear eye protection[8]. No vaccine is currently available to prevent COVID-19 and evidence indicate that human Coronavirus may potentially remain infectious in the air for at least 3 hours and on inanimate surfaces for 2-3 days up to 9 days, and perhaps longer[9]. Viable SARS COVID 2 RNA has been isolated from respiratory, blood, urine, and stool specimens[10]. The mainstay of treatment is supportive care. Various therapies are under investigation and under development which included antiviral drugs (lopinavir and ritonavir), immune-modulators like interleukin-6 inhibitors (sarilumab), antimalarial (hydroxychloroquin), and corticosteroids. Lopinavir and Ritonavir, combined with Chinese herbal medicines, were used in preliminary clinical studies[11,12].

In this connection, *in silico* technique was used to identify potential leads for the treatment for COVID-19. The crystallized form of SARS-CoV-2 main protease (Mpro) is demonstrated to be a novel therapeutic drug target according to current research. The present study was conducted to evaluate the efficacy of few plant-based bioactive compounds against COVID-19 Mpro by molecular docking study against COVID-19.

Materials and Methods

The structures of the compounds were procured from the authors' earlier research on plant-based isolation and characterization of drugs. 2D structures were analyzed in PubChemand PDB files of ligands were generated using OpenBabel software[13,14]. Energy minimization was carried out using PRODRG server and PDB file with known inhibitors were converted into PDBQT file format using Auto Dock Tool (ADT)[Autodock Vina] for further analysis[15].

The atomic coordinates of COVID-19 Mpro main were retrieved from the RCSB PDB (https:// www.rcsb.org/pdb/home/home.do). Before docking analysis, the structure was emended by removing co-crystallized heteroatoms and water molecules using SPDBV software, followed by the addition of polar hydrogen and Gasteiger charges using Auto Dock Tool (ADT). Then structures were saved in PDBQT files, for further analysis.

Binding mode and interaction of COVID-19 Mpro with ligands were performed using Auto dockVina on Ubuntu Linux platform[16]. This Program requires a pre-calculated grid box, which serves as a frontier of active pocket amino acids in the receptor by attaining XYZ coordinates. The active pocket amino acid residues were identified using PoSSuM server (http://possum.cbrc.jp/PoSSuM/). The grid optimization was carried out using the auto grid and the grid box was placed such that it covers all amino acids of an active pocket. The grid box size was set at 16, 16, and16 and centred on mass centre 3.753, 15.918, and 9.96 for x, y, and z co-ordinate respectively with space-separated by 1.0 Å (grid-point spacing). Docking was performed using Auto Dock program and ten different modes of conformations were generated with their respective binding energy/affinity. The lowest binding energy results were considered for further post docking analysis. Docked complex of protein and ligand with good binding affinity were visualized using maestro and a chimera. LigPlot 1.4.5 was used to generate a two-dimensional docking for the analysis of hydrogen bond and hydrophobic interaction[17].

Results and Discussion

Recently, COVID-19, a rapidly spreading new strain of Coronavirus has received worldwide attention. The first Indian case of COVID-19 was found in Kerala on 30 January 2020. There onwards the increase in the cases in India is given in Fig 1. It has affected more than 176 countries and due to the lack of efficacious drugs or vaccines against SARS-CoV-2, it has further worsened the situation everywhere. There was an increase in cases of COVID-19 from 1 to 4281 [06.04.2020] with 111 death and 318 recovered patients. Whereas the total number of global cases was 1328150 with 73750 death and 277285 recovered patients in the month of

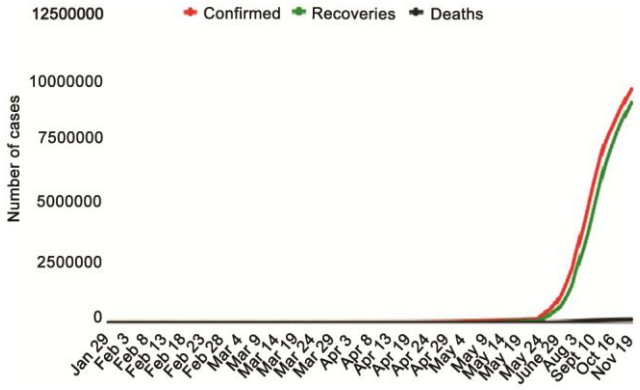

Fig. 1 — Number of COVID cases in India with confirmed, recoveries and death.

April[18,19] and presently as of 8.12.2020, the total number of global cases is 68,084,568 with 1,555,774 deaths and 47,160,070 recovered patients[20].

In the present scenario as no treatment is available, so natural sources may come as a saviour for this deadly disease. Natural product treatment will be cheap and with fewer side effects. As per WHO, 80% of the world population relies on natural products for their ailments. In this connection, we have chosen plants that are frequently used in Indian cooking like Turmeric (*Curcuma longa*), Black pepper (*Piper nigrum*), Zinger (*Zingiber officinale*), Tulsi (*Ocimum sanctum*), Liquorice (*Glycyrrhiza glabra*), Kalonji (*Nigella sativum*) etc. The basis for choosing the phytoconstituents is their reported usage in respiratory, asthma and for immunity booster and numerous other disorders[21-26]. *In silico* modelling was done with azadirachtin, β-sitosterol, curcumin, gingerol, glycyrrhizin, piperine, quercetin, thymoquinone, ursolic acid against the synthetic drugs viz., hydroxychloroquine, lopinavir, nelfinavir, and ritonavir.

The binding energies obtained and hydrogen bonds formed from the docking of 6LU7 are presented in Table 1. Ritonavir has formed 4 hydrogen bonds followed by Curcumin and Glycyrrhizin. In terms of binding energy also, these three require minimum energy [-6.4; -6.6 and -6.5 Kcal/mol] respectively and in turn, seem to be more efficient compared to other compounds. The standard drugs nelfinavir and hydroxychloroquine have formed 2 hydrogen bonds by using binding energy [-6.0 and -5.0 Kcal/mol] respectively slightly more than that of the above-mentioned values. Fig 2 shows the superimposition of all ligands with COVID-19 Mpro whole complex. Fig 3 shows 2D interaction of ligands with amino acids of COVID-19 Mpro whole complex. Detailed hydrogen bond formation of the ligand with amino acids of COVID-19 Mpro whole complex of ligands showing best conformation (Curcumin, glycyrrhizin, ritonavir) are given in Fig. 4.

Molecular docking investigations were performed by removing C Chain of COVID-19 as it is present as a peptide near the active site of the protein. The

Table 1 — Showing binding energy and hydrogen bonds formed with COVID-19 Mpro whole complex and ligands.

Ligands	Affinity (Kcal/mol)	H bonds	H bond interaction
Azadirachtin	-5.4	-	-
Beta_Sitosterol	-5.0	-	-
Curcumin	-6.6	3	Cys 145(A); Ser 144(A); His 163(A)
Gingerol	-6.0	1	Cys 145 (A)
Glycyrrhizin	-6.5	2	Thr 24(A); Gln 189(A)
Hydroxychloroquine	-5.0	2	Cys 145 (A); Thr 24 (A)
Lopinavir	-6.4	2	2-Gln 189(A)
Nelfinavir	-6.0	2	-
Piperine	-5.9	1	Gly 143(A)
Quercetin	-5.8	-	-
Ritonavir	-6.4	4	Asn 119(A); Glu 166(A); Cys 145(A); Thr 26(A)
Thymoquinone	-4.3	2	Gly143 (A); Thr 26(A)
Ursolic acid	-5.9	-	-

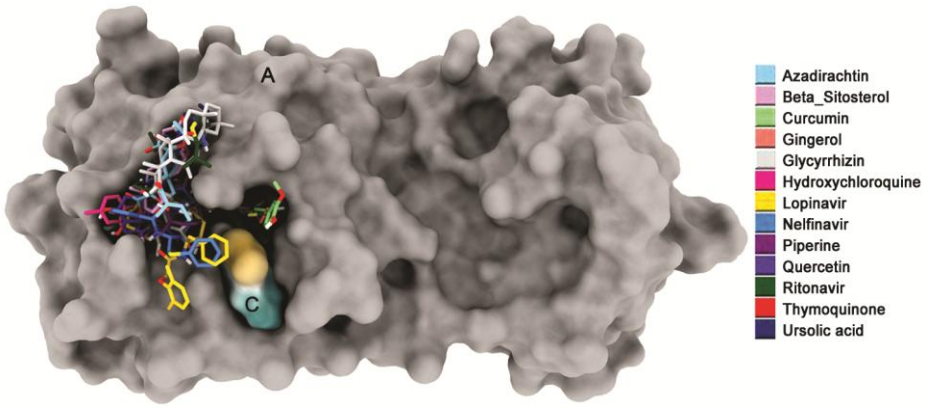

Fig. 2 — Superimposed image representing docking conformations of all ligands with COVID-19 Mpro whole complex.

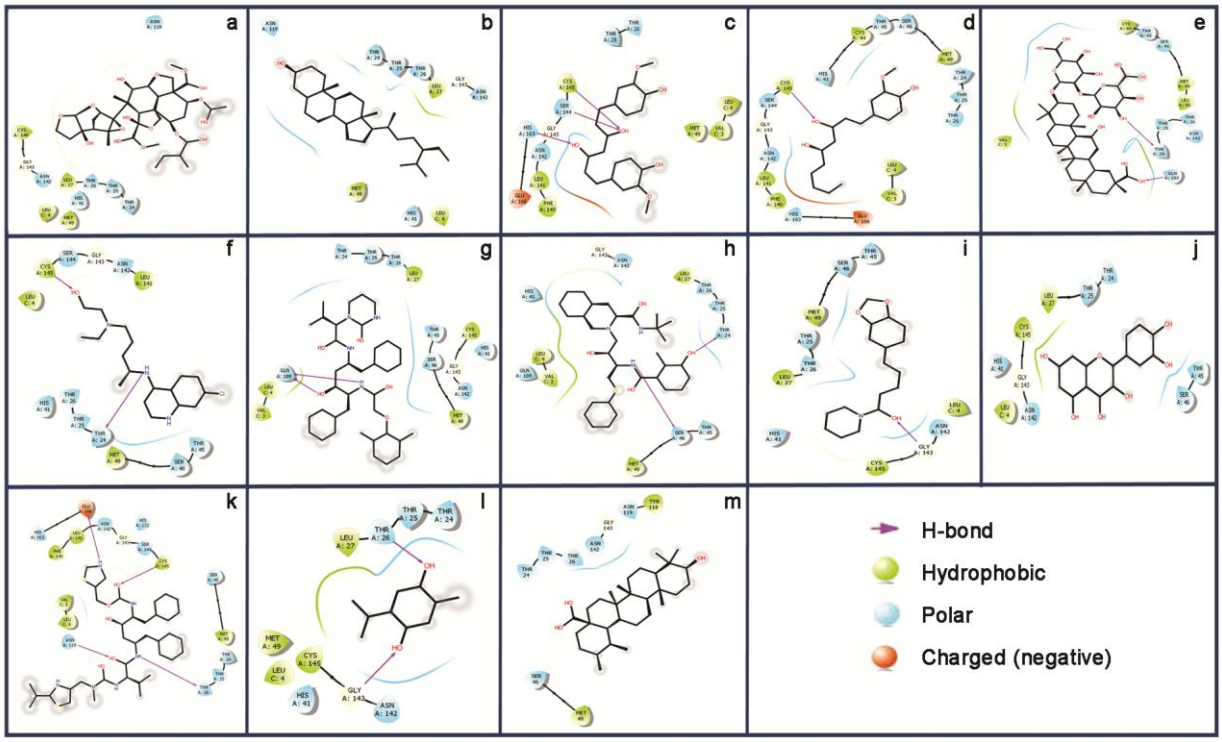

Fig. 3 — Showing 2D interaction of ligands with amino acids of COVID-19 Mpro whole complex. a) Azadirachtin,b) Beta_Sitosterol, c) Curcumin, d) Gingerol, e) Glycyrrhizin, f) Hydroxychloroquine, g) Lopinavir, h) Nelfinavir, i), Piperine, j) Quercetin, k) Ritonavir, l) Thymoquinone, and m) Ursolic acid.

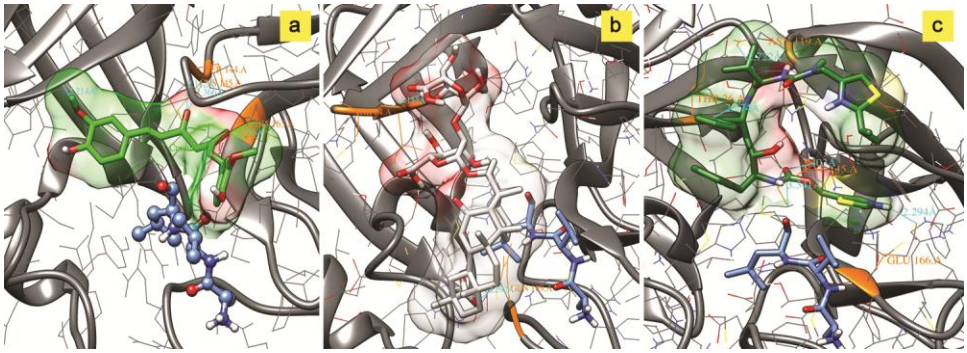

Fig. 4 — Showing detailed hydrogen bond formation of a ligand with amino acids of COVID-19 Mpro whole complex. a) Curcumin, b) Glycyrrhizin, and c) Ritonavir.

binding energies obtained and hydrogen bonds formed from the docking of 6LU7 in absence of C Chain are presented in Table 2. Ritonavir formed 2 hydrogen bonds with -7.1 Kcal/mol of binding affinity. This is followed by curcumin and glycyrrhizin. In terms of both hydrogen bonds and binding energy, ritonavir was seen to be more efficient compared to other compounds. The standard drug nelfinavir has shown-8.3 Kcal/mol of binding affinity with no hydrogen bonds, whereas another standard drug hydroxychloroquine had formed 1 hydrogen bond with -6.3 Kcal/mole as binding energy which is slightly more than that of the above-mentioned values. Fig. 5 shows the superimposition of all ligands with COVID-19 Mproin absence of C chain. Fig. 6 shows 2D interaction of ligands with amino acids of COVID-19 Mpro in the absence of C chain. Detailed hydrogen bond formation of the ligand with amino acids of COVID-19 Mpro whole complex of ligands showing best conformation (Curcumin, Glycyrrhizin, Piperine, Ritonavir) are represented in Fig. 7.

The Catalytic dyad present between the cleft of domain 1 and 2 which is a substrate-binding site is

Table 2 — Showing binding energy and hydrogen bonds formed with COVID-19 Mpro (absence of C chain) and ligands.

Ligands	Affinity (Kcal/mol)	H bonds	H bond interaction
Azadirachtin	-5.9	-	-
Beta_Sitosterol	-7.1	-	-
Curcumin	-7.1	2	Ser 144(A); Gly143(A)
Gingerol	-5.7	2	His164(A); Arg188(A)
Glycyrrhizin	-8.0	1	Phe140(A)
Hydroxychloroquine	-6.3	1	Ser144(A)
Lopinavir	-7.7	-	-
Nelfinavir	-8.3	-	-
Piperine	-7.0	2	Cys145(A); Ser144(A)
Quercetin	-7.5	1	His163(A)
Ritonavir	-7.1	2	Gly143(A); Gln189(A)
Thymoquinone	-5.0	-	-
Ursolic acid	-7.6	1	Leu141(A)

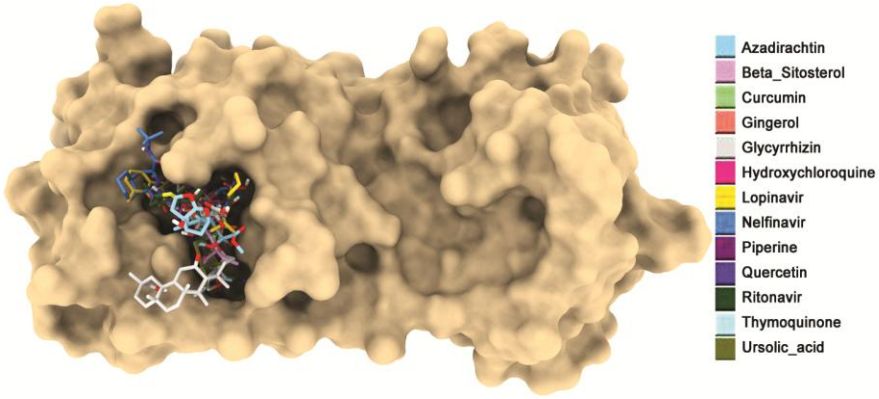

Fig. 5 — Superimposed image representing docking conformations of all ligands with COVID-19 Mpro (absence of C chain).

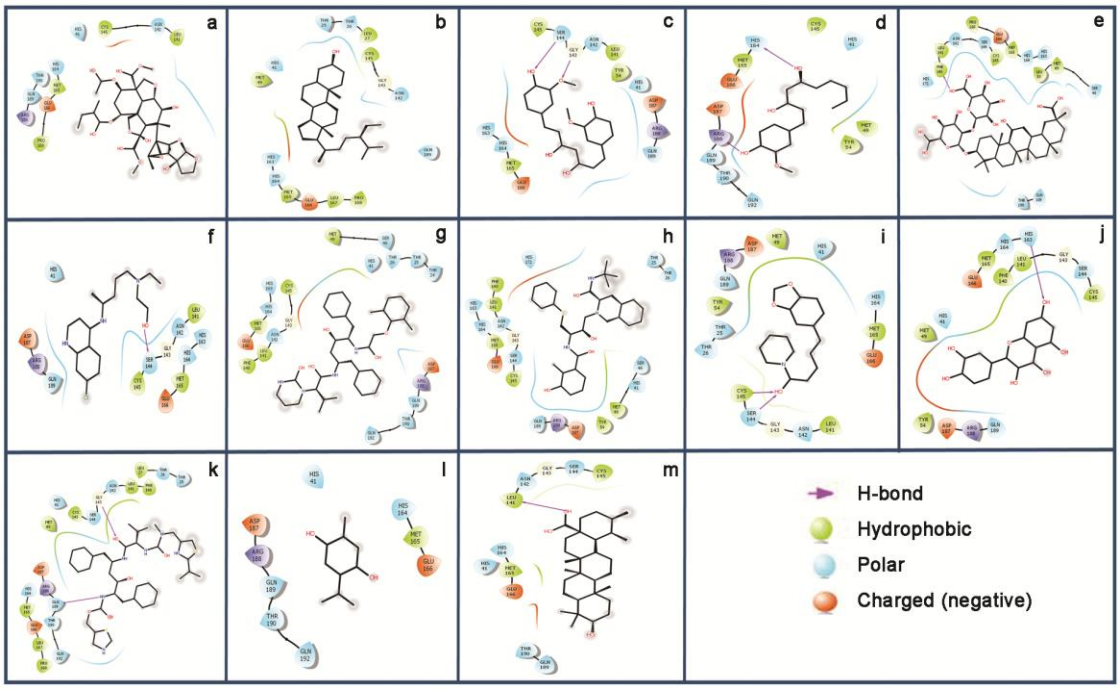

Fig. 6 — Showing 2D interaction of ligands with amino acids of COVID-19 Mpro (absence of C chain). a) Azadirachtin, b) Beta_Sitosterol, c) Curcumin, d) Gingerol, e) Glycyrrhizin, f) Hydroxychloroquine, g) Lopinavir, h) Nelfinavir, i) Piperine, j) Quercetin, k) Ritonavir, l)Thymoquinone, and m) Ursolic acid.

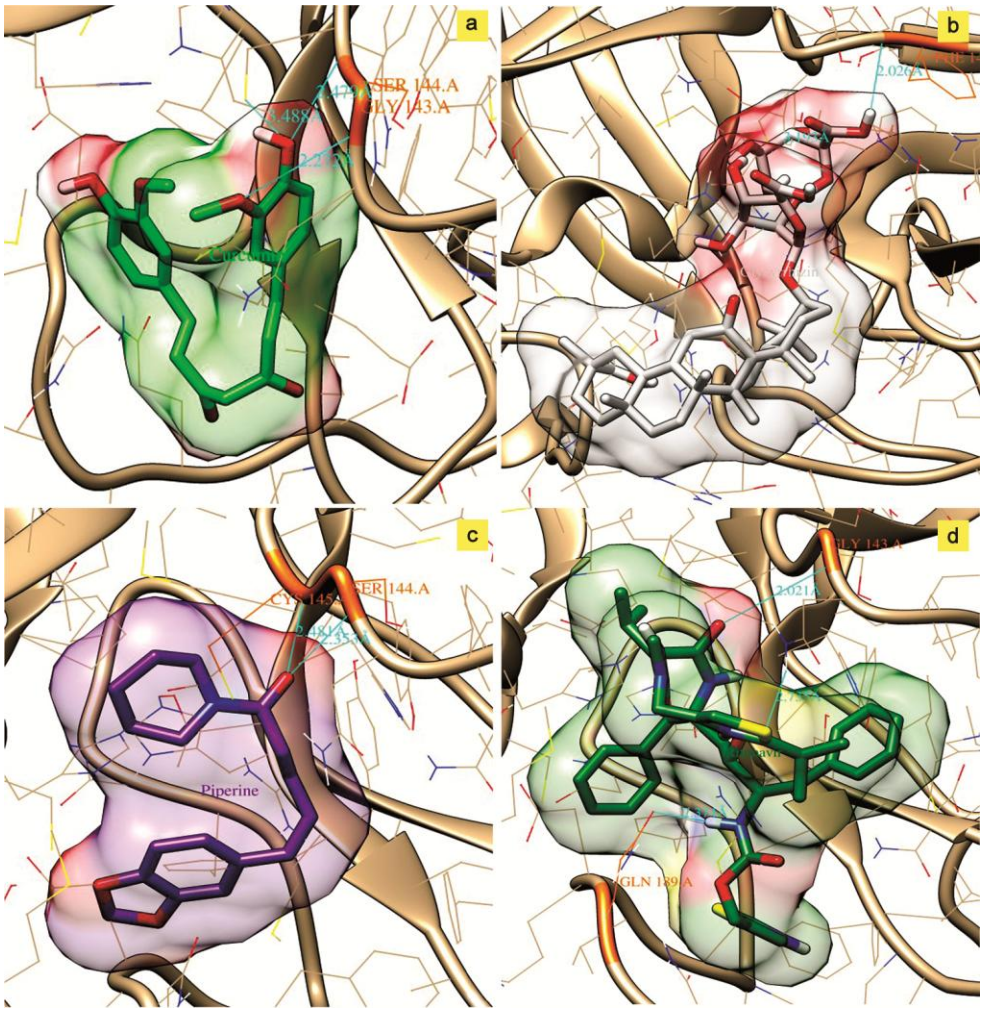

Fig. 7 — Showing detailed hydrogen bond formation of a ligand with amino acids of COVID-19 Mpro whole complex. a) Curcumin, b) Glycyrrhizin, c) Piperine, and d) Ritonavir.

mainly composed of His41 and Cys145. Ritonavir and curcumin molecules showed interaction with these two residues.

From the above results, it is clear that curcumin, glycyrrhizin, and piperine showed activity against the COVID-19. This can be attributed to the traditional as well as scientific uses of the herbals containing these phytoconstituents. For instance, turmeric which contains curcumin as major constituents possess therapeutic activities as an anti-diabetic, hypolipidemic, anti-inflammatory, anti-diarrhoeal, hepatoprotective, anti-asthmatic, and anti-cancerous drug and is widely used in cosmetology[21]. Curcumin has shown to alleviate macrophages activation and lung inflammation induced by influence virus[27] and curcumin was found to inhibit SARS-CoV in the range of 3–10 µM[28].

G. glabra possesses antibacterial, antioxidant, antimalarial, antispasmodic, anti-inflammatory, anti-hyperglycemic properties, antiulcer, antiviral, antihepatotoxic, antifungal, and herpes simplex inhibitory activities[25]. In an earlier study, glycyrrhizin was found to be most active in inhibiting replication of the SARS-associated virus[29]. Prepared derivatives of glycyrrhizin have shown 10-fold increased anti-SARS-CoV activity compared to glycyrrhizin. Amides of glycyrrhizin and conjugates of glycyrrhizin with two amino acid residues and a free 30-COOH function presented up to 70-fold increased activity against SARS-CoV[30].

Piper nigrum, commonly known as black pepper is reported to possess antiapoptotic, antibacterial, anti-colon toxin, antidepressant, antifungal, antidiarrhoeal, anti-inflammatory, antimutagenic, anti-metastatic activity, antioxidative, antispasmodic, antispermatogenic, antitumor, antithyroid, gastric ailments, hepatoprotective, insecticidal activity, intermittent

fever, larvicidal activity, protection against diabetes-induced oxidative stress, analgesic, anti-inflammatory, anticonvulsant, antimalarial, antifiliarial, and antifertility activities[22]. It is very well in accordance with the present results of *in silico* technique.

Conclusion:

6LU7 is docked with different compounds and compared with standard drugs prescribed so far for coronavirus. Docking capability was evaluated both during the presence and absence of C chain (peptide) which is present at one of the active pocket. According to the results obtained, ritonavir and curcumin were found to be more effective on COVID-19 than nelfinavir which is an anti-HIV drug. This is followed by glycyrrhizin and piperine, which correlates with COVID-19 as a whole complex and also in the absence of Chain C. This study will pave a way for performing more advanced experimental research and to evaluate the natural compounds to cure COVID-19.

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Ethical issues

There are none to be applied.

Conflict of interest

None to be declared.

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