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SHORT COMMUNICATION

In silico approach for identification of natural compounds as potential COVID 19 main protease (M^{pro}) inhibitors

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Abstract With the recent pandemic outbreak and subsequent worldwide spread of COVID-19 from Wuhan city of China, millions of infections and lakhs of deaths have resulted. No registered therapies have been developed to treat infection with COVID-19. The present study was conducted to evaluate the efficacy of herbal drugs as drug target molecules against COVID-19 by molecular docking. The inhibitory effects of natural compounds were analyzed against COVID-19 main protease (M^{pro}). The inhibition of M^{pro} prevents the virus replication. In the current study forty eight compounds were screened with AutoDock 4.2. Discovery Studio has visualised the interaction between targeted protein amino acids and ligands. The potent phytochemicals inhibitors were identified based on the binding energy with the targeted protein. Phytochemicals such as Fagaronine, Isoboldine, Sageone, Lycorine and Wogonin were noted as potential inhibitors whereas the docking study demonstrated the significant binding energy with the target enzyme, viz. − 6.21, − 5.99, − 5.97, − 5.86 and − 5.62 Kcal / Mol respectively. These lead compounds can be used against SARS-CoV-2 infections for drug development.

Keywords Molecular docking · Covid-19 · Phytoligand · M^{pro}

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Introduction

Dramatic changes to the recent exceptional corona viral situation have been declared pandemic by the World Health Organization. Highly contagious respiratory disease COVID-1 (Coronavirus Disease-2019) outbreaks begin from Wuhan, China in December 2019. It is the result of a new strain of SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2). At current scenario no effective drug has been developed, several works is ongoing worldwide. According to WHO 150,989,419 cases of COVID-19 were reported including 3,173,576 of confirmed deaths have been reported worldwide till May 1, 2021 [1]. Day by day COVID-19 infected cases are increasing tremendously. It is important to know the virus structure, active site for drug binding, mechanism for the production of different drugs for the virus. SARS-CoV-2 origins are actually unclear, although there is evidence that it is perhaps of animal type [2]. SARS-CoV-2 belongs to Coronaviridae family having positive single-stranded RNA (Ribonucleic acid) molecule with spike glycoprotein envelope on their surface. SARS-CoV-2 starts its life cycle in host when S protein attached to the angiotensin-converting enzyme 2(ACE2) cellular receptor [3]. After entry of the virus into the host cell the replication takes place in host with the synthesis of polyproteins. One of the proteins, main protease of coronavirus is responsible for new virus replication [4].

Indian medicinal herbs are a promising area for the treatment of different diseases [5]. Phytocompounds of medicinal herbs can be effectively characterized that can help to alleviate the infection. Practices of Ayurveda and Siddha emerged in India and are still commonly used by the Indian community. Secondary metabolites are developed from primary plant metabolism and share common precursors [6, 7]. Plants face virus infections and it is

therefore not surprising that a number of plants have antiviral-secondary metabolites. Similar structures can be useful for secondary metabolites of plant viruses that combat human pathogens, as plant and animal viruses share the same structure. The extracts of medicinal plants or independent plant secondary metabolites have been used to control viral infection [8, 9]. The numbers of study reported that plant secondary metabolites are effective as antiviral agent like buchapine, colchicines alkaloids against Human Immunodeficiency virus-1(HIV-1). Flavonoids such as ternatin, lignans, peltatin, podophyllotoxin and derivatives are effective against *Rhabdoviridae* family viruses [8]. Flavonoids like naringenin, ternatin, galangin, quercetin, genistein used against Human simplex virus (HSV) [9]. Chloroquine an antimalarial drug recently being given to COVID-19 patients [10]. Antimalarial alkaloid quinine was earlier reported as antiviral activity against Dengue and Human simplex virus [11]. The molecular docking study was carried out on 48 protease inhibitors compounds as antiviral agents from plants against COVID-19 main protease (M^{pro}).

The COVID-19 main protease (M^{pro}) was used as the receptor. The PDB ID: 6LU7 structures was acquired from PDB (https://www.rcsb.org/), in pdb format. The 6LU7 protein is a homodimer of A and B chains. A Chain having 306 amino acid residues were taken as protein for *in-silico* analysis. A total of 30 recorded antiviral agents from the literature quest were chosen to conduct molecular docking, screen and classify potent antiviral agents specifically for COVID-19. The structures of the ligands were depicted in Supplementary Table 1. Ligands from Pubchem were extracted and converted into PDB format by open babel. Molecular docking performs significant role in drug rational design by recognizing the essential interactions of amino acids between the protein selected and the produced low energy conformation ligands. Autodock 4.2 has been used for in-silico analysis. Using Autodock Methods, the position of the amino acids as active sites in the target where the ligand was docked is determined. Determination of the ligand's docking conformation was done by choosing the pose with highest affinity (most negative of the free energy Gibbs). Hydrophobic and hydrogen interaction between docked products were visualized by Discovery studio a commercial graphics visualization tool used to view and analyse protein ligand interaction predictions.

Adsorption, Distribution, Metabolism and Excretion was used to analyse the pharmacodynamics of the molecule. SWISS-ADME (http://www.swissadme.ch/) that enables the user to draw their possible ligand or drug molecule and it offers the parameter such as lipophilicity, water solubility, druglikeness rules. The prediction of compound toxicities is an important part for drug discovery. Toxicity analysis was carried out by using PROTOX (http://tox.charite.de/protox_II/) and pkCSM (http://biosig.unimelb.edu.au/pkcsm/prediction) webserver. It predict the degree of tolerability of the small molecule formerly being injected into the human and animal models. Various toxicology effects like AMES Toxicity, LD_{50} and maximum resistance dosage for humans, Hepatotoxicity etc. Target Prediction is useful for understanding the molecular mechanisms underlying a given phenotype or bioactivity, for rationalizing potential side effects, for predicting of target, and for determining the potential of compounds that are therapeutically important. Swiss target prediction (http://www.swisstargetprediction.ch/) is an online resource for predicting the macromolecular targets of bioactive small molecules (proteins from humans, cat, and rats) etc.

Out of 30 selected phytoligands, 24 phytoligands have higher binding affinity than standard Nelfinavir. The 2D interaction of all top 5 phytoligands and the standard Nelfinavir have been shown in Fig. 1a-f. Molecular docking study revealed that the phytochemical Fagaronine showed the highest binding energy of − 6.21 Kcal/Mol with single hydrogen bond with LYS positioned at 197 followed by Isoboldine with binding energy of − 5.99 Kcal/Mol. The binding energy, inhibition constant with LD_{50}values of top 5 phytoligands with M^{pro} have been shown in Table 1. The Sageone and Wogonin showed the three hydrogen bonds with binding energy of − 5.97 and − 5.62 Kcal/Mol respectively. Lycorine showed the five hydrogen bond interaction with binding energy of − 5.86 Kcal/Mol. The binding energy value of standard Nelfinavir was reported − 3.84 Kcal/Mol. Hydrophobic interaction plays important role in ligand and target binding. Supplementary Fig. 1 showed the hydrophobic graph of Fagaronine. The occurrence of discontinuous hydrophilic and hydrophobic waves in the graph of all proper distribution of hydrophilic and hydrophobic bonds in the protein provides an optimal conformation of side chains for a steady structure. The docking results showed the binding energy of Fagaronine was the highest but other four compounds also showed the good binding energy.

The online SWISSADME database was used for the ADME (Adsorption, Distribution, Metabolism and Excretion) analysis of the top five plant ligand. Table 2 demonstrates and explains the physiochemical properties such as number of high atoms, number of accepter of hydrogen bonding agents, number of donor of hydrogen bonding substances, and molar refractive effects as well as the topologic of the polar region (TPSA). It also described about the lipophilicity, gastrointestinal absorption, water solubility and druglikeness factors. All the five compounds follow Lipinski rule of drug likeness and with high gastrointestinal absorption.

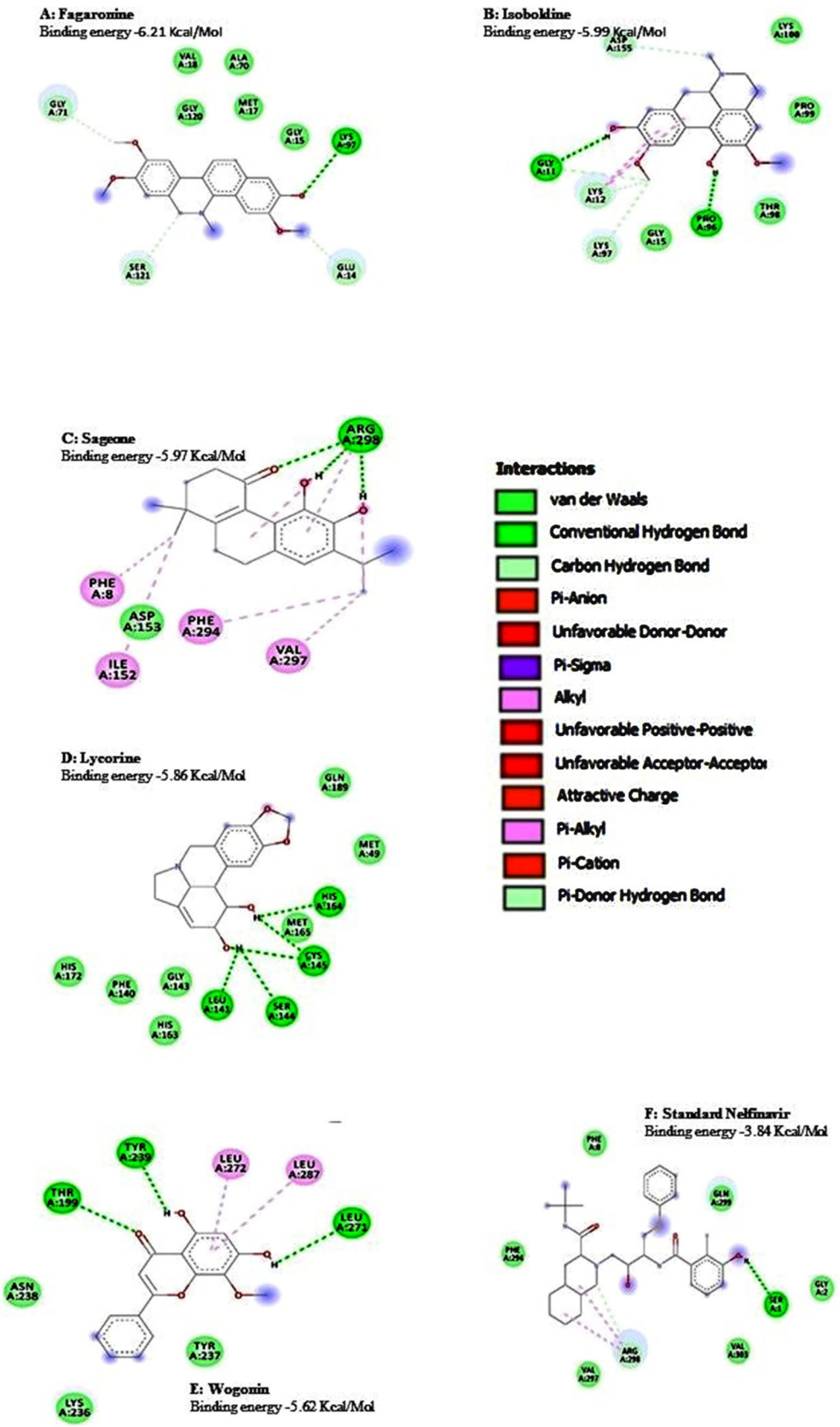

Fig. 1 Showing 2D image of top five phytoligand **A** Fagaronine, **B** Isoboldiene, **C** Sageone, **D** Lycorine, **E** Wogonin, **F** Nelfinavir

Table 1 Showing binding energy, inhibition constant with LD_{50} value of top 5 phytoligands with M^{pro}:

Compound	Binding Energy (Kcal/Mol)	Inhibition Constant (uM)	Hydrogen bond	Bond length (Å)	Amino acid involved
Fagaronine	− 6.21	27.85	1	3.13	LYS 197
Isoboldine	− 5.99	42.70	2	2.92, 2.00	GLY11, PRO 96
Sageone	− 5.97	42.10	3	2.85, 2.63, 2.17	ARG 298
Lycorine	− 5.86	50.68	5	2.54, 2.64, 3.27, 2.73, 1.94	HIS164, MET165, CYS145, SER144, LEU141
Wogonin	− 5.62	75.59	3	3.12, 2.32, 3.08	THR199, TYR239, LEU271

*LYS = lysine, GLY = glycine, PRO = proline, ARG = arginine, HIS = histidine, MET = methionine, CYS = cysteine, SER = serine, LEU = leucine, THR = threonine, TYR = tyrosine

Table 2 Showing ADME prediction values of top 5 phytoligands

Compound	HA	HBD	HBA	MR	TPSA(Å)	Ilogp	GI	Log S	Lipnski rule Follow
Fagaronine	26	1	4	104.05	51.80	− 0.55	High	− 5.09	Yes
Isoboldine	24	2	5	96.00	62.16	3.18	High	− 3.52	Yes
Sageone	22	2	3	89.49	57.53	1.78	High	− 4.15	Yes
Lycorine	21	2	5	78.40	62.16	2.06	High	− 1.82	Yes
Wogonin	21	2	5	78.46	79.90	2.55	High	− 4.23	Yes

*HA = No. of heavy atom, HBD = No. of hydrogen bond donor, HBA = No.of hydrogen bond acceptor, MR = Molar refractiveness, TPSA = Topological polar surface area, iLOGP = liphophilicity city, GI = Gastrointestinal absorption, Log S = Water solubility, Lipnski rule = Druglikeness factor

The toxicity predicted for these five phytoligands were displayed in Supplementary Table 2. These include the Ames toxicity, acute oral rat toxicity, chronic oral rat toxicity, hepatotoxicity, LD_{50} value. Fagaronine having 640 mg/kg value for LD_{50} and maximum tolerated dose in human is 0.479 (log mg/kg/day) with no hepatotoxicity. The potential sites of target to which the ligand binds were determined by the software on the basis of high target attraction towards the specific binding site. The target prediction analysis was done for these phytoligands from the online web server for top 5 predictions. Supplementary Fig. 2 showed the Fagaronine target prediction result as a pie chart which reflects that it has 8 % targets for protease enzyme.

COVID-19 M^{pro} is main chymotrypsin-like protease enzyme essential for corona virus life cycle was selected for docking study. In the study of molecular docking, the strong and stable interactions between ligands and the viral target molecule have been demonstrated by low binding energy and hydrogen bond. The analysis of the study showed the least binding energy in Fagaronine, Isoboldine, Sageone, Lycorine and Wogonin. Hence, these phytoligands have been described to display antiviral potential againstCOVID-19 M^{pro}. Fagaronine is an alkaloid which inhibits retrovirus reverse transcriptase activity isolated from *Fagara zanthoxyloides* Lam which belongs to family Rutaceae. Isoboldine is also an alkaloid which has been reported for antiviral activity against polio virus. Sageone is a constituent of *Salvia officinalis* commonly called as sage which is an aromatic herb used in traditional medicine to treat viral infections. Earlier study reported that it exhibit anti HIV-1 activity as it prevents the virus enter in target cell. The antiviral activity of *S. officinalis* is most probably mediated by safficinolide and sageone, two diterpenoids which are found in its aerial parts. Lycorine has good antiviral activity against many viruses like flaviviruses, bunyaviruses, alphavirus. It has activity against Zika virus by inhibiting RNA replication and protein synthesis [12, 13]. In recent years, Wogonin, (flavonoid) has drawn more and more scientific interest because of the strong anti-tumor activity of the flavonoid isolated from *Scutellaria baicalensis*. Wogonin was used for treatment of influenza A and B viruses [14].

In earlier studies it was concluded that the compounds present in citrus fruits like limes, lemons, oranges and grapefruit etc. showed the inhibition effect against COVID M^{pro}[15]. Hesperedin also showed the inhibition effect against SARS-CoV-2 target such as spike protein binding, ACE2 binding, SARS-COV-2 protease binding. Many reports on molecular docking between compounds from medicinal plants and $3CL^{pro}$ show negative binding energy with Catechin, Epicatechin-gallate, Theaflavin, 3,3′-di-*O*-gallate from green tea, Andrograpanin, Andrographiside present in Andrographis [12]. Rosmarinic acid is a primary

active component of rosemary. It is also found in other herbs such as peppermint, spearmint, thyme, sage and oregano show potential binding with $3CL^{pro}$ binding [14]. In another docking study the binding energies found from the docking of 6LU7 with inherent ligand, nelfinavir, lopinavir, curcumin, luteolin-7-glucoside, oleuropein, demethoxycurcumin, catechin, apigenin-7-glucoside and epicatechin-gallate appeared to have the best potential to act as COVID-19 M^{pro} inhibitors [15].

It is concluded that the natural products can be effective source for drug against COVID-19. Hence Fagaronine, Sageone, Lycorine and Wogonin may be used as potential antiviral drugs against COVID-19. The therapeutic applications of medicinal plants containing bioactive compounds must be explored in future pharmacological research.

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