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Research Article

COMBATING SARS-COV-19 BY PHYTOCHEMICALS: AN *IN SILICO* STUDY

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ABSTRACT

Objective: The objective of the study was to identify the potential lead phytochemical compounds that can be targeted against the severe acute respiratory coronavirus, 2019 (SARS-CoV-19) main protease (6LU7).

Method: A total of 191 phytochemicals from the Phytochemical and Drug Target Database (PDTDB) were retrieved and screened against SARS-CoV-19 protease by employing computational molecular docking studies.

Results: Our molecular docking results reveal that compounds ergosterol peroxide, punicalin, oleanolic acid, naringin, and diosmin are showing a very good affinity for coronavirus disease, 2019 (COVID-19) main protease and can be explored further as the lead candidate.

Conclusion: Computational studies of phytochemicals library against 6LU, a SARS-CoV-19 main protease led to the determination of compounds with the best theoretical affinity. Further, more target information of these compounds is needed for drug intervention, rational drug design, *in vitro* and *in vivo* evaluations, and final preparation in terms of clinical trial so that they can be used to combat the novel virus SARS-CoV-19.

Keywords: Coronavirus, COVID-19 main protease, Phytochemicals, Molecular Docking, Receptor-ligand Interaction AutoDock Vina, Chimera 1.13.1, Discovery studio.

INTRODUCTION

One of the worst viral outbreaks that have become a reason for everyone's concern is contagiously spreading coronavirus disease, 2019 (COVID-19). Severe acute respiratory coronavirus, 2019 (SARS-CoV-19), a zoonotic virus having an incubation period of 2–14 days, was reported causing a cluster of pneumonia cases in Wuhan city, Hubei Province of China. The outbreak has caused havoc globally by affecting 213 countries and territories along with two international conveyances. Worldwide, a total of 5,403,979 COVID-19 cases were reported as of May 24, 2020, and 343,975 people have died. Symptoms of COVID-19 include respiratory problems, fever, cough, shortness of breath, and dyspnea. In more severe cases, the infection can cause pneumonia, severe acute respiratory syndrome, kidney failure, and even death [1]. Unfortunately, there are no specific effective therapies available for treating COVID-19.

Genetic sequence analysis reveals that it is closely related to coronavirus found in bats [2]. Coronavirus (CoVs) belongs to the Coronaviridae family of the order Nidovirales which are divided into four genera (α, β, γ, and δ) [3]. Viruses of the family Coronaviridae possess a single-stranded, positive-sense RNA genome ranging from 26 to 32 kb in length [4]. CoVs contain four structural proteins: Spike (S) protein, envelope (E) protein, membrane (M) protein, and nucleocapsid (N) protein [5]. The known proteases of SARS-CoV-19 include viral papain-like protease (PLpro), main protease (3CLpro, also named as 3-chymotrypsin-like protease), RNA-dependent RNA polymerase (RdRp), helicase, and spike [6]. One of the best strategies to treat SARS-CoV-2 is to target the main proteases encoded by it. Among all of the proteases found in SARS-CoV-19, 3CLpro, the main protease plays an important role in the viral invasion by aiding in the maturation of non-structural protein, making it an attractive target for COVID-19 drug development. The main protease 6LU7 is a 3CLpro monomer and it consists of three domains, domain I (residues 8-101), domain II (residues 102-184), and domain III (residues 201-303), and a long loop (residues 185-200) that connects domains II and III. The active site of 3CLpro is located in the gap between domains I and II and has a CystHis catalytic dyad (Cyst145 and His41, as shown in Fig. 1) [7]. This catalytic site is of significance and can be used for finding small molecules that can bind and further, prevent viral molecular pathology.

Treatment using natural products remains a plausible option in terms of a control mechanism as specific vaccines and antiviral agents will take time because of epitope shifting in SARS-CoV-2. The effectiveness of herbal treatment to control contagious disease was demonstrated during the 2003 SARS outbreak [8] Shuanghuanglian, a Chinese herbal medicine that contains extracts from the dried fruit Lian Qiao (*Forsythia Fructus*), has been reported for treating infections for more than 2000 years [9]. Hence, this study focuses on the importance of medicinal plants and their constituents to identify the lead compound for treating COVID-19.

METHODOLOGY

The 3D structure of 191 phytochemicals was downloaded from the Phytochemical and Drug Target Database (PDTDB) [10] and screened by employing a structure-based virtual screening method. The X-ray crystal structure of SARS-CoV-19 main protease, a potent target for coronavirus disease, was retrieved from Protein Data Bank (http://www.rcsb.org/structure/6LU7). Target receptors were prepared for docking by removing undesirable bound ligand and water molecules using UCSF Chimera [11]. Hydrogen atoms, Gasteiger charges, were added to every atom of the receptors and finally converted into PDBQT format for docking. The structure of investigated phytochemicals was minimized using Open Babel software [12] and for final conversion into PDBQT format for docking. The virtual screening was performed using AutoDock Vina [13] wizard of PyRx [14]. Docking was performed using Lamarckian genetics as a scoring algorithm. For each docked molecule, AutoDock Vina showed different binding positions so, among them, we have selected the best conformation with the best energy score, i.e., low binding affinity (Kcal/mol). The result of docking and protein-ligand interaction was visualized using the Biovia Discovery Studio software [15]. This software helps in 3D visualization

of amino residue interaction, binding pocket, and the forces involved in the ligand binding.

RESULTS AND DISCUSSION

The emergence and contagious spread of COVID-19 have posed complex challenges to the public health, research, and medical communities which have led to attempts for combined drug therapy directed against its various targets but, unfortunately, there is no registered medical treatment for the zoonotic virus as of now. Various computational studies have been carried out and are believed to be of immense potential. Especially, the use of phytochemicals as a target against SARS-CoV-19 remains a point of interest due to their good medical history. Our high throughput virtual screening study done using 191 phytochemicals against COVID-19 protease (6LU7) reveals that 13 compounds are showing a binding affinity of ≤−8 Kcal/mol (the binding affinity of top 20 compounds is shown in Table 1).

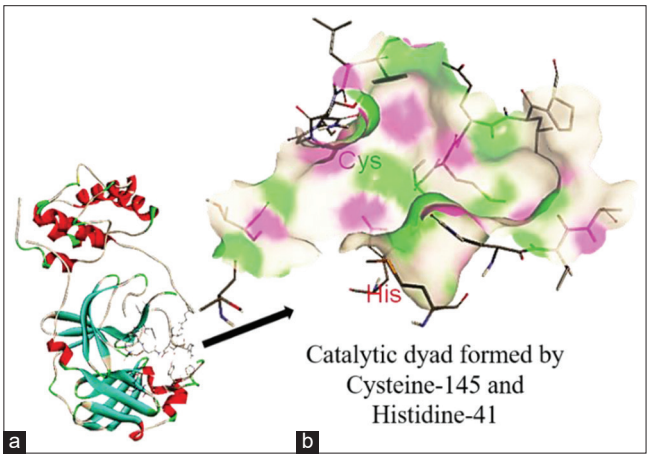

Fig.1: (a) Crystal structure of SARS-CoV-19 main protease (PDB ID-6LU7); (b) representation of catalytic dyad formed by cysteine-145 and histidine-41 residues in the binding pocket of SARS-CoV-19 main protease

Further, we have also tried to explore the molecular interactions involved in the binding of the top 5 phytochemicals (ergosterol peroxide, punicalin, oleanolic acid, naringin, and diosmin) with the amino acid residues present in the binding pocket of the SARS-CoV-19 main protease. Hydrogen bond, van der Waals force, and pi-pi interaction were the main interaction forces involved in the binding, as summarized in Figs. 2 and 3.

Docking studies constitute an important role in finding out the binding affinity of the ligand to a particular target, the conformation of the ligand bound to a macromolecule, and the appropriate target binding site, thus increasing the accuracy which forms the basis of rational drug design. Several studies highlighting the importance of phytocompounds and natural herbs have been reported in the literature. A randomized, double-blind study was conducted for evaluating the effect of natural herbal medicine composition on SARS and included herbs such as *Gypsum fibrosum, Bupleurum chinense, Gardenia jasminoides,* and *Siler divaricatum* [16]. Deng-hai Zhang *et al.* reported more than 20 Chinese herbal medicines showing antiviral and inflammatory responses using an *in silico* study to combat SARS-CoV-19 [8]. Indian herbal

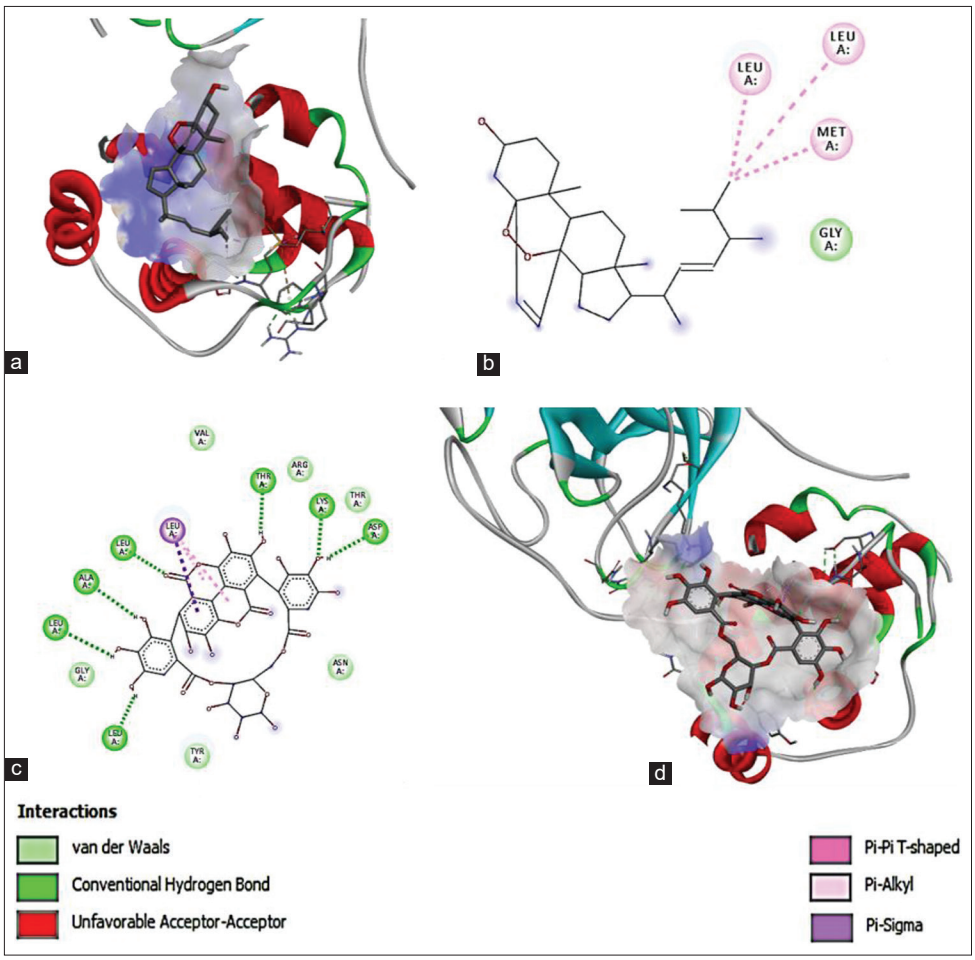

Fig. 2: Interaction of phytochemicals with the active site residue of SARS-CoV-19 main protease (6LU7). (a) Ergosterol peroxide. (b) 2D interaction diagram of ergosterol peroxide. (c) Punicalin. (d) 2D interaction diagram of punicalin

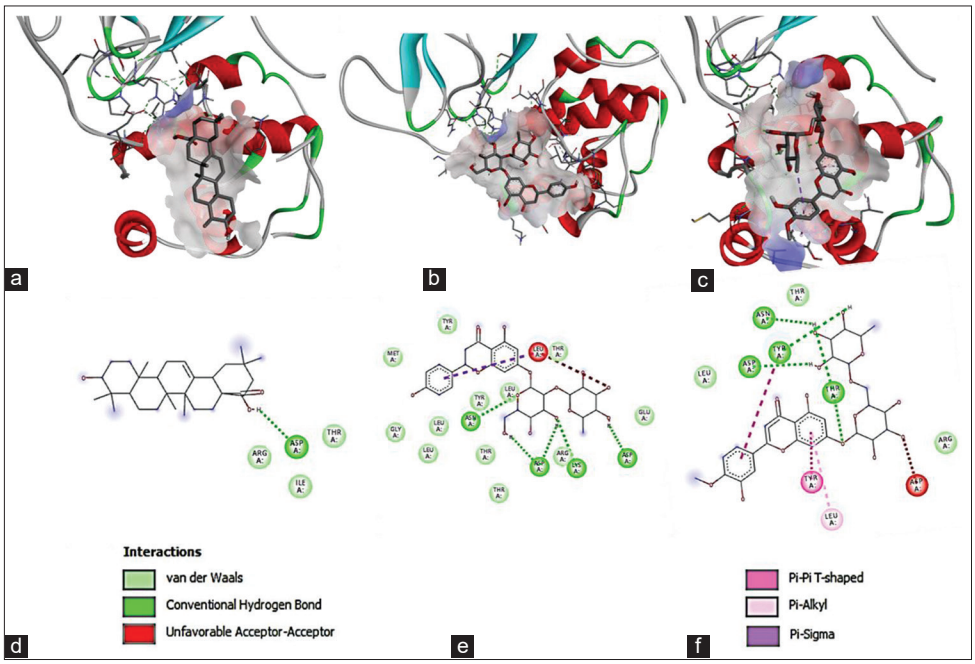

Fig. 3: Interaction of phytochemicals with the active site residues of SARS-CoV-19 main protease (6LU7). (a) Oleanolic acid. (b) Naringin. (c) Diosmin. (d) 2D interaction diagram of oleanolic acid. (d) 2D interaction diagram of naringin. (f) 2D interaction diagram of diosmin

Table 1: Binding affinity of top 20 phytochemicals against SARS-CoV-19 main protease (6LU7)

S. No.	Phytochemical compound	Binding affinity (Kcal/mol)
1.	Ergosterol peroxide	−8.8
2.	Punicalin	−8.6
3.	Oleanolic acid	−-8.5
4.	Naringin	−8.5
5.	Diosmin	−8.3
6.	Guaijaverin	−8.2
7.	β-Amyrin& 6''-O-(Pelargonidin 3-O-[2''-O-(beta-D-xylopyrano,hexose-hexose-hexose-Bayogenin	−8.1
8.	Campesterol	−8
9.	Ursolic acid	−7.9
10.	Avicularin	−7.8
11.	Deoxycalyxin A	−7.7
12.	Procyanidin B2	−7.6
13.	Riboflavin	−7.5
14.	Leucocianidol	−7.4
15.	Vasicinol	−7.3
16.	(+)-delta-Cadinene	−7.2
17.	2,5-Dihydroxybenzoic acid	−7.1
18.	Pyridoxine	−7
19.	Menthofuran	−6.9
20.	Geranic acid	−6.8

medicines have always been relied on as the representatives of the field of traditional medicines [17,18]. Recently, the Ministry of AYUSH has also emphasized on developing drugs using natural compounds from ayurvedic medicinal herbal plants for treating COVID-19. By conducting this study, we have been successful in identifying five natural compounds showing good binding affinity against the SARS-CoV-19 protease (6LU7).

Lead phytochemicals and their medicinal use

The top 5 potential lead phytochemicals include ergosterol peroxide, punicalin, oleanolic acid, naringin, and diosmin. Ergosterol peroxide is a C28 sterol, isolated from *Ganoderma lucidum* and *Cordyceps sinensis* which are a type of fungus [19]. Several beneficial biological effects of this compound, such as antimicrobial, cytotoxic, and immunosuppressive activities, have been reported [19]. Its role as a metabolite, an antineoplastic agent, an antibacterial drug, and a trypanocidal drug, has also been mentioned. Punicalin is found in edible sources such as *Punica granatum,* i.e., pomegranate along with plants such as *Combretum glutinosum* and *Terminalia catappa* which is used to treat dermatitis and hepatitis [20]. It has been used to treat several complications including parasitic and microbial infections, diarrhea, hemorrhage, and respiratory problems [21]. The rich source of oleanolic acid is *Olea europaea,Rosa woodsii* (leaves), *Prosopis glandulosa* (leaves and twigs), and *Phoradendron juniperinum* (whole plant) [22]. Oleanolic acid exhibits hepatoprotective, antitumor, and antiviral properties [19]. Another compound with beneficial biological properties is naringin which is a flavone glycoside found in grapes and citrus fruits. It also has a role as an anti-inflammatory agent, antineoplastic agent, and blood lipid-lowering agent [23]. Diosmin is a natural flavone that is abundant in the pericarp of various citrus fruits. It acts as an antioxidant, antihyperglycemic, anti-inflammatory, antimutagenic, and antiulcer agent [24,25]. These compounds showing good binding affinity have been known for their various therapeutic and medicinal properties and our study highlighted their potential to conquer SARS-CoV-19 by interacting with its main protease.

CONCLUSION

Several attempts have been made to treat COVID-19 by combined drug therapy directed against its targets and also various clinical trials have been initiated. The use of natural compounds has a successful history in treating SARS which occurred in 2003. Hence, virtual screening of the natural compounds was really of great significance in discovering the compound with the best binding affinity against one of the important targets of SARS-CoV-19 main protease. Our virtual screening shows that ergosterol peroxide, a compound from fungal origin, has the potential to be tested further by *in vitro* and *in vivo* studies for the treatment of COVID-19.

CONFLICTS OF INTEREST

There are no conflicts of interest.

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