MOLECULAR DOCKING STUDY TO IDENTIFY POTENTIAL INHIBITOR OF COVID-19 MAIN PROTEASE ENZYME: AN *IN-SILICO* APPROACH

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ABSTRACT

Coronavirus became pandemic very soon and is a potential threat to human lives across the globe. No approved drug is currently available therefore an urgent need has been developed for any antiviral therapy for COVID-19. For the molecular docking study, ten herbal molecules have been included in the current study. The three-dimensional chemical structures of molecules were prepared through ChemSketch 2015 freeware. Molecular docking study was performed using AutoDock 4.2 simulator and Discovery studio 4.5 was employed to predict the active site of target enzyme. Result indicated that all-natural molecules found in the active site of enzyme after molecular docking. Oxyacanthine and Hypericin (-10.990 and -9.05 and kcal/mol respectively) have shown good binding efficacy among others but Oxyacanthine was the only natural product which made some of necessary interactions with residues in the enzyme require for target inhibition. Therefore Oxyacanthine may be considered to be potential inhibitor of main protease enzyme of virus but need to be explored for further drug development process.

Keywords: Coronavirus, COVID-19, Molecular docking study, Main Protease, Natural Ligands

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Introduction

SARS-CoV-2 (Severe acute respiratory syndrome coronavirus-2) initially designated as 2019 novel corona virus (2019nCoV) is the latest zoonotic coronavirus after SARS-CoV (severe acute respiratory syndrome coronavirus) extended from 2002-2003 and MERS-CoV (Middle East respiratory syndrome coronavirus) occurred in 2012; has been declared Pandemic by World Health organization on March 12, 2020. Similar to first two coronavirus outbreaks SARS-CoV and MERS-CoV, bats have been denoted as primary reservoir of SARS-CoV-2. However, information about intermediary reservoir is still unknown [1]. Coronaviruses (CoVs) are the enveloped positive stranded RNA viruses, supposed to be possessing largest viral RNA genome known till date [2, 3]. As per the recent situational report released by World Health Organization (WHO) on March 28, 2020, 571678 COVID-19 cases have been confirmed globally, including 62514 new cases and total 26494 deaths [4].

SARS-CoV-2 a highly infectious and pathogenic coronavirus has become global concern [5]. Recently sequence analysis confirmed that the SARS-CoV-2 belongs to the class of β-coronaviruses including SARS-CoV and MERS-CoV. [6)]. Since, SARS-CoV-2 is novel pathogenic coronavirus, till date no effective antiviral therapy is available. Preventive and supportive therapies are main approach for management of COVID-19. Around the globe researchers are endeavoring for the development of effective prevention and treatment strategies for COVID-19. Preliminary studies have suggested potential repurposing of available anti-viral drugs like protease inhibitors lopinavir/ritonavir, nucleoside analogues, neuraminidase inhibitors, remdesivir, umifenovir (arbidol), tenofovir disoproxil (TDF), and lamivudine (3TC), etc. for the treatment of COVID-19 infected patients [7]. In another study Camostat mesylate, a clinically approved TMPRSS2 inhibitor was found to block SARS-CoV-2 entry to human cells, indicating its therapeutic potential as a drug against COVID-19 [8]. Xu et al. (2020) in another investigation screened 1903 approved drugs by homology modeling, molecular docking and binding free energy calculation and suggested nelfinavir as potential inhibitor against SARS CoV-2 [9].

A sizeable body of data demonstrates anti-coronaviral effect from several natural products and herbal medicines. In separate investigations Phenolic compounds of *Isatisindigotica*, Amentoflavone isolated from *Torreya nucifera* and *Houttuyniacordata* water extract have

shown SARS-CoV 3CL protease enzyme inhibitory potential against SARS-CoV.(10-12) Since the Main Protease M^{pro}or Chemotrypsin-like-protease (3CL) which is highly conservable among SARS CoV and SARS-CoV-2 proved by sequential analysis; is suggested to be potential target to fight SARS-CoV-2 [6]. Recently, Khaerunnisa et al, reported M^{pro}Inhibitory potential of kaempferol, quercetin, luteolin-7- glucoside, demethoxycurcumin, naringenin, apigenin-7-glucoside, oleuropein, curcumin, catechin, and epicatechin-gallate against COVID-19 [13]. Prompted by this, in present study we investigated Caffeine, Capsaicin, Hypericin, Gossypol, Luteolin, Berberine, Embelin,Oxyacanthine, Sanguinarine and Emodin as potential inhibitor candidates for SARS-CoV-2 $M^{pro}$with the help of molecular docking study.

Material and Methods

The crystal structure of the molecular target, protease enzyme (COVID-19 $3cl^{pro}$ /M ^{pro} (PDB ID: 6LU7), was retrieved from RCSB protein data bank (https://www.rcsb.org/) [14]. Target needs to be prepared before starting the molecular docking process, which involves removal of the water molecules and native ligand attached with target and other heteroatoms which may provide hindrance in the simulation. Besides, hydrogen atoms were added into target. These all processes were carried out in the Auto Dock window execution file. Investigational ligands were designed using ChemSketch (ACD 2012) and optimized for energy minimization using MM2 force field and saved in.mol format subsequently converted into .pdb format by Open Bable -2.3.2 software. The investigation ligand was loaded and their torsion salong with rotatable bonds are assigned and the files are saved as ligand. PDBQT. In the current study, identification of binding modes of the herbal molecules with targetwas identified using Auto Dock 4.2 software program. Moreover, to confirm actual binding interaction with targets blind docking was performed and the best conformers were represented with lowest binding energy (-kcal/mol) which might pave the way to disclose the mode of actions of these ligands. The docking parameters were defined as coordinates of the center of binding site with x = 126, y = 126, z = 126 and binding radius = 0.375 Å. All AutoDock output file (.dlg) were then analyzed through Analysis option provided in MGLTools-1.5.6 rc3. Top-scoring molecules in the largest cluster were analyzed. Conformers of the ligand were automatically docked to the enzymes and most stable conformer in terms of binding affinity (most negative) was used for post-docking analysis.

Results and Discussion

Table1 shows the ligands structures and necessary hydrogen bond formation by ligands with targets required for inhibition of target enzyme of covid-19. The rationale of choosing these ligands is only that they have shown efficacy in preclinical experimental models in different diseases. The SARS coronavirus main peptidase (SARS-CoVMpro) plays an essential role in the life cycle of the virus and is a primary target for the development of effective antiviral agents in case of COVID-19 infection. Figure 1 indicates the active site of target to confirm the binding site of investigational ligands if they were interacted with the amino acids in the active site of target or found to be attached any other site inside the target. The active site of this target enzyme comprises The active site of this target enzyme is composed of HIS41, MET 49, PHE140, LUE141, ASN142, GLY143, HIS163, HIS164, MET165, GLU166, LEU167, PRO168, HIS172, GLN189, THR190, ALA191 (Figure 1). The best poses of natural ligands and enzyme complexes are described, and the number of hydrogen bonds formed by both proteins with the drug are displayed in figure 2(a-j) as well as in the table1.

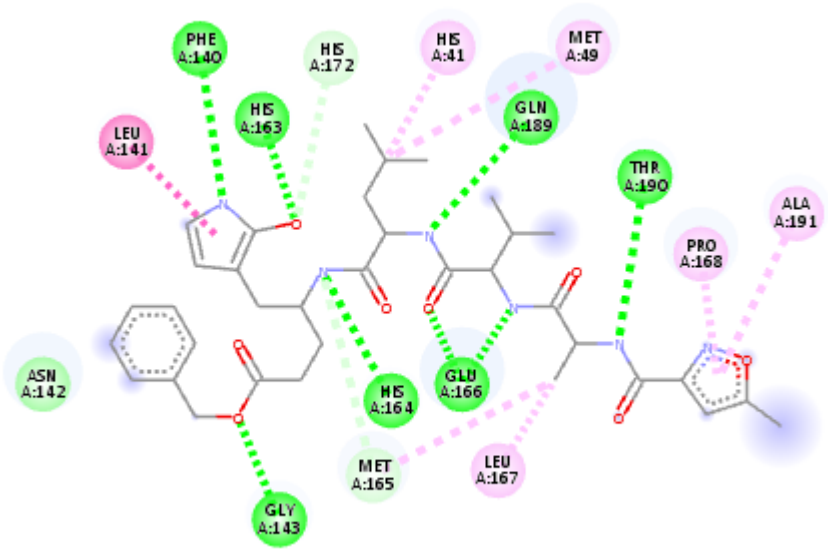
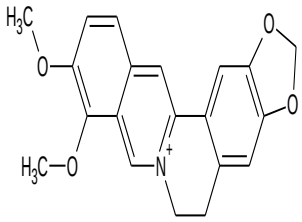
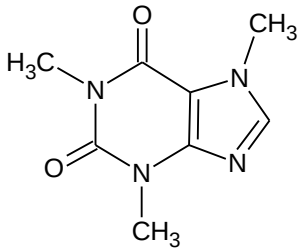
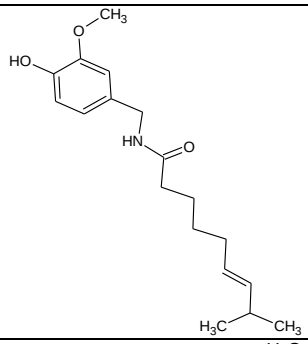
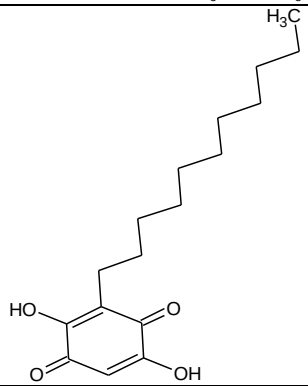
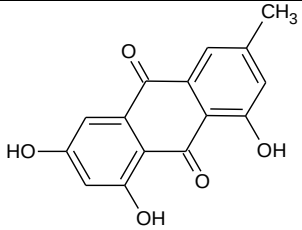
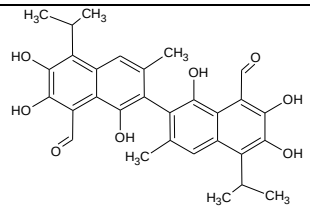
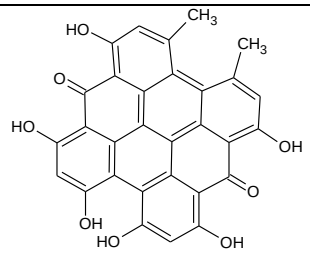
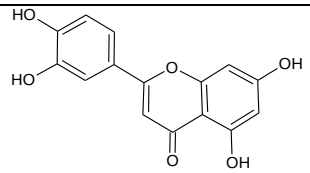
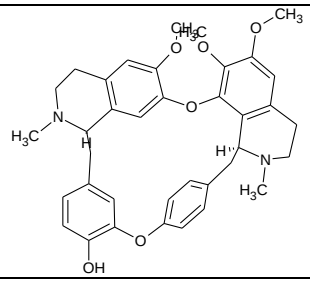
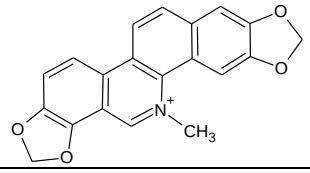


Figure1: Active site of target enzyme.

Table: 1 Structures of ligands and Hydrogen bond formation between ligands and target

S.N.	Ligand	Binding Energy	Structure of ligands	H-bond formed b/w ligand and receptor
1	Berberine	-7.910		Berberine-2:: LIG1:O,6lu7:A:ASP187:O
2	Caffeine	-4.520		6lu7: A:GLY143:N,caffeine-2: :LIG1:O,6lu7:A:GLY143:HN 6lu7: A:HIS163:NE2,caffeine-2: :LIG1:N,6lu7:A:HIS163:HE2 6lu7_3: A:GLY143:N,caffeine-2: :LIG1:O,6lu7_3:A:GLY143:HN 6lu7_3: A:HIS163:NE2,caffeine-2: :LIG1:N,6lu7_3:A:HIS163:HE2
3	Capsaicin	-5.510		Capsaicin-2:: LIG1:N,6lu7:A:GLU166:O,Capsaicin-2: :LIG1:H
4	Embelin	-4.940		6lu7: A:GLY143:N,Embelin-2: :LIG1:O,6lu7:A:GLY143:HN 6lu7: A:CYS145:N,Embelin-2: :LIG1:O,6lu7:A:CYS145:HN 6lu7: A:HIS163:NE2,Embelin-2: :LIG1:O,6lu7:A:HIS163:HE2
5	Emodin	-6.490		6lu7: A:GLU166:N,emo :LIG1:O,6lu7:A:GLU166:

6	Gossypol	-6.290		6lu7: A:SER144:N,Gossypol-2: :LIG1:O,6lu7:A:SER144:HN Gossypol-2:: LIG1:O,6lu7:A:SER144:OG,Gossypol-2: :LIG1:H 6lu7: A:HIS163:NE2,Gossypol-2: :LIG1:O,6lu7:A:HIS163:HE2 6lu7: A:HIS163:NE2,Gossypol-2: :LIG1:O,6lu7:A:HIS163:HE2
7	Hypericin	-9.050		Hypericin-2:: LIG1:O,6lu7:A:ASN142:OD1,Hypericin-2: :LIG1:H Hypericin-2:: LIG1:O,6lu7:A:HIS164:O,Hypericin-2: :LIG1:H Hypericin-2:: LIG1:O,6lu7:A:HIS164:O,Hypericin-2: :LIG1:H
8	Luteolin	-6.890		Luteolin-2:: LIG1:O,6lu7:A:ASP187:O,Luteolin-2: :LIG1:H 6lu7: A:GLN192:N,Luteolin-2: :LIG1:O,6lu7:A:GLN192:HN
9	Oxyacanthine	-10.990		6lu7: A:GLY143:N,Oxyacanthine-2: :LIG1:O,6lu7:A:GLY143:HN
10	Sanguinarine	-7.720		No Hydrogen bond formed

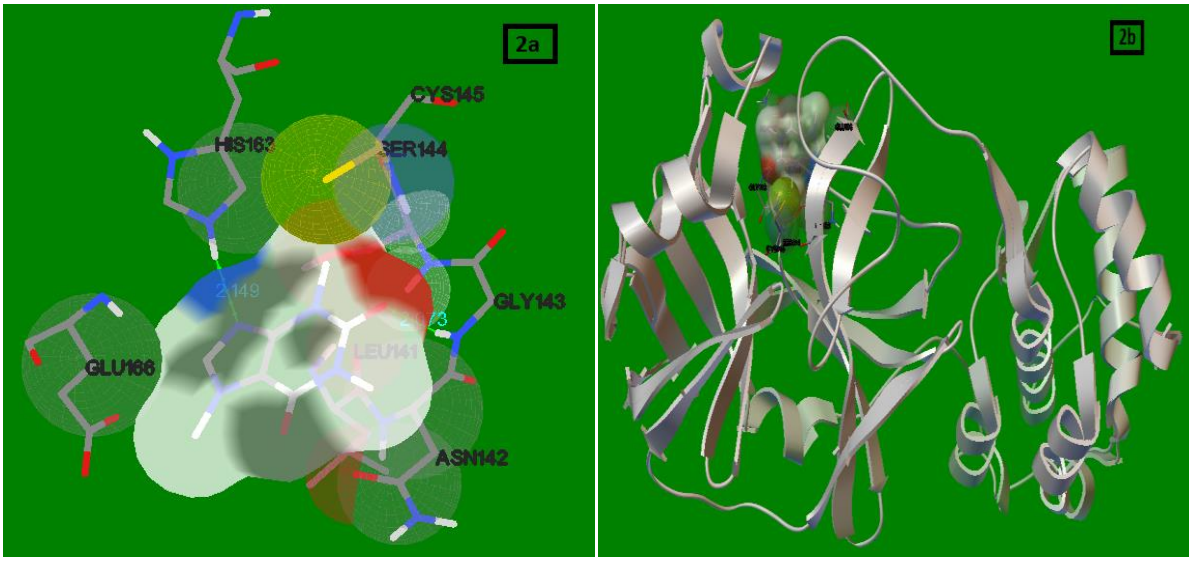


Figure 2a and 2b: Visualization of interactions between Caffeine and target enzyme

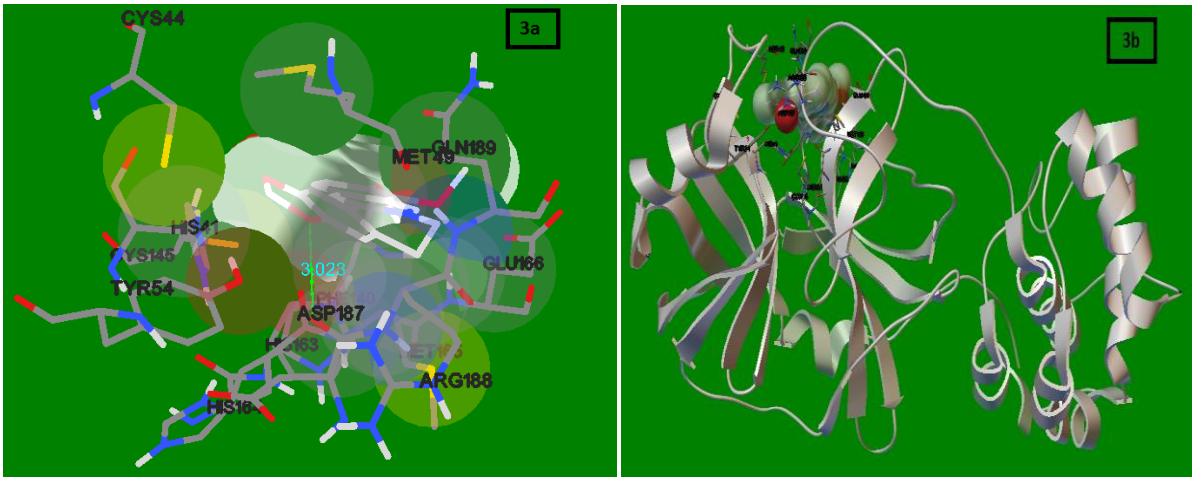


Figure 3a and 3b: Visualization of interactions between Berberine and target enzyme

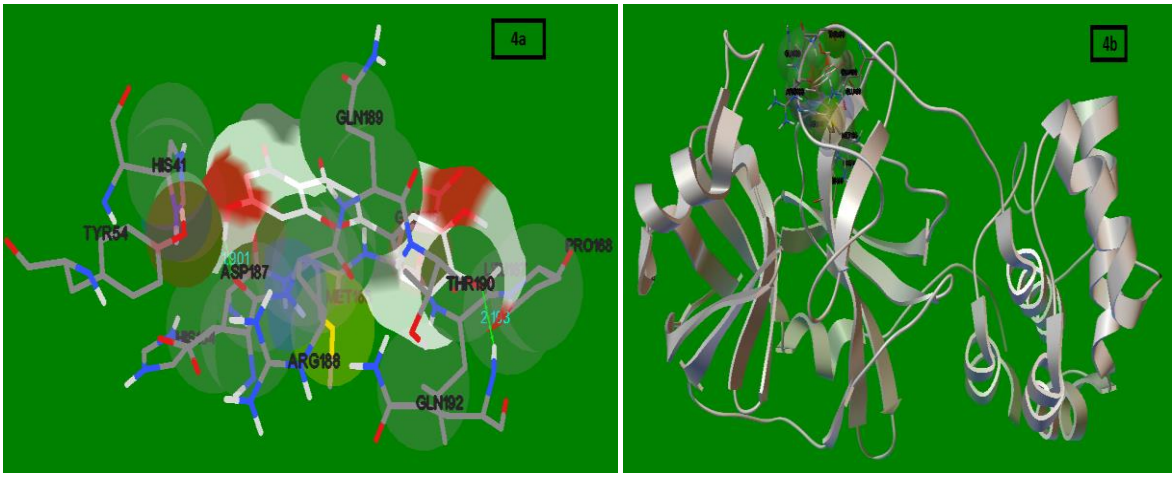


Figure 4a and 4b: Visualization of interactions between Luteolin and target enzyme

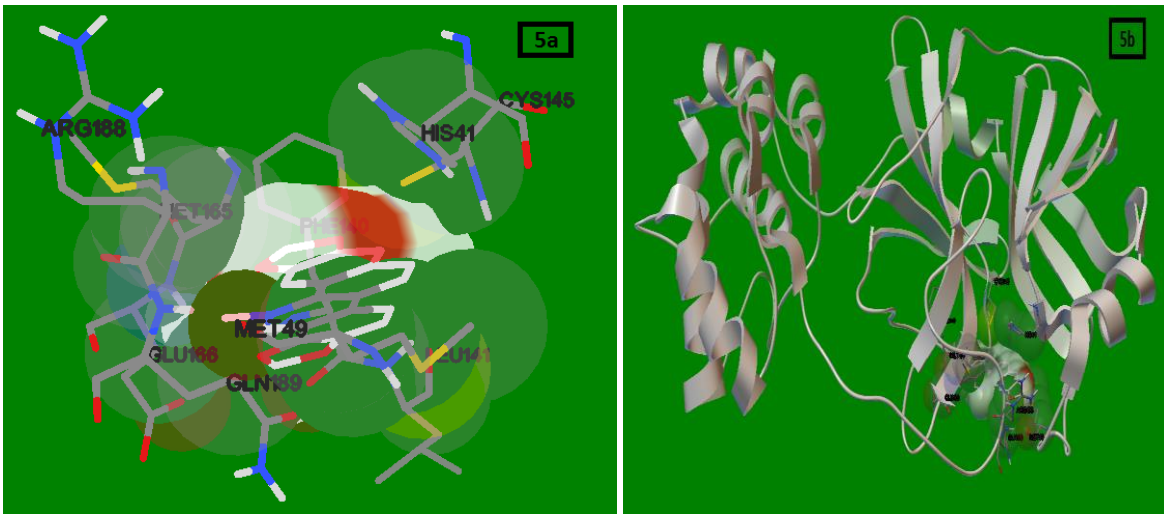


Figure 5a and 5b: Visualization of interactions between Sanguinarine and target enzyme

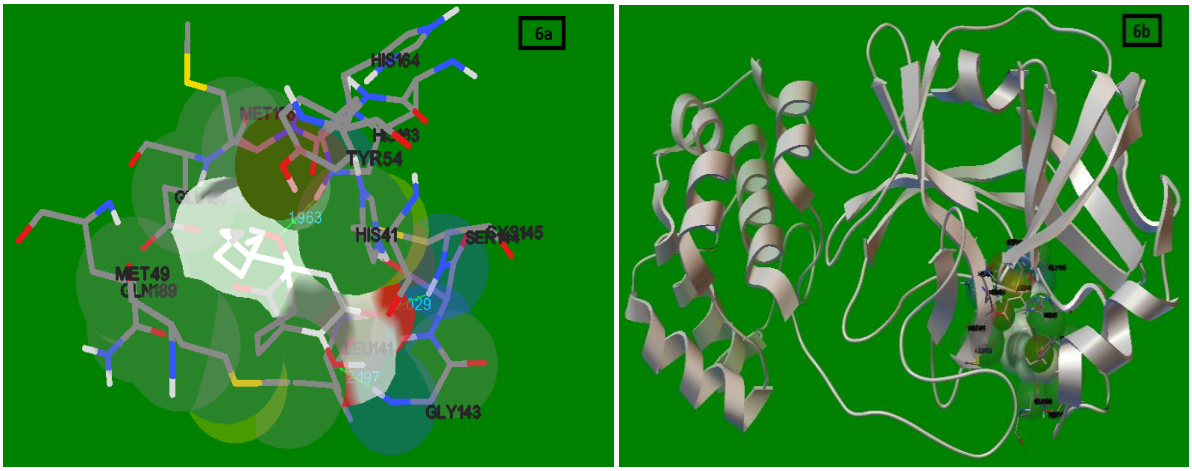


Figure 6a and 6b: Visualization of interactions between Embelin and target enzyme

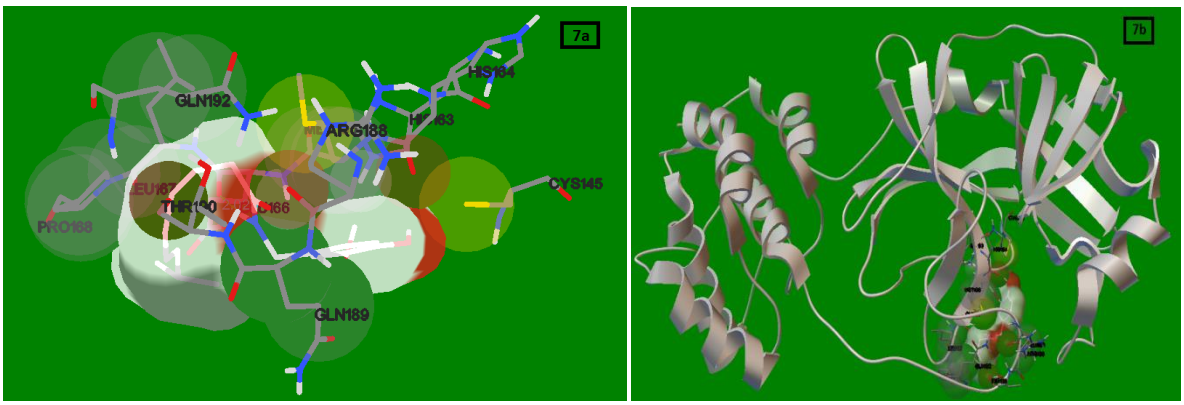


Figure 7a and 7b: Visualization of interactions between Capsaicin and target enzyme

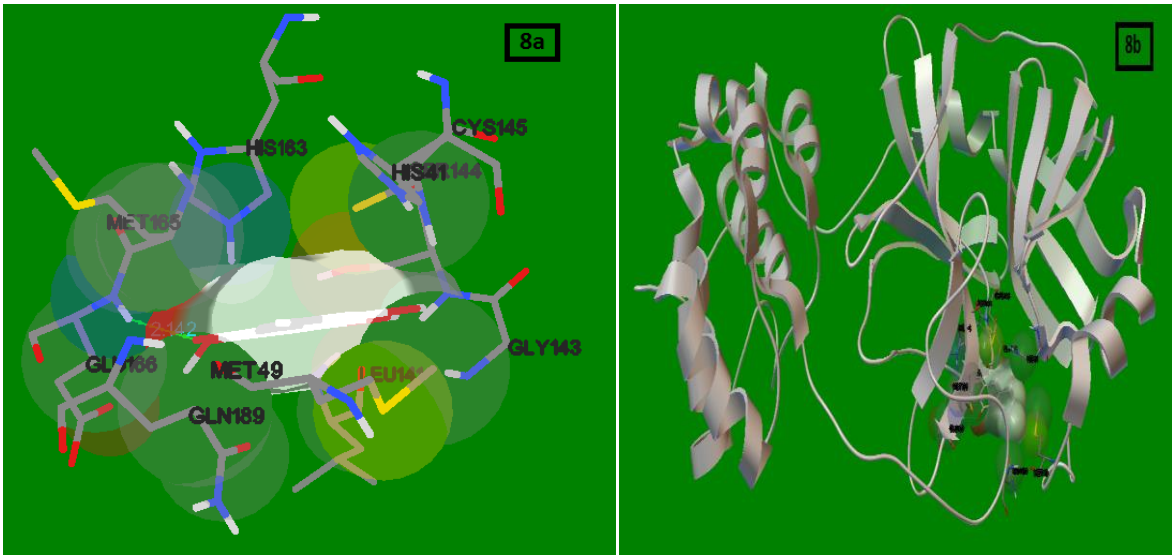


Figure 8a and 8b: Visualization of interactions between Emodin and target enzyme

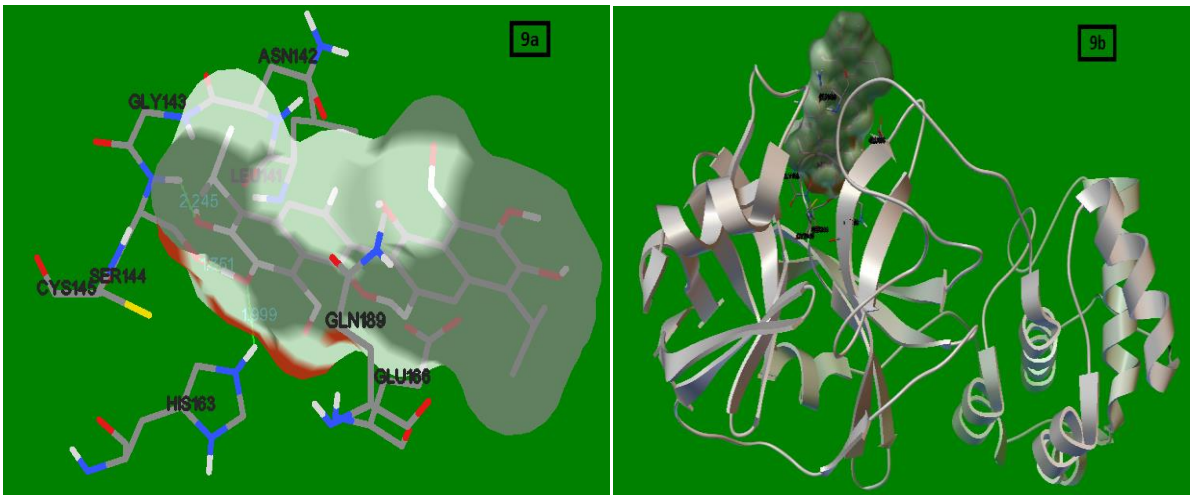


Figure 9a and 9b: Visualization of interactions between Gossypol and target enzyme

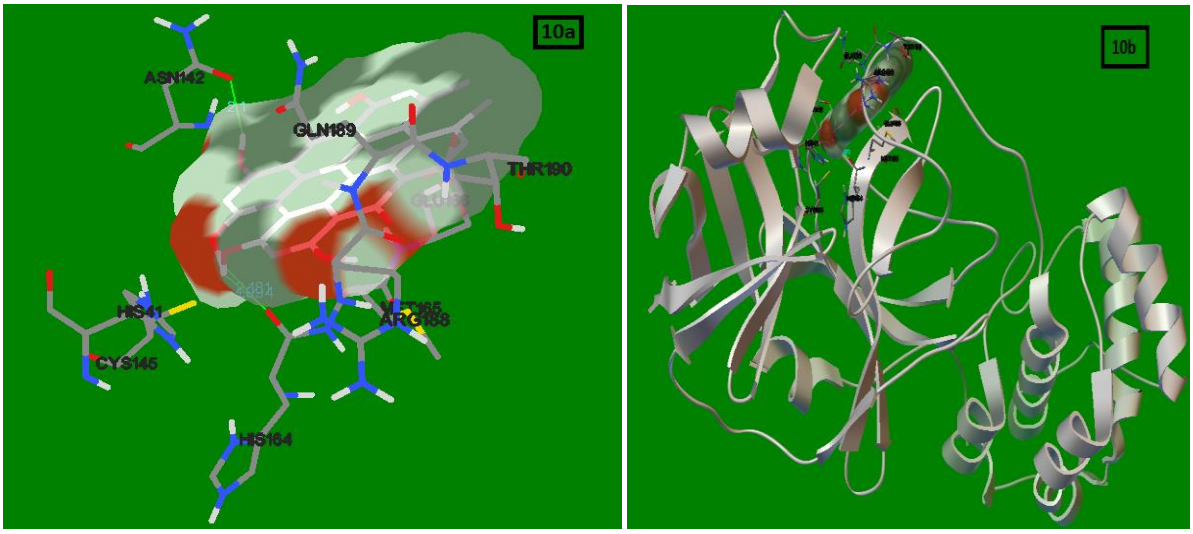


Figure 10a and 10b: Visualization of interactions between Hypericin and target enzyme

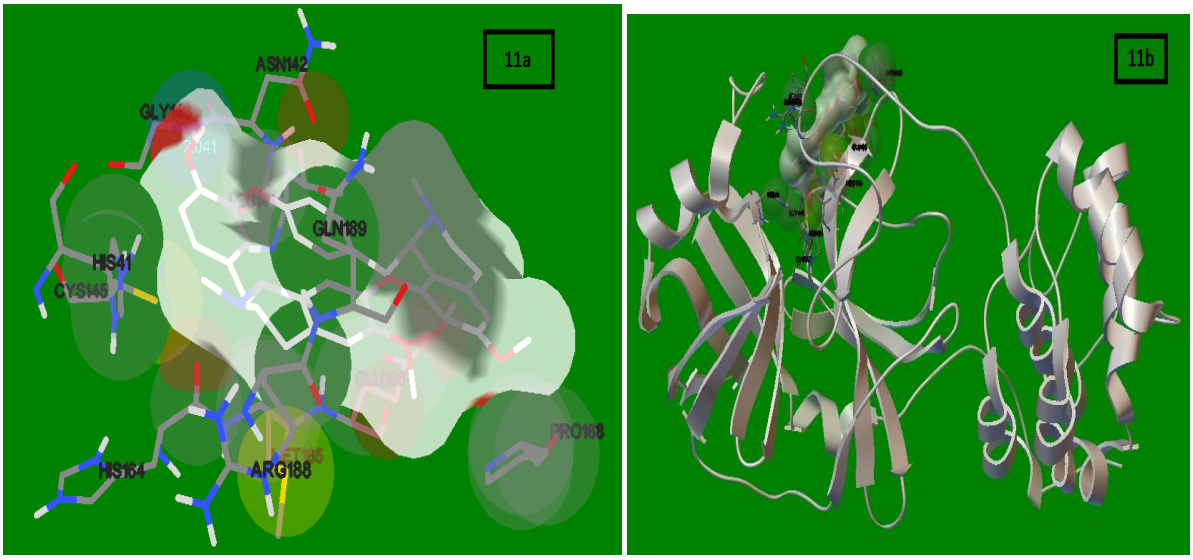


Figure 11a and 11b: Visualization of interactions between Oxyacanthine and target enzyme

DISCUSSION

The main protease enzyme Mprois a chymotrypsin-like cysteine proteaseplays an important role in mediating the replication and transcription of virus therefore this enzyme has been prime target for discovery of antiviral agents. The existence of the coronavirus Mpro was originally predicted by sequence analysis of IBV replicase polyprotein in 1989 [15]. We decipher here the mechanism of the most potent molecule in terms of binding affinity and necessary hydrogen bond formation responsible for inhibition of target enzyme. This study might be paving the way for the treatment of future similar viral infections caused by which have similarities with SARS or COVID-19. The native ligand attached in the main protease enzyme is showing its inhibitory activity by forming hydrogen bonds with PHE140, GLY143, HIS163, HIS164, GLU166, GLU189 and THR190. In the current study, molecular docking analysis reveals that although all-natural ligands interacted in the active site of target enzyme but some of them could not interact through hydrogen bonds with target which are required for enzyme inhibition.Caffeine is a CNS stimulant, exhibited least binding affinity (-4.520 kcal/mol) towards enzyme but could afford to form required some hydrogen bonds with GLY143 and HIS163 amino acids residues of enzyme. Berberine is an alkaloid, showed slightly good binding energy (-7.910 kcal/mol) than that of Caffeine but could interact with enzyme through different amino acid viz. ASP187 and produced different conformational changes in the target enzyme after binding than that of caffeine (see figure2 b and 3b). Although Luteolin exhibited moderate binding affinity (-6.890 kcal/mol) to target but could not make mandatory hydrogen bonds with enzyme for demonstrating inhibitory activity and

made hydrogen bonds with ASP187 and GLU192 amino acids residues of enzyme and revealed quite similar conformational changes in the target enzyme (see figure 4b). Therefore, Berberine and Luteolin could not be used as inhibitors of main protease enzyme. Sanguinarine also demonstrated a good binding energy but could not afford any hydrogen bond with virus enzyme. Embelin, Capsaicin, Emodin and Gossypol were found to be in the active site and interacted with enzyme through requisite hydrogen bonds but could not exhibit good binding affinity toward target enzyme (-4.940 kcal/mol, -5.510 kcal/mol, -6.490 kcal/mol and -6.290 kcal/mol respectively). Hypericin is a naphthodianthrone, an anthraquinone derivative expressed better binding affinity i.e. -9.050 kcal/mol but made unsuitable hydrogen bond with ASN142 residue in the active site of target enzyme also made a necessary hydrogen bond with HIS 164 residue required for enzyme inhibition.Oxyacanthine displayed the highest binding affinity those of among other ligands involved in the study i.e. -10.990 kcal/mol and produced in the significant changes in the conformations of main protease enzyme (see figure.11b).

CONCLUSION

A new strain of coronavirus has now been extended from Wuhan city of China to the other parts of world for which not any approved medications are existed. Therefore, from the current molecular docking study it has been concluded that Oxyacanthine can act as Mpro inhibitor but need to be explored for more experimental work regarding development of anti SARS- Mpro inhibitor.

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REFERENCES

1. Lee PI , Hsueh PR. Emerging threats from zoonotic coronaviruses-from SARS and MERS to 2019-nCoV. J Microbiol Immunol Infect 2020; 20:30011-30016. doi: 10.1016/j.jmii.2020.02.001.
2. Lu R, Zhao X, Li J, Nui P et al. Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding. The Lancet 2020; 395(10224): 565-574 doi: 10.1016/S0140-6736(20)30251-8.

3. Paules CI, Marston HD, Fauci AS. Coronavirus Infections—More Than Just the Common Cold. JAMA 2020; 323(8):707–708 doi:10.1001/jama.2020.0757
4. World Health Organization (WHO), "Coronavirus disease 2019 (COVID-19) Situation Report – 58, 18 March. pp. 1–9, 2020. Available at https://www.who.int/emergencies/diseases/novel-coronavirus-2019/situation-reports
5. Cai G. Cui X, Zhu X, Zhou J. A Hint on the COVID-19 Risk: Population Disparities in Gene Expression of Three Receptors of SARS-CoV. *Preprints* 2020 doi:10.20944/preprints202002.0408.v1
6. Chen Y, Liu Q, Guo D. Emerging Coronaviruses: genome structure, replication, and pathogenesis. J Med Virol. 2020; 92(4):418-423 doi: 10.1002/jmv.25681
7. H. Lu. Drug treatment options for the 2019-new coronavirus (2019-nCoV). Biosci. Trends 2020, doi: 10.5582/bst.2020.01020.
8. Hoffmann M, Kleine-Weber H, Schroeder S, Kruger N, et al., SARS-CoV-2 Cell Entry Depends on ACE2 and TMPRSS2 and Is Blocked by a Clinically Proven Protease Inhibitor. Cell 2020; 181:1-10 doi:10.1016/j.cell.2020.02.052
9. Xu Z, Peng C, Shi Y, Zhu Z, Mu K, Wang X. Nelfinavir was predicted to be a potential inhibitor of 2019-nCov main protease by an integrative approach combining homology modelling, molecular docking and binding free energy calculation. bioRxiv2020 doi: 10.1101/2020.01.27.921627
10. Lin CW, Tsai FJ, Tsai CH, Lai CC, Wan L, Ho TY, et al. Anti-SARS coronavirus 3C-like protease effects of Isatisindigotica root and plant-derived phenolic compounds. Antivir Res. 2005;68:36–42. doi:10.1016/j.antiviral.2005.07.002
11. Ryu YB, Jeong HJ, Kim JH, Kim YM, Park JY, Kim D, et al. Biflavonoids from Torreya nucifera displaying SARS-CoV 3CL (pro) inhibition. Bioorg Med Chem. 2010;18:7940–7. doi: 10.1016/j.bmc.2010.09.035.
12. Lau KM, Lee KM, Koon CM, Cheung CS, Lau CP, Ho HM, et al. Immunomodulatory and anti-SARS activities of Houttuynia cordata. J Ethnopharmacol. 2008;118:79–85. doi: 10.1016/j.jep.2008.03.018
13. Khaerunnisa S, Kurniawan H, Awaluddin R, Suhartati S, Soetjipto S. Potential Inhibitor of COVID-19 Main Protease (M^{pro}) From Several Medicinal Plant Compounds by Molecular Docking Study. Preprints 2020; 2020030226 doi: 10.20944/preprints202003.0226.v1
14. https://www.rcsb.org/. Last accessed on 20/3/2020
15. Yang H, Bartlam M, Rao z. Drug Design Targeting the Main Protease, the Achilles' Heel of Coronaviruses. Current Pharmaceutical Design 2006; 12: 4573-4590 doi: 10.2174/138161206779010369