Searching inhibitors for three important proteins of COVID-19 through molecular docking studies

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Abstract

After the first infected patient detected in Wuhan, China in December, 2019 with the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) virus, the COVID-19 (‘CO’ means corona, ‘VI’ is virus, ‘D’ is disease, ‘19’ is the year of discovery) disease spread globally due to its high contagious nature and became an ongoing pandemic. Also, the lack of vaccines and efficacious drugs to treat infected patients is a great problem to cope this pandemic. The approved drugs for similar health problems, reported potential drugs but not yet approved clinically or under clinical trial, and molecules from medicinal plants extracts are investigated randomly to deal with the COVID-19 infection. Molecular docking, one of the best approach to search therapeutically potent drugs/molecules in real time with possible hope to apply on COVID-19. In this communication, molecular docking studies of 18 ligands were carried out with the three important proteins of SARS-CoV-2, i.e., RNA-dependent RNA polymerase (RdRp), angiotensin-converting enzyme 2 (ACE2) and spike glycoprotein (SGp). From the obtained results, we observed that all the tested molecules showed better dock score in compared to the hydroxychloroquine claimed to be effective against COVID-19. Combining the dock score and other medicinal properties, we believe the limonin can be further explored for potential use against COVID-19.

Keywords: Coronavirus; COVID-19; Molecular docking; Limonin.
1. Introduction

The virus strain severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a single-stranded RNA virus caused deadly novel coronavirus disease (COVID-19), a respiratory malady with the common symptoms of shortness of breath, cough and fever [1-4]. This disease also reported to show other symptoms like muscle aches, loss of smell, fatigue and abdominal pain. The SARS-CoV-2 virus attacks the T lymphocytes of a lung that destroy the protecting proteins and builds up unwanted fluids within the lung causing difficulty in breathing [5]. After the first infected patient detected in Wuhan, China in December, 2019 with COVID-19, the virus spread globally due to its high contagious nature and became an ongoing pandemic. The virus generally spread from the infected person through close contact along with the droplets spilled during talking, coughing and sneezing [6]. After infection with the virus, the symptoms likely to appear within two to fourteen days that depends on the age of person and weak immunity due to other illness like diabetes, asthma, heart ailment etc. [7]. The outbreak of SARS-CoV-2 was declared as a public health emergency of international concern (PHEIC) and a pandemic respectively on 30\textsuperscript{th} Jan 2020 and 11\textsuperscript{th} March 2020 by World Health Organization (WHO) [8]. The confirmed COVID-19 cases by 15\textsuperscript{th} April 2020 reported to 1914916 across 210 countries and territories with a total deaths of 123,010 [9].

The non-availability of recommended drugs or vaccines to deal with the COVID-19 along with the quick human to human transmission is the main cause of this pandemic. Therefore, at present scenario, efforts have been made to identify the infected persons through rapid diagnosis followed by quarantine them to stop the further spread of this disease. Also, other recommended steps such as using masks, washing hands with soap and maintaining social distancing are suggested to control the spread of this virus. Simultaneously, the approved drugs for similar health problems, reported potential drugs but not yet approved clinically or under clinical trial, and molecules from medicinal plants extracts are investigated randomly to deal
with the COVID-19 infection. In searching drugs/molecules from a library that contained in lakhs, the computational approaches like molecular docking, screening, simulations etc. can save money and expedite the research on drug discovery for COVID-19 [10]. In this work, we have performed molecular docking studies of total 18 molecules with the three important proteins of SARS-CoV-2. The main drug hydroxychloroquine studied here is claimed effective against the SARS-CoV-2 [11] and therefore, its dock score compared with the other tested molecules. From the obtained results, we observed that all the tested molecules showed better dock score in compared to the hydroxychloroquine and overall, the molecule from limonoid family (i.e. limonin) can be further explored for potential use against COVID-19.

2. Experimental

2.1. Preparation ligands and protein structures for docking

To study the protein-ligand interactions, we retrieved ligands in ‘.sdf’ format directly the PubChem (National Library of Medicine) [12] and converted in to ‘.pdb’ format using the Gaussian tool. The crystallographic 3D protein structures of SARS-CoV-2 RdRp (RNA-dependent RNA polymerase) in complex with cofactors and PDB ID: 6M71, spike glycoprotein with PDB ID: 2GHV, Angiotensin Converting Enzyme 2 (ACE2) with PDB ID: 6M1D were collected from RSCB protein data bank (http://www.rscb.org). The 3D protein structures were analysed for torsion angles and missing residues using Biovia Drug Discovery Tool for plotting Ramchandram plot. Protein-ligand preparation was done using Autodock 4.2 Software for removing water molecules, added hydrogen’s, confirming torsion angles and added Kollman charges [13].

2.2. Procedure for molecular docking

All molecular docking were done by using Autodock 4.2 tools to predict protein-ligand interaction and active sites. We generated grid files to localise the binding positions for random docking. We had applied Lamarckian Genetic Algorithm (LA) protocol to perform molecular
docking. The polar contacts, Van der Waals forces and optimal protein-ligand docking site confirmations were calculated and predicted using autodock computational method. The output files generated were converted to protein-ligand complex as .pdb file and screened using BioVia Discovery Studio Software for better visualization.

3. Results and discussion

3.1. Target proteins and ligands for docking studies

SARS-CoV-2 is a single-stranded virus which is pleomorphic i.e., spherical in shape with enveloped RNA covered with club-shaped glycoprotein with bulbous surface projections. The virus is about 60-140 nm in diameter with spike-shaped proteins about 9-12 nm [14]. The internal envelope of the virus consists of a lipid bilayer on which membrane, envelope and spiked structural proteins are anchored. In the Fig. 1 showing ACE2 and TMPRSS2 on host cellular membrane interact with viral receptor spike glycoproteins containing docking site and activator sites that plays a crucial role in entering into the host cells. The blockage of these receptors could stop the entry of virus into the host cells. RdRp is a RNA replication tool which plays crucial role in multiplying RNA for viral proliferation. So, the proteins RdRp, ACE2, and spike glycoprotein were chosen as therapeutic targets to search inhibitors for COVID-19.

![Fig. 1. SARS-CoV-2 initial interactions with host cell receptors ACE2 and TMPRSS2.](image)
To search potential inhibitors for COVID-19, total 18 molecules were selected for molecular docking studies with the proteins RdRp, ACE2, and spike glycoprotein (Table 1). We had mainly screened phytochemicals that showed some biological activities, and the results were compared with the hydroxychloroquine and paracetamol pretended to be a temporary therapeutic targets. We have selected the phytochemicals by considering their biological activities like antiviral, anticancer, antioxidant agent, anti-inflammatory agent, anti-dengue, antibacterial and antimicrobial. The 2D structures of some of the important ligands that showed good dock score including hydroxychloroquine are shown in chart 1.

![Chart 1. Structure of the ligands (a. Limonin, b. Ellagic acid, c. Baicalin, d. Scopadulcic Acid, e. Nimbolide) that showed high docking score with the three proteins of COVID-19 and (f) hydroxychloroquine claimed to be effective for COVID-19.](image)

| PubMed ID | Ligands     | Mol. Wt. (g/mol) | Target-1 (ACE2) | Target-2 (RdRp) | Target-3 (SGp) |
|-----------|-------------|------------------|-----------------|-----------------|----------------|
| 179651    | Limonin     | 470.5            | -8.9            | -9.0            | -8.4           |

Table 1. Comparative dock score of the ligands on COVID-19 enzymes.
|     | Compound                     | Mass   | LogP   | LogD   | LogS   |
|-----|------------------------------|--------|--------|--------|--------|
| 5281855 | Ellagic acid                | 302.19 | -8.4   | -8.1   | -7.5   |
| 64982  | Baicalin                    | 446.4  | -8.3   | -8.1   | -7.6   |
| 11729855 | Scopadulcic Acid     | 438.6  | -8.2   | -8.6   | -8.8   |
| 12313376 | Nimbolide               | 466.5  | -8.0   | -7.6   | -7.9   |
| 22215841 | Dammarenolic acid    | 458.7  | -7.9   | -7.2   | -6.7   |
| 5280343 | Quercetin                  | 302.23 | -7.9   | -7.3   | -7.1   |
| 1742129 | Tocopherol                 | 430.7  | -7.8   | -5.5   | -6.0   |
| 122685 | 1,5-Dicaffeoylquinic acid  | 516.4  | -7.6   | -6.9   | -7.0   |
| 5280863 | Kaempferol                 | 286.24 | -7.6   | -7.4   | -7.2   |
| 5322078 | 5,7,4'-Trihydroxy-8-methoxy flavone | 302.26 | -7.4   | -7.1   | 7.0     |
| 638024  | Piperine                    | 285.34 | -7.1   | -7.4   | -7.2   |
| 637760  | Chalcone                   | 208.25 | -6.9   | -6.6   | -6.1   |
| 445354  | Retinol                    | 286.5  | -6.6   | -6.8   | -7.2   |
| 1983    | Acetaminophen(Paracetmol)  | 151.16 | -6.5   | -7.8   | -8.2   |
| 68077   | Tangeretin                 | 372.4  | -6.4   | -6.9   | -6.4   |
| 370     | Gallic Acid                | 170.12 | -6.4   | -5.9   | -5.7   |
| 3652    | Hydroxychloroquine         | 335.9  | -6.2   | -6.0   | -5.8   |
3.2. Docking results

The molecular docking results showed best dock score with limonin with all three proteins (Table 1), which is a good indication to propose limonin for further investigation in developing inhibitors against COVID-19. Limonin is the first isolated tetranortriterpenoid that cause bitterness in citrus and one of the limonoids known for a wide range of biological activities like antimalarial, anticancer, antiviral, antibacterial, antifungal along with other pharmacological activities on humans [15].

The RdRp is a catalytic RNA containing hydrophobic cavities at active N-terminal and C-terminals involved in catalysis of RNA replication. The docking of RdRp with the tested 18 molecules gave best dock score with limonin. Limonin docked with RdRp at β-sheet ChainA-NSP12 RNA polymerase residues (Fig. 2). The docked structure showed limonin formed different non-covalent interactions like alkyl and π-alkyl interactions with the amino acids Pro323, Pro322, Phe321, Pro461 at the active sites of NSP12, Van der Waals interactions to Arg349, Phe396, Cys395, Trp268, Thr252, Arg553, Tyr619, Gly616, Asp618 and conventional hydrogen bonds to Ser255, Trp617, Trp800. These bonded amino acids residues showed a perfect hydrophobic cavity to encapsulate limonin, and may be the inhibitory site of NSP12. Limonin binding with other confirmations predicted at chain B-NSP7 part of NSP8 RNA polymerase showing alkyl and π-alkyl interaction to Leu122, Ala150, Leu122, π-sigma interaction to Tyr149 and conventional hydrogen bonds to chainA NSP12 at Lys267, Trp268. All these non-covalent interactions between limonin and RdRp resulted in the minimum binding energy -9.0 kcal/mol upon docking studies and therefore, this molecule can be studied further to inhibit the functions of RdRp.
Fig. 2. (a) 2D animated pose showing non-covalent interactions between limonin and RdRp, (b) 3D representation showing the position of limonin within the cavity of RdRp and (c) the Ramachandran plot of corresponding docked ligand-protein complex. Ramachandran plot showing RdRb protein containing non-glycine and non-proline residues are 980 and the total number of residues are 1077. The most favoured regions are 856 residues, additionally allowed regions are 123 residues and disallowed regions are none. The Average G-factor score of RdRp (6M71.pdb) is 0.13.

The homo sapiens angiotensin-converting enzyme 2 (ACE2) is a membrane protein that facilitates the binding of chainB domain with spike glycoprotein. Limonin shows minimum binding energy -8.9 kcal/mol by actively targeting arginin and histidin residues at active site, which may involve in reduction of catalysis. It is binding to chainB residues such as Arg514, Tyr196 via conventional hydrogen bonds (Fig. 3). The non-covalent interactions are abundant due to multiple rings with oxygen-atoms in limonin showing π-alkyl interactions with Trp203 and Van der Waals interactions with Glu398, Ser511, Asp206, Gln102, Gly205, Tyr202,
Tyr199, Lys187, Asp509, Tyr510. These docking results predicts the hydrophobic interactions in the active site of ACE2 scoring minimum binding energy. ACE2 is responsible for differing substrate and the inhibitor sensitivities by binding to specific cavities especially chainB: His195, Asn103, Asn194, Gln98, Asn210, Ser105, Ser106, Lys74, π-alkyl interactions to Gln102, Leu73, Leu100 and Van der Waals sites are Tyr196, Gln102, Glu101, Gln81, Glu208 residues can effectively reduce the binding capabilities to parasite and acts as a potent antioxidant compound having inhibitory functions.

\[\text{(a)}\] 2D animated pose showing non-covalent interactions between luminon and ACE2, \[\text{(b)}\] 3D representation showing the position of limonin within the cavity of ACE2 and \[\text{(c)}\] the Ramachandran plot of corresponding docked ligand-protein complex. Ramchandram plot showing ACE2 protein containing non-glycine and non-proline residues are 314 and the total number of residues are 366. The most favoured regions are 276 residues, additionally allowed regions are 35 residues and disallowed regions are none. The Average G-factor score of ACE2 (2GHV.pdb) is -0.04.
The spike glycoproteins (SGp) having multiple chains in which we focused on a specific chain played a vital role in binding with host ACE2 receptor and bind to TMPRSS2 receptor to activate the virus-host interactions showing in Fig. 1. Spike protein1, this helps in attaching the virus to the cell membrane of the human receptor ACE2 and CLEC4M/DC-SIGNR. This also internalizes the virus into the endosomes where the conformational changes take place in the spike glycoprotein. Limonin showing good interactions to SGp chainE residues with \( \pi \)-alkyl interactions to Val394, Arg395, chainC: pro399, Tyr367. There are conventional hydrogen bond interactions to chainE: Thr363, Ser358, Lys365, chainC: Gln401, and Van der Waals interactions with chainE: Tyr494, Gly391, Ile489, Ser362, chainC: Ala398 (Fig. 4). These interactions may collectively inhibit the functions of spike1. By achieving the blockage of spike1 functional protein will suppress the activity of binding to host. These binding sites having hydrophobic surfaces including chainE: Gly368, Tyr494, Asn424, Thr359, Trp423, Ser358, Phe360, Tyr367, Phe361, Van der Waals interactions, the minimum binding energy -8.4 kcal/mol indicates the limonin plays a crucial role in inhibiting the spike glycoproteins.
Fig. 4. (a) 2D animated pose showing non-covalent interactions between luminon and SGp, (b) 3D representation showing the position of limonin within the cavity of SGp and (c) the Ramachandran plot of corresponding docked ligand-protein complex. Ramchandran plot showing SGp protein containing non-glycine and non-proline residues are 2400 and the total number of residues are 2706. The most favoured regions are 1975 residues, additionally allowed regions are 410 residues and disallowed regions are 5. The Average G-factor score of SGp (6M1D.pdb) is -0.17.

4. Conclusions

In summary, we have performed the molecular docking studies of phytochemicals chosen randomly with three important proteins (RdRp, ACE2, and spike glycoprotein) and compared the dock score results with the hydroxychloroquine. Our results revealed that all the tested phytochemicals showed higher dock score than the hydroxychloroquine, with the maximum dock score shown by the limonin. Limonin, the first isolated limonoid found in citrus fruits with bitter taste is known to inhibit the replication of retroviruses like HTLV-I and HIV-
1. Other important limonoid like azadirachtin is found in neem leaves and seeds. In Traditional Indian medicine, neem leaves are largely used for various medications (exceptionally some people shows allergy due to the bitter taste). Many other limonoids are also known for their potential biological activities. Therefore, with the combined docking results and the medicinal importance of limonin, we propose that the limonin and other limonoids can be studied further with the hope to get suitable inhibitors against the COVID-19.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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