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Molecular Docking study to Identify Potent Fungal Metabolites as Inhibitors against SARS-CoV-2 Main Protease Enzyme

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Abstract: Severe acute respiratory syndrome (SARS) is a viral respiratory disease caused by a SARS-associated coronavirus and SARS-CoV-2 has proven to be a pandemic worldwide. Coronaviruses are a type of enveloped virus. They are basically single-stranded and positive-sense RNA viruses which belongs to the subfamily *Coronavirinae*. Structure of SARS-CoV-2 is predicted to be the same as SARS-CoV due to high sequence similarity. SARS-CoV-2 is proven to be a major pandemic creator and affected the world at an exponential rate. The genome of COVID19 codes for the main protease 6LU7, is essential for viral replication and multiplication. To get a possible antiviral drug(s), nowadays is the major concern. In our study we screened ten fungal metabolites such as Aspirochlorine, Aflatoxin B1, Alpha-Cyclopiazonic acid, Sporogen, Asperfuran, Aspergillomarasmine A, Maltoryzine, Kojic acid, Aflatrem and Ethyl 3-nitropropionic acid against main protease 6LU7. These molecules were of fungal origin from *Aspergillus flavus* and *Aspergillus oryzae*. Aspergillomarasmine A exhibited the docking score of – 6.02 Kcal/mol, almost nearer to presently used drug Chloroquine (-6.29 Kcal/mol). Second highest docking score was found for Asperfuran (-5.5 Kcal/mol), whereas Aflatoxin B1 provided docking score was -5.0 Kcal/mol. We found similar docking score -5.4 Kcal/mol for Asperfuran, Maltoryzine and Kojic acid. Aspirochlorine and Ethyl 3-nitropropionic acid exhibited docking score were -5.3 Kcal/mol and -5.1 Kcal/mol respectively. These natural bioactive compounds could be tested in near future for their ability to inhibit viral growth both in *invitro* as well as *invivo* study.

Keywords: SARS-CoV-2, Main Protease Enzyme 6LU7, Aspirochlorine, Aflatoxin B1, Alpha-Cyclopiazonic acid, Sporogen, Asperfuran, Aspergillomarasmine A, Maltoryzine, Kojic acid, Aflatrem.

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1. INTRODUCTION

Viral infections are amongst the most common diseases which may affect people worldwide. The year 2020 was a global tragedy year in the form of a natural pandemic, named COVID-19. It was a highly pathogenic and transmittable viral infection causing the severe acute respiratory syndrome (SARS)[1]. As per the WHO report, it originated in Wuhan, China, and from there spread rapidly to other parts of the world. SARS-associated coronavirus may cause Severe acute respiratory disease caused by a SARS-associated coronavirus. Coronaviruses are enveloped. They are single stranded with positive-sense RNA viruses which belong to the subfamily *Coronavirinae*. There are seven human coronaviruses strain such as HCoV-229E, HCoV-OC43, HCoV-229E, HCoV-OC43, SARS-CoV, HCoV-NL63 and HCoV-HKU1, other novel human coronaviruses have also been discovered in recent years[2]. SARS can spread through small droplets of saliva from one to another in a similar way to cold and influenza. The coronavirus family has the four subgroups such as alpha (α), beta (β), gamma (γ) and delta (δ) coronavirus[3]. Pathogenicity of coronaviruses associated with mammals and birds may cause respiratory, gastroenteritis, reproductive and generalized infections. SARS-CoV2 pathogenicity shows bronchial epithelial cell peeling, cilia damage, the formation of multinucleated giant cells, squamous cell aplasia, alveolar interstitial fibre cell hyperplasia, and fibrotic lung disease[4,5]. There are three domains in the COVID-19 main protease (2019-nCoV) that help to form a catalytic dyad consisting of two amino acids histidine and cysteine mainly[6]. When we analysed the structure of the active site, we found that the protease is located between the two domains I and II. It is well known now that COVID-19 is a mutated form of SARS - CoV-1, only one amino acid at position 46 i.e., Ser46 varies between the two proteases in the area of the active site and thus their proteases exhibit sequence similarity of about 96%[7]. Structure of SARS-CoV-2 is predicted to be the same as SARS-CoV due to high sequence similarity. Structurally, SARS-CoV-2 has four main structural proteins including spike (S) glycoprotein, small envelope (E) glycoprotein, membrane (M) glycoprotein, and nucleocapsid (N) protein, and also several accessory proteins [5,8]. Therefore, the inhibitors of SARS protease are likely to exhibit varied binding strategies for COVID-19 main protease. The main protease in COVID-19 helps in assemble and multiplication of the virus and that can be one of the ideal targets without causing any harm to the host. It has an active site for the inhibitors where compounds are able to interact at this active site of the enzyme and therefore interfere with the viral replication process at a different level[9]. The spikes which are mainly glycoproteins, responsible for the attachment of the viruses on the surface and their subsequent entry into the host cells[10]. Thus, developing drugs against spike glycoproteins are the major target. So far, no specific drug against COVID-19 has been approved by the US Food and Drug Administration Agency. The use of computer-assisted structure-based drug design (SBDD) or the computer-aided drug design (CADD) approach to combat this problem over the conventional method of drug discovery and it is the prime concern. For thousands of years, bioactive components of natural products have been used to make traditional medicine to treat various diseases in many developing countries. Bioactive compounds are naturally derived metabolites which are actual by-products from microorganisms, plants, or animals[11]. In our study, we focused on structure-based computational modelling of ligand-receptor interactions. The present study deals with the *in-silico* study of various fungal secondary metabolites as potential bioactive components (to be used as drugs) against COVID-19 protease enzyme 6LU7 as the receptor.

2. MATERIALS AND METHODS

The in- silico study for receptor-ligand docking was carried out by using Patchdock online server as well as Molegro virtual docker. Protein preparation was carried out by using Biovia discovery studio (free software) also by using inbuilt protein preparation method of Molegro virtual docker. Ligand preparation (Lig Prep) was carried out by using Open babel software as well as the ligand preparation method of Molegro virtual docker. For knowing ADME of fungal metabolites, used SWISS ADME prediction server [5-13].

2.1 Protein and chemical compounds

All ten fungal secondary metabolites (ligands) were down loaded by using the following URL of NCBI available at http://www.ncbi.nlm.nih.gov/pccompound. Fungal chemical compounds were taken from literature[11]. The X-ray crystal structure of COVID-19 main protease 6LU7 in complex with an inhibitor N3, having 2.16 Å resolution was retrieved from the Protein Data Bank (PDB) [12].

2.2 Protein Preparation

The 6LU7 protein was prepared for a docking study by using Biovia discovery studio software, where the ligand was prepared by adding hydrogen atoms, removing unwanted molecules, generating ionization states at pH7, tautomer, geometric characteristics, and low-energy ring conformations[12].

2.3 Grid generation and docking study

The Docking process is managed via the Docking Wizard of Molegro virtual docker system. The wizard allows us to select which structures to include in the simulation. Followed by choosing the potential binding region (software automatically displays potential binding pockets). After that search, algorithm properties were configured along with clustering and data logging. Additional constraints were also managed by inspecting warnings about unlikely preparations and missing structural information (e.g. unknown residues). Molegro virtual docker was used to predict the binding affinity, ligand competence, and inhibitory candidate to the protein by performing rigid, flexible docking. Molecular docking was conducted with fungal secondary metabolites In the present study, we prepared ten ligands (fungal chemical compounds produced as secondary metabolites) obtained from the fungi *Aspergillus flavus* and *A. oryzae*, which were docked with generated Grid of receptor protein PDB ID: 6LU7. The optimal ligand selection for the receptor was done based on the docking score. After docking, we also continued ADME prediction of fungal metabolites by using SWISS ADME prediction server [13,14].

3. RESULTS AND DISCUSSION

The 6LU7 is a ~312 amino acid long main protease of 34.51 KD, the crystal structure with a resolution of 2.16 Å has been elucidated here [15]. The 6LU7 enzyme is the best target for inhibiting the SARS - CoV [15,16,17]. These proteases cleave

the spikes and further penetrate that protein in a better way [18,19]. This study was performed to identify possible compounds that can bind to the 6LU7, which may be used as a potential drug for COVID 19 in near future. Among the different compounds tested, we found ten fungal-derived molecules that can bind with the 6LU7 with a docking score ranging from -6.0 to -4.8 Kcal/mol (Table 1). We observed out of all the compounds studied, Aspergillo marasmine A (Fig 5, Table 1), had a higher docking score and the best binding affinity with main protease 6LU7 than the other ligands. Aspergillo marasmine A is a polyamino acid. It has been reported that it can inhibit two carbapenemase proteins in bacteria. The molecule has a tetracarboxylic acid with four -COOH groups and one section of the molecule is the amino acid aspartic acid. This has basically two alanine molecules attached. Ligands used in this study are obtained from NCBI. All these are secondary metabolites produced by fungus *A. flavus and A. oryzae*, which can be synthesized by culturing organisms in submerged liquid media under controlled environment[20]. Second highest docking score was found for Asperfuran (-5.5 Kcal/mol) (Fig 4, Table 1), whereas Aflatoxin B1 (Fig 1 and Table 1) provided docking score was -5.0 Kcal/mol. We found a similar docking score -5.4 Kcal/mol for Asperfuran (is a novel antifungal dihydrobenzofuran derivative), Maltoryzine (basically a toxic metabolite) (Fig 9, Table1) and Kojic acid (a chelation agent) (Fig 8, Table 1). Aspirochlorine and Ethyl 3-nitropropionic acid (role as a neurotoxin) exhibited docking scores were -5.3 Kcal/mol (Fig 6, Table 1) and -5.1 Kcal/mol (Fig 7, Table 1) respectively. Aspiro chlorine molecule belongs to the gliotoxin family and reported to have both antibacterial [21] and antifungal activity [22,23]. The other compounds (Table 1) also show a significant strength of interaction with the 6LU7 protein. Chloroquine (basically a terpenoids) is being used as a drug along with several antiviral molecules during the global outbreak of COVID 19 (Fig 11, Table 1). We also tried to predict binding mode and binding energy of this drug with respect to 6LU7 protein. Our all compounds, likely to inhibit the process of viral replication and translation and may have much significant impact on controlling the viral load in infected individuals. Aspergillomarasmine A exhibited the docking score of – 6.02 Kcal/mole, almost nearer to presently used drug Chloroquine (-6.29 Kcal/mol). Here all the used fungal metabolites pass Lipinski rule of five and therefore can be used as potential drugs (Table 2).

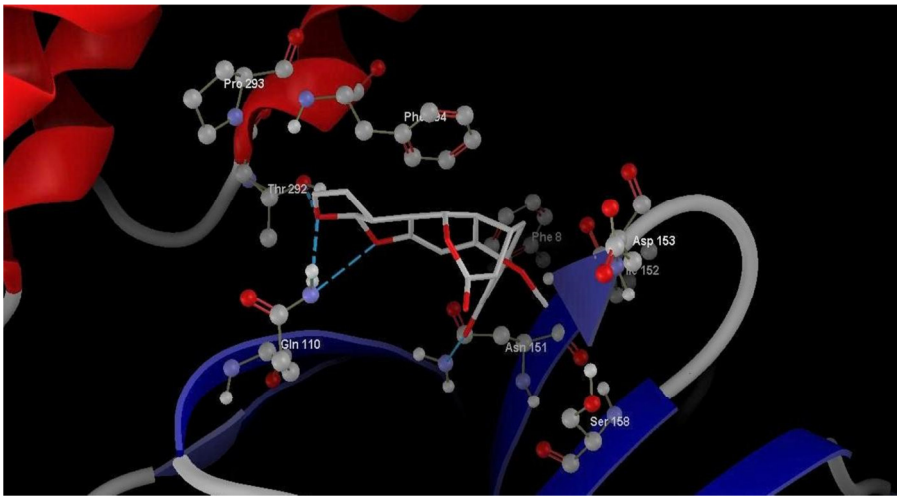

Fig 1: 3D structure of docking (Aflatoxin B1); Docking energy -5.0 Kcal/mol; Interaction Residues: Gln110, Asn 151 and Thr 292.

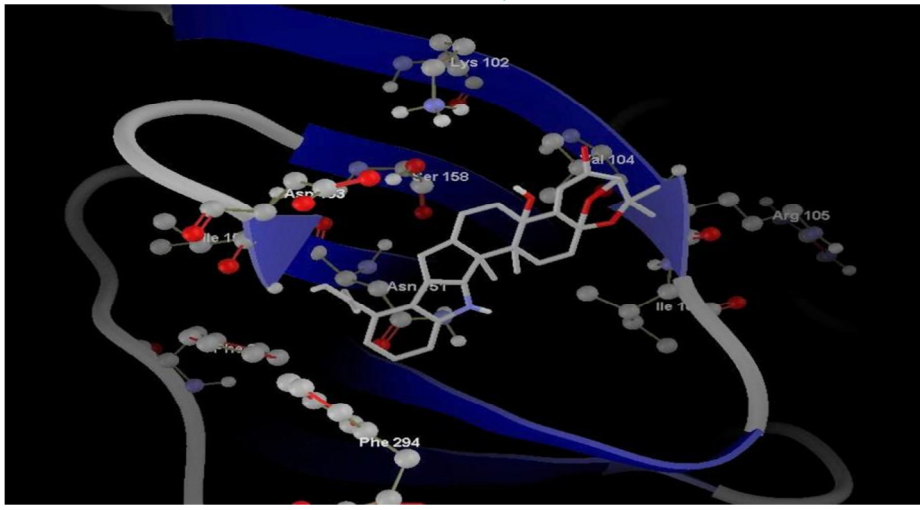

Fig 2: 3D structure of docking (Aflatrem); Docking energy -4.8 Kcal/mol; Interaction Residues: Val104, Arg105, Gln110 and Asp153.

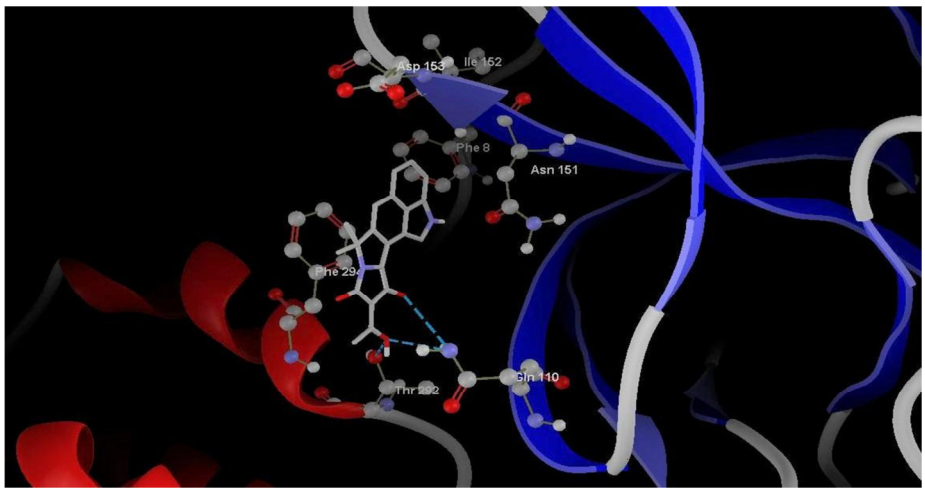

Fig 3: 3D structure of docking (Alpha-Cyclopiazonic acid); Docking energy -5.2 Kcal/mol; Interaction Residues: Gln110 and Asn151.

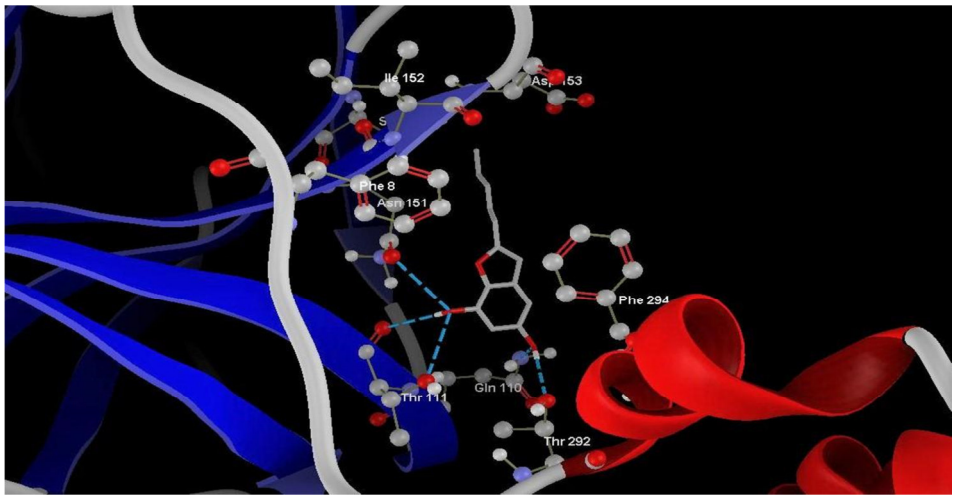

Fig 4: 3D structure of docking (Asperfuran); Docking energy -5.5 Kcal/mol; Interaction Residues: Thr111, Gln110, Thr292 and Asn151.

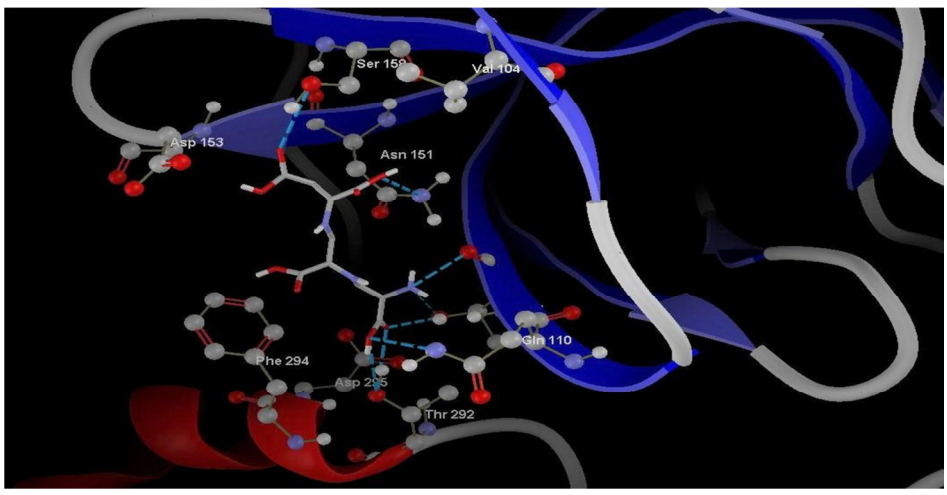

Fig 5: 3D structure of docking (Aspergillomarasmine A); Docking energy -6.0 Kcal/mol; Interaction Residues: Ser158, Thr292, Gln110, Asp295, Thr111and Asn151.

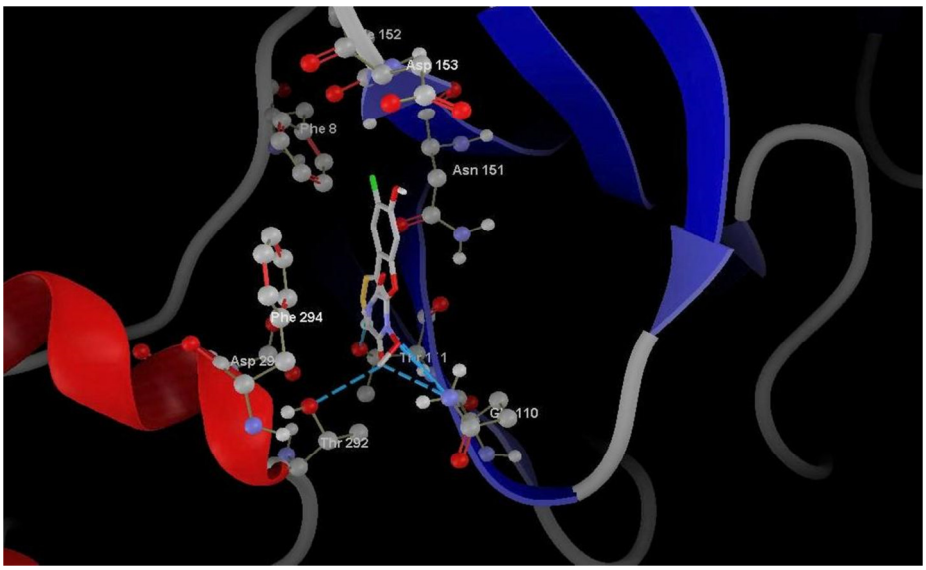

Fig 6: 3D structure of docking (Aspirochlorine); Docking energy -5.3 Kcal/mol; Interaction Residues: Thr292, Thr111, Gln110, Ile152 and Asn151.

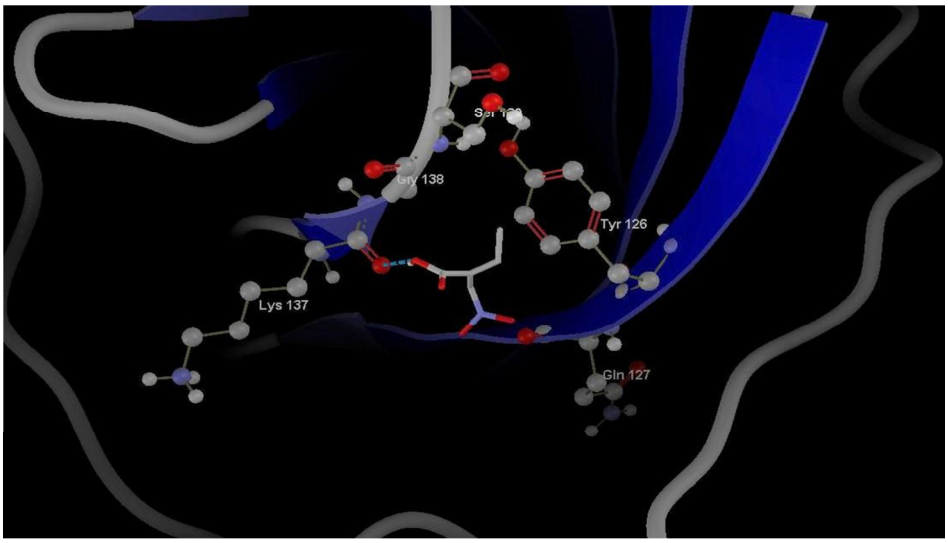

Fig 7: 3D structure of docking (Ethyl 3-nitropropionic acid); Docking energy -5.1Kcal/mol; Interaction Residues: Thr111, Thr292 Gln110 and Phe294.

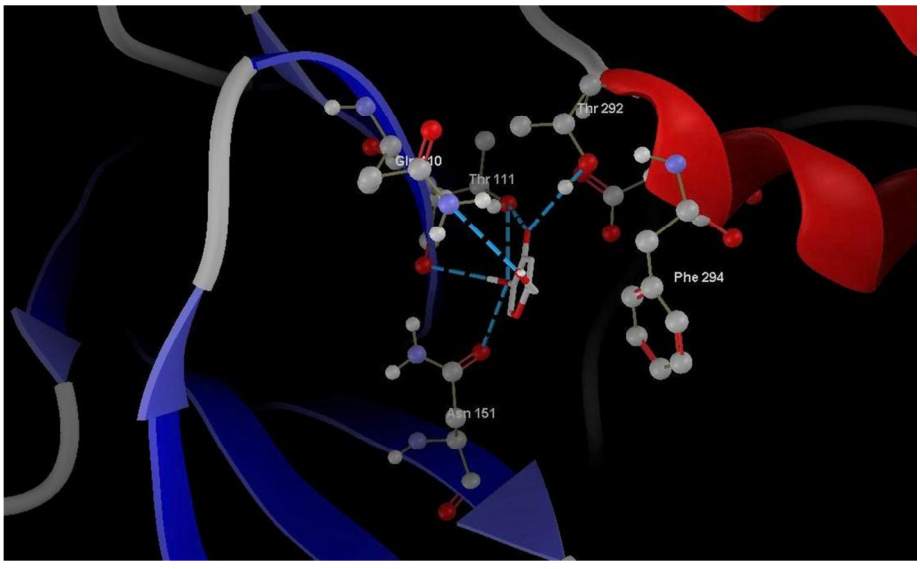

Fig 8: 3D structure of docking (Kojic acid); Docking energy -5.4 Kcal/mol; Interaction Residues: Thr292, Thr111, Asn151 and Gln110.

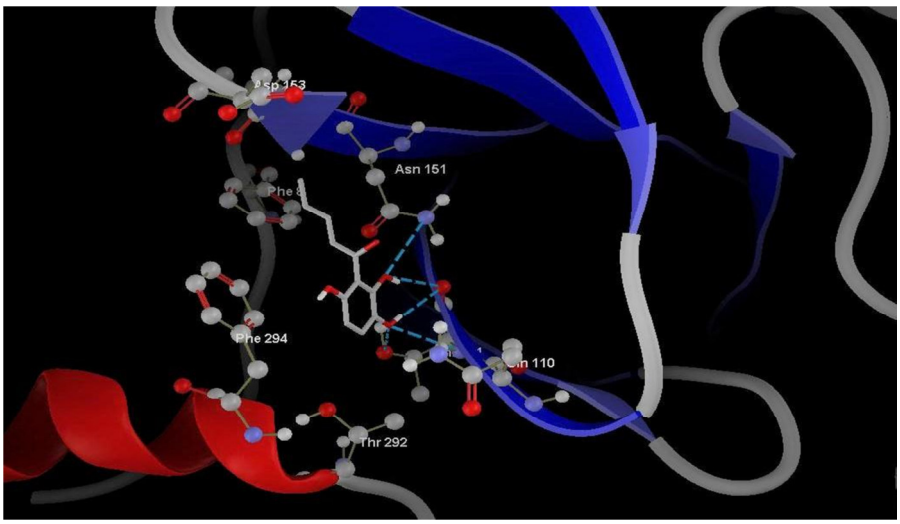

Fig 9: 3D structure of docking (Maltoryzine); Docking energy -5.4 Kcal/mol; Interaction Residues: Leu141, Ser144, Cys145, Met 165, His 164, His163, His172, Phe140 and Glu166.

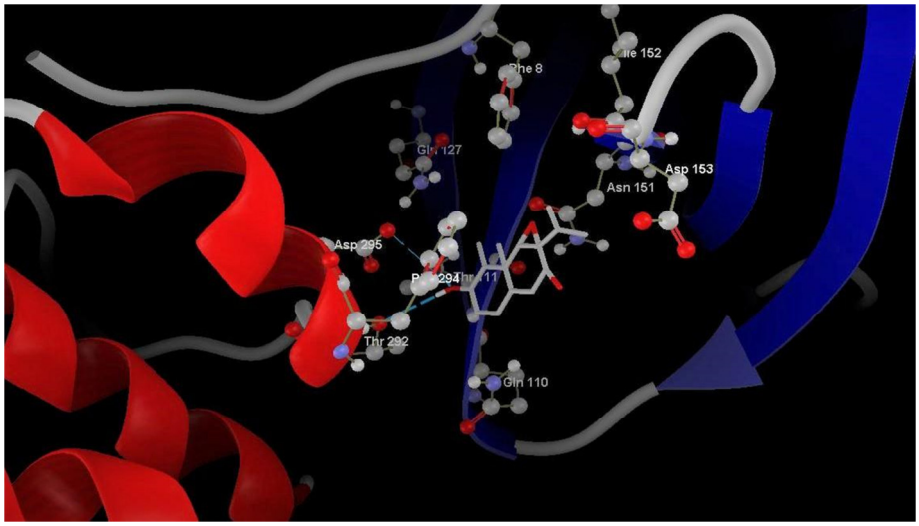

Fig 10: 3D structure of docking (Sporogen); Docking energy -5.4 Kcal/mol; Interaction Residues: Thr111, Thr292, Asp295 and Asn151.

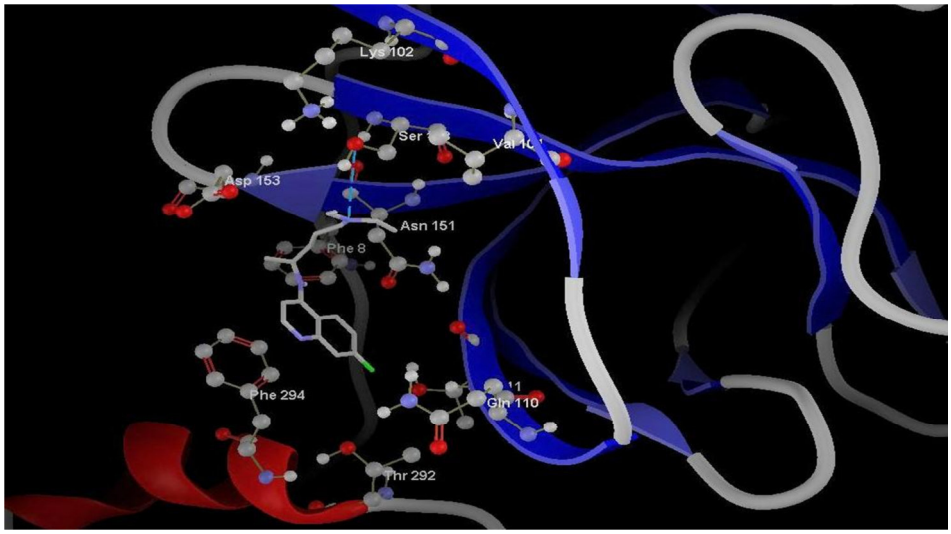

Fig 11: 3D structure of docking (Chloroquine); Docking energy -6.29 Kcal/mol; Interaction Residues: Phe140, His172, Glu166 and Leu141.

Table 1: Ten fungal inhibitors and potential known drug chloroquine of COVID-19 protease enzyme 6LU7.

Sl No	Compound	Docking energy	Interaction residue	No. of hydrogen bonds
1	Aflatoxin B1	-5.0 Kcal/mol	Gln110, Asn 151 and Thr 292	2
2	Aflatrem	-4.8 Kcal/mol	Val104, Arg105, Gln110 and Asp153	3
3	Alpha-Cyclopiazonic acid	-5.2 Kcal/mol	Gln110 and Asn151.	2
4	Asperfuran	-5.5 Kcal/mol	Thr111, Gln110, Thr292 and Asn151.	3
5	Aspergillomarasmine A	-6.0 Kcal/mol	Ser158, Thr292, Gln110, Asp295, Thr111and Asn151	5
6	Aspirochlorine	-5.3 Kcal/mol	Thr292, Thr111, Gln110, Ile152 and Asn151.	3
7	Ethyl 3-nitropropionic acid	-5.1 Kcal/mol	Thr111, Thr292 Gln110 and Phe294	3
8	Kojic acid	-5.4 Kcal/mol	Thr292, Thr111, Asn151 and Gln110	4
9	Maltoryzine	-5.4 Kcal/mol	Leu141, Ser144, Cys145, Met 165, His 164, His163, His172, Phe140 and Glu166.	3
10	Sporogen	-5.4 Kcal/mol	Thr111, Thr292, Asp295 and Asn151	3
11	Chloroquine	-6.29 Kcal/mol	Phe140, His172, Glu166 and Leu141.	2

Table 2: ADME prediction information of all fungal metabolites.

Sl No	Molecule	Mol.Wt (g/mol)	No. of H acceptors	No. of H donors	MLOGP	Lipinski
1	Aflatoxin B1	312.27	6	2	1.22	0 violation
2	Aflatrem	501.66	4	2	3.87	1 violation
3	Alpha-Cyclopiazonic acid	336.38	3	2	1.42	0 violation
4	Asperfuran	218.25	3	2	1.76	0 violation
5	Aspergillomarasmine A	307.26	11	7	1.29	1 violation
6	Aspirochlorine	360.69	5	2	0.09	0 violation
7	Ethyl 3-nitropropionic acid	147.13	4	2	-0.49	0 violation
8	Kojic acid	142.11	4	2	-1.69	0 violation
9	Maltoryzine	208.21	4	3	0.75	0 violation
10	Sporogen	248.32	3	1	1.66	0 violation

Here entire results in this article are based on completely computer based virtual screening. In this study, based on the results of bioinformatics analysis, we found all the screened molecules with the hydrogen bond(s) and steric interaction got bound to the active pocket of 6LU7 protein and made it inactive. Similar activity found with chloroquine. The potential binding compounds found in this study for these targets might be a good start point. Currently, numerous fungal-derived metabolites such as lovastatins, antibiotics and antifungal agent griseofulvin are present on the drug markets[24]. Fungal-derived compounds have not been approved for antiviral treatment so far. However, as per various researchers in their studies have found many of them exhibiting potential antiviral activity. So, it is just a matter of time before some molecules will be taken for clinical testing [25-26]. However, most of the above compounds were not predicted to bind with the binding interface of the Spike-ACE2 complex. The effective antiviral fungal compounds showing the best ADME (pharmacokinetic characteristics adsorption, distribution, metabolism and excretion) in vitro will be taken for animal testing in vivo. Currently no registered therapies for treating coronavirus infections are available. Any new drug development process consumes lots of time. Therefore, drug transposing may be the only solution to the epidemic of sudden contagious diseases. Fungi speak for a boundless of bioactive molecules, which could assuredly be used as antivirals in the future. Here, we have summarized the current evidence of fungi as creator of antiviral compounds and discuss their potential applications. Especially, we have scrutinized how the antiviral action has been levied and what is known about the biomolecular mechanisms and actual targets. These natural bioactive compounds could be tested in near future for their propensity to impede viral growth both in *invitro* as well as *invivo* study.

4. CONCLUSION

Our screen identified ten bioactive compounds of *Aspergillus* with the potentialities to bind the main protease enzyme (6LU7) of the COVID-19 virus. Aspergillomarasmine A exhibited the docking score of – 6.02 Kcal/mole, almost nearer to presently used drug Chloroquine (-6.29 Kcal/mol). Second highest docking score was found for Asperfuran (-5.5 Kcal/mol), whereas Aflatoxin B1 provided docking score was -5.0 Kcal/mol. We found a similar docking score -5.4 Kcal/mol for Asperfuran, Maltoryzine and Kojic acid. Aspirochlorine and Ethyl 3-nitropropionic acid exhibited docking scores were -5.3 Kcal/mol and -5.1 Kcal/mol respectively. These molecules may have antiviral activities against COVID19. Further simulation study needs to be conducted before proceeding for invitro and in vivo experiments.

5. CONFLICT OF INTEREST

Conflict of interest declared none.

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