Original Research Paper

Volume - 10 | Issue - 6 | June - 2020 | PRINT ISSN No. 2249 - 555X | DOI : 10.36106/ijar

Life Sciences

PHYTOCHEMICALS AS THERAPEUTIC AGENTS FOR TREATING COVID19

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ABSTRACT Severe Acute Respiratory Syndrome – Corona Virus 2 (SARS_CoV_2) is a deadly and new pandemic, which became diagnosed typically as a virus in Wuhan, China in the year 2019 . In current remedy protocols, there is no specific inhibitor for SARS _CoV_2 main protease, thereby offering an opportunity to find an inhibitor. Homology model was constructed by SWISS modelling, utilising gene sequence from NCBS database —— and the active site was determined by SWISS Prosite and Molegro Virtual Docking ——. Computational analysis of five phytochemicals Allicin, Alliin, rosmarinic acid, Carvacrol, and Methyl eugenol; ligands were retrieved from PubChem and were analyzed by Swiss ADME for drug likeness and pharmacokinetics —— .Docking and visualization was performed by Molegro Virtual Docking. The results were as follows for carvacrol: -61.634, allicin: -63.078, methyl eugenol: -72.594, alliin: -83.518, rosmarinic acid: -98.038.

KEYWORDS : Sars_cov_2, Covid-19, Phytochemicals, Allicin, Therapeutic, Molecular Docking.

INTRODUCTION

SARS_CoV_2 irruption was situated to be in city Wuhan of China, the World Health Organization (WHO) counsel the name COVID-19. 80 thousand confirmed cases and more than 2,700 deaths reported by WHO worldwide [1]. The unfold of COVID-19 was through sternutation from human to human, analysis of infected patients provided an insight – that the viral load was higher in nasal. In analysis of 23 patient cohort, the viral load peaked during the first week, it was conjointly with age as a factor. The immunoglobulin G and M was noticed to have increased from normal levels indicating the infection after the onset of 10 days incubation, the analysis reported that the viral load was high in older patients not only due to low immunity but also due to high ACE2 (Angiotensin Converting Enzyme 2) receptor, which provides the higher chance of infection [15,16]. Hong Kong studies show symptoms and clinical manifestations such as diarrhoea and asymptomatic sputum of a child which indicated COVID-19 RNA, conjointly glass opacities in lung imaging and was severed in older patients [1]. The replication of COVID-19 has a certain physical impact on the lungs and were analysed through CT (Computerized Tomography) scans to understand if COVID-19 also had similar patterns with other respiratory viruses, the CT scan analysis reported the presence of Ground glass opacities and consolidation with or without vascular enlargement, also were detected with halo sign as an exceptional feature [17]. A correspondence experiment analyses the stability of the artificially sneezed COVID-19 aerosol through a nebulizer, on various materials such as plastic, stainless steel, copper and cardboard, using Bayesian regression model. The results showed that COVID-19 was quite stable on plastic and stainless steel when compared to copper and cardboard, the COVID-19 was in a detectable range even after 72 hours. The stability of the virus was also studied with physical factor such as temperature and pH. The studies showed that the virus was stable at 4°C and the stability reduced at 70°C, and the inactivation time was down to 5 minutes, also COVID-19 was quite stable between a range of 3-10 pH, at room temperature. The viricidal effects of various disinfectants were also analysed and found out that after 5 minutes there was no detectable infectious viral particle incubated at room temperature [18,19].

Replication cycle of COVID-19 is through transmission of respiratory sneezed aerosol, once the virus has entered the cell, the positive mRNA strand (genetic material) is released into the endoplasmic reticulum, where the positive mRNA strand is utilized to produce certain viral replication machinery such as replicase. The replicase considers the positive mRNA strand as a template and polymerizes the negative strand and thereby increasing the viral genetic material. Once the viral genetic material is expressed in sufficient quantities, polyprotein is produced from the open reading frame, later which are converted to active viral particles by the viral main proteases. Then with the assembly of the viral active particles the cell is lysed and the viral particles emerge to infect further cells and multiply [20]. These features and diagnosis help scientist and radiologists provide an opportunity to find a target-specific drug treatment for COVID-19.

METHODS AND MATERIALS

Target Macromolecule

COVID-19 3C-like protease genome was extracted from the NCBI nucleotide database (https://www. ncbi.nlm.nih.gov/) in FASTA format. The sequence was then utilized to create homology model through SWISS-MODEL (https://swissmodel.expasy.org/), SWISS-MODEL is a completely automated online user interface homology modelling integrated server assists in building homology models at different levels of complexity [2–6]. The 3C-like protease contains two chains A and B, Chain A was used for macromolecular preparation. Also, crystal (https://www.rcsb.org/) structure retrieved from PDB database was utilised in docking analysis.

Target Active Site Determination

To determine the amino acid residue in the active site/cavity of the homology model were determined through SWISS Prosite [7–11] and Biovia Discovery Studio version 4.5 (Dassault Sytemes Biovia., 2020). The cavity was determined to confine the docking parameters used to analyse the grid box and docking evaluation.

Phytochemical Ligand

PubChem is a database of biologically active chemical structures repository and provides both 2-dimensional and 3-dimensional structures. Structure of drug was retrieved from PubChem [21] in 3-Dimension (3D) (https://pubchem.ncbi.nlm.nih.gov/) in .sdf format Drug like properties were determined by using Lipinski's standard rule of five: passage and oral ingestion have molecular weight >500, ClogP> 5, with cohesion to Lipinski's rule of five, were calculated utilizing SWISSADME analysis [12–14]. The ligands used are Chloroquine (CID_2719), Carvacrol (CID_10364), Allicin (CID_65036), Methyl Eugenol (CID_7127), Alliin (CID_87310) and Rosmarinic Acid (CID_5281792).

Molecular Docking

The receptor was auto optimized and docked by SWISS DOCK (http://www.swissdock.ch/docking) [22,23] and manual optimization was performed to minimize the energies of the receptor utilizing Molegro Virtual Docking software.

RESULTS

Figure 1 shows the representation of crystal structure of 3C_Like protease (3CL protease), also represents the active site/ catalytic site of the enzyme in the ball and stick format, the residues in the active site are 25Thr, 26Thr, 27 Leu, 41His, 44Cys, 45Thr, 46Ser, 49Met, 52Pro, 54Tyr, 140Phe, 141Leu, 142Asn, 143Gly, 144Ser, 145Cys, 163His, 165Met 166Glu, 167Leu, 168Pro, 172His, 187Asr, 188Arg, 189Gln, 190Thr, and 192Gln. The active amino acid that play the catalytic role is 41His and 145Cys. The crystal and homology model had similarities and were used for docking with allicin, alliin, rosmarinic acid, carvacrol, and methyl eugenol, considering chloroquine as comparable drug molecule, tabulated in Table 1. The docking results depict that chloroquine had one hydrogen bond interaction, when compared with other phytochemical drug molecule had varied number of hydrogen bond formation

possibilities with steric interactions in the active site, while carvacrol having 3 bonds, allicin with 2 bonds, methyl eugenol 2 bonds, alliin 6 bonds and rosmarinic acid 6 bonds of hydrogen bonding possibilities. The active site of the enzyme was validated with the Swiss Prosite scan and the active site domain and amino acid residue match the active site visualized with Molegro Virtual Docking version 4.5. Figure 2 represents the active site residues and the domain. According to Lipinski's rule in the shortlisted ligands did not violate any parameters and also suggest that they are a good agent to be considered as a therapeutic. The docking was performed twice on 5 shortlisted phytochemicals were validated, highly similar values were observed in Molegro Virtual Docking, the Lipinski's rule of five results are tabulated in Table 2. Comparative analysis of ligand interacting with the 3C_Like protease, the selected ligands carvacrol, allicin, methyl eugenol, alliin, and rosmarinic Acid have specific interactions with the active amino acid that is involved in the catalytic domain. The ligands have higher possibility to form a hydrogen bond with cystine 145 amino acid, which is represented in Figure 3 and adding with steric interactions would thereby acts as a potent inhibitor for 3C Like protease. The physical characters of the ligands play a very important role, when considered to be used as drug or therapeutic.

DISCUSSION

The previous data on beta coronavirus has led to insight and to screen certain potential key targets for the treatment of COVID-19. An enzyme called 3-chymotrypsin like protease/ Main protease is involved in the cleavage of the polyprotein (PP) -PP1a and PP1ab to active viral particles, this makes it a great target to be studied for the inhibition of the virion formation [24]. The phytochemicals have a history of ethnobotanical medicinal values, treating as a prophylactic agent for several ailments [25]. Even after so many advances in science and technology there is morbidity and mortality seen in viral infections. Phytochemicals have been tested in-vitro and in-vivo for antiviral activity and it is suggested for consideration by the pharmaceutical industries [26]. Chloroquine is an anti-malaria drug that contains a 4 -aminoquinoline scaffold, the drug being highly efficient and target specific to inhibit the activity of the malaria parasite, as well as cost-effective synthesis. Currently there is no specific pharmacological drug that is specific to SARS_CoV_2, it is the ongoing treatment which has no recorded side effects and mortality evidenced [27,28].

Carvacrol showing anti-pathogenic and anti-inflammatory activity against *Campylobacter jejuni*, the analysis was performed on the murine model to understand the effect of carvacrol treatment. The carvacrol treat murine group did survive post-infection and the placebo murine group did suffer from enterocolitis, it was observed that the effect of carvacrol did not restrict to gut but also had an effect on kidneys and liver by affecting the IFN-γ, TNF, MCP-1 and IL_6, it was also observed that there was reduced dissemination of pathogen [29].

In vivo assay was carried out in systemic inflammation rat models. Lung histology evidenced that rosmarinic acid treated thermal models showed an effective reduction of neutrophil accumulation in the peri-alveolar zone [30]. In asthmatic murine model exhibited high levels of IFN-gamma, IL_4, IgE, PLA2, and total protein, post-treatment of rosmarinic acid, levels of IFN-gamma, IL-4, IgE, PL2 and total protein significantly reduced [31]. Japanese encephalitis virus has high infection and mortality rate, murine model studies showed that rosmarinic acid treatment reduced the mortality rate also reduced the pro-inflammatory cytokines such as IFN gamma, IL-6, IL-12, TNF-alpha, MCP-1 and significantly reduced the viral load [32]. Studies have to be established for immunomodulatory and anti-inflammatory activity of methyl eugenol. Proinflammatory responses play a major role in controlling the effect of inflammatory cytokine outburst during infection [33]. Chicken in vivo studies for the infection of the Reticuloendotheliosis virus showed immune suppression and promoted the production of IL-1, IL-6, IL-10, IFN-, and TNF-. In Allicin treated chicken, allicin reversed the promotion of reticuloendotheliosis virus-mediated cytokines and elevated the production of IFN-, IFN, IL-2, also suppressing the toll-like receptors, MDA5 gene, MAPK and nuclear factor kappa B p65 which was promoted by the virus. Phosphorylation of JNK, ERK and p38 inhibit the MAPK pathway and modulate the cytokine-mediated immune response; whereas allicin suppresses phosphorylation of JNK, ERK and p38 factors that play a major role in MAPK pathway and thereby enhancing the immune modulation to overcome the viral infection [34]. Oxidation of exposed cysteine in enzymes can lead to a permanent change in the structure leading to loss of function, allicin being a reactive sulphur, also act as an oxidizing agent, react with thiols and exposed cysteine residues of enzyme and inhibit the function; when comparatively analysed in the situation of COVID-19, the main protease of COVID-19 active site is a cysteine residue, therefore allicin medication is suggested for the treatment of COVID-19 [35].

CONCLUSION

The ongoing pandemic COVID-19 does not possess a target-based treatment or therapy. The study aimed to analyse various phytochemicals compounds for their antiviral properties, the analysis predicted that not every phytochemical can be considered as potential drug therapy for COVID-19. But it has identified two potential phytochemicals, that is effective as a phytochemical and is suitable to be considered as a drug for their various properties, the phytochemicals are rosmarinic acid and allicin. These phytochemicals can be considered as potent enough to inhibit the main protease of COVID-19.

Declaration of Interest

The author reports no conflict of interest. The author alone is responsible for the content and writing of this article.

Acknowledgement

The author acknowledges that there was no financial support provided from any funding agencies to in carrying out this work.

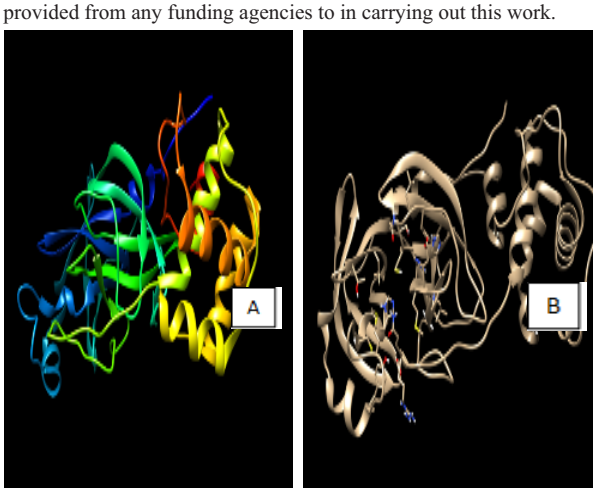


Figure 1: SWISSMODEL–A: Homology Structure of 3CL-protease. B: the active site of the enzyme is represented in ball stick format of amino acids.

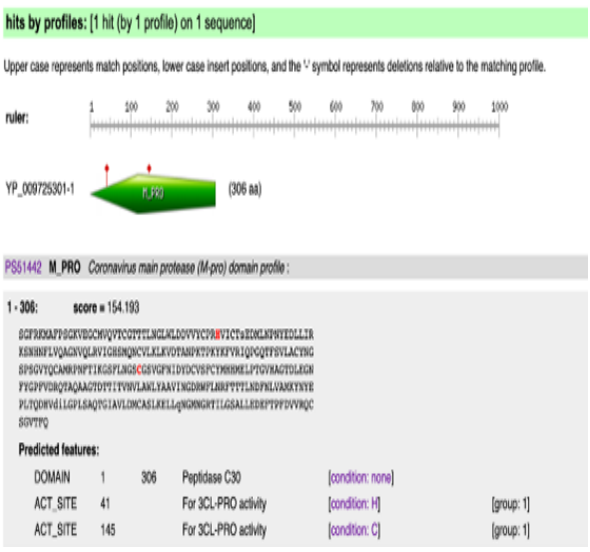


Figure 2 : The green colour mass representing the domain and the red colour indicates the active site of the enzyme, as indicated by Swiss Prosite.

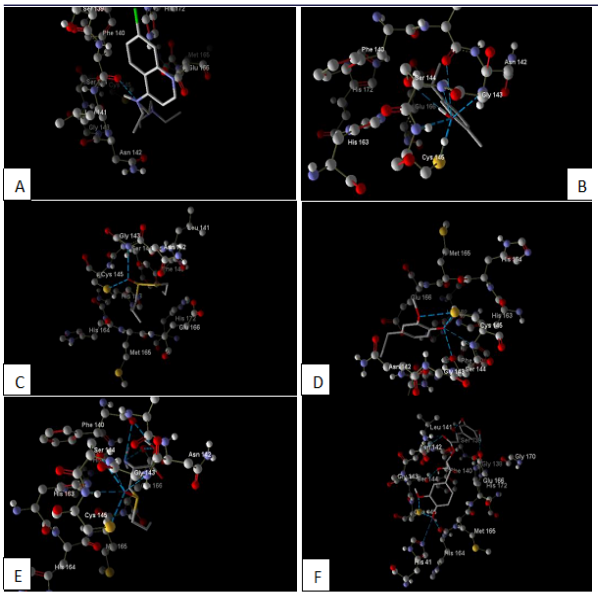


Figure 3: Poses of Drug docked in the active site of 3C-Like Protease. A: Chloroquine, B: Carvacrol, C: Allicin, D: Methyl Eugenol, E: Alliin, F: Rosmarinic Acid. The Blue colour dotted line indicate hydrogen bond interacting with the amino acid residues

Table 1: Tabulation of Molecular Docking Results. The 2-dimensional structure of the ligand and the amino acid residues of the active site interaction showing active site with dotted blue line.

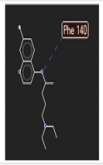
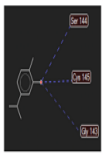
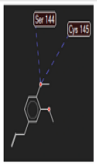
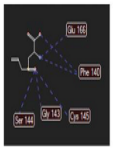
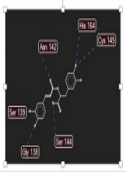
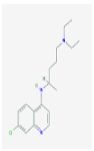
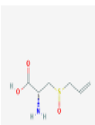
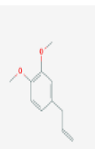
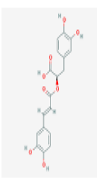
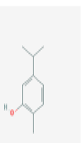
Ligand-Chemical Structure	Protein-Ligand Interaction - 2D	Docking Score	Molecular Weight
$C_{18}H_{26}ClN_3$ (Chloroquine)		-70.6224	319.872
$C_{10}H_{14}O$ (Carvacrol)		-61.6341	150.218
$C_6H_{10}OS_2$ (Allicin)		-63.0781	162.273
$C_{11}H_{14}O_2$ (Methyl Eugenol)		-72.5943	178.228
$C_6H_{11}NO_3S$ (Alliin)		-83.5183	177.221
$C_{18}H_{16}O_8$ (Rosmarinic Acid)		-98.0385	360.315

Table 2: Lipinski's five rules for molecules to be considered as drugs. The phytochemicals selected and docked, does not violate any rules of Lipinski's.

Ligand	Chemical structure	Lipinski's five rules			
		Molecular weight <500 Da	LogP<5	H bond Donor 5	H bond Acceptor <10	Violations
Chloroquine		319.87 g/mol	4.15	1	2	0
Allicin		162.27 g/mol	1.61	0	1	0
Alliin		177.22 g/mol	-1.33	2	4	0
Methyl eugenol		178.23 g/mol	2.58	0	2	0
Rosmarinic Acid		360.31 g/mol	1.52	5	8	0
Carvacrol		150.22 g/mol	2.82	1	1	0

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