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# nCOV-19 peptides mass fingerprinting identification, binding, and blocking of inhibitors flavonoids and anthraquinone of *Moringa oleifera* and hydroxychloroquine

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**ABSTRACT**

An rare pandemic of viral pneumonia occurs in December 2019 in Wuhan, China, which is now recognized internationally as Corona Virus Disease 2019 (COVID-19), the etiological agent classified as Severe Acute Respiratory Syndrome Corona Virus 2 (SARS-CoV-2). According to the World Health Organization (WHO), it has so far expanded to more than 213 countries/territories worldwide. Our study aims to find the viral peptides of SARS-COV-2 by peptide mass fingerprinting (PMF) in order to predict its novel structure and find an inhibitor for each viral peptide. For this reason, we calculated the mass of amino acid sequences translated from the SARS-CoV2 whole genome and identify the peptides that may be a target for inhibition. Molecular peptide docking with *Moringa oleifera,* phytochemicals (aqueous and ethanolic) leaf extracts of flavonoids (3.56 ± 0.03), (3.83 ± 0.02), anthraquinone (11.68 ± 0.04), (10.86 ± 0.06) and hydroxychloroquine present therapy of COVID-19 in Pakistan for comparative study. Results indicate that 15 peptides of SARS-CoV2 have been identified from PMF, which is then used as a selective inhibitor. The maximum energy obtained from AutoDock Vina for hydroxychloroquine is −5.1 kcal/mol, kaempferol (flavonoid) is −6.2 kcal/mol, and for anthraquinone −6 kcal/mol. Visualization of docking complex, important effects are observed regarding the binding of peptides to drug compounds. In conclusion, it is proposed that these compounds are effective antiviral agents against COVID-19 and can be used in clinical trials.

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SARS-CoV-2; COVID-19; peptide mass fingerprinting; hydroxychloroquine; flavonoids; anthraquinone; AutoDock Vina

## 1. Introduction

Identified cases of trigger pneumonia were recorded in Wuhan City by the Health Commission of Hubei province, China, in December 2019, having clinical manifestation with viral pneumonia.(Chan et al., 2020; Chen et al., 2020; Kumar, 2020; Lu et al., 2020; Tahir Ul Qamar et al., 2019; Umesh et al., 2020). Initially, 27 patients were registered, and the number increased to 41 on 11 January 2020 (Chan et al., 2020). Finally, World Health Organization (WHO) declared it as Corona Viruses Disease 2019 (COVID-19). As of April 21, 2020 about 250,000 Laboratory confirmed cases have been identified worldwide. Of these, 170,000 died (Fahmi, 2020). As data compiled by the WHO, some of the worst-affected nations such as China in the western Pacific, have 84,237 confirmed cases with 4,642 deaths. The situation in the European region worsens with 195,944 cases in Spain, 1,78,972 in Italy, and 120,071 confirmed cases in the United Kingdom. The United States of America has already been seriously hit by 7,23,605 confirmed cases with high mortality rates. In addition, many developing countries are struggling with this health issue. Specifically, In Pakistan, 8,418 cases and 176 deaths have been confirmed (Culp, 2020). Next-Generation Sequencing detected the novel human viral infection that shows it is somehow similar to Severe Acute Respiratory Syndrome Corona Virus (SARS-CoV) that affects the Chinese horseshoe bat, which is a novel strain not previously reported in humans (Chan et al., 2020; Fahmi, 2020). The pathogen was identified as a novel enveloped RNA subtype of Betacoronavirus (Kumar, 2020) and referred to as SARS-CoV-2 (Severe Acute Respiratory Syndrome Corona Virus 2) by the International Committee on Virus Taxonomy (ICVT) based on Phylogenetic similarities (Al-Khafaji et al., 2020;

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Islam et al., 2020; Tahir Ul Qamar et al., 2019; Wahedi et al., 2020). This renders 74.5% of the genome equivalent to SARS-CoV (Huang et al., 2020).

The third most virulent disease of the 21st century triggered by the MERS-COV and SARS-COV has a significant fatality rate (Khan, Zia, et al., 2020). Coronavirus is a nonsegmented, enveloped, positive-stranded RNA virus with an RNA genome that ranging 26 to 32 kb in length (Chen et al., 2020; Sinha et al., 2020; Wu et al., 2020). The coronae family has four main genetic subgroups, namely alpha coronavirus genus, beta coronavirus genus, delta coronavirus genus, and gamma coronavirus genus. There are several types of human coronavirus, particularly, HCoV-HKU1, HCoV-229E, HCoV-NL63, and HCoV-OC43 which flow into the human body and induce lenient respiratory infection (Enayatkhani et al., 2020). Since in the epidemic of SARS-COV in 2002 MERS-CoV in 2012 identified extremely pathogenic properties of human coronavirus causing extreme respiratory syndrome accumulating 10,000 cases over the last two decades with a 10% death rate for SARS-CoV and 37% for MERS-CoV (Elfiky & Azzam, 2020). SAR-COV-2 is spread via the respiratory tract and therefore causes pneumonia; thus, a molecular diagnosis with oral swabs had been provided for the approval of this disease. It may also be spread by infected intestines via oral fecal route (Khan, Jha, et al., 2020; Zhang et al., 2020).

The Structure of SARS-CoV-2 consists of three major viral proteins namely Spike protein (S) that causes viral infection by binding to the host receptor. A Membrane protein (M) and envelope protein (E) (Abdelli et al., 2020; Boopathi et al., 2020; Sarma et al., 2020; Wahedi et al., 2020).

ACE2 is the essential receptor that interacts with spike protein on the host cell membrane (Hasan et al., 2020). The common signs and symptoms of coronavirus include, fever, trouble coughing, difficulty in breathing, pyrexia, potentially lethal pneumonia, more severe types of kidney failure, fatigue, diarrhea, rhinorrhea, vomiting, and nausea. Senior citizens are prone to be infected by this disease (Chen et al., 2020; Gyebi et al., 2020; Kumar et al., 2020; Lobo-galo et al., 2020).

In this study, the peptide mass fingerprinting (PMF) technique was conducted on SARS-CoV-2. Peptide mass fingerprinting is one of the significant methods used to identify peptides, especially through the deployment of molecular masses of peptides. PMF is chosen on the basis of simple and most effective process for the detection of peptides with specific masses and is one of the key technologies driving the growth of proteomics (Henzel et al., 2003). In this technique, first, the protein of interest is digested into small fragments called peptide with the aid of endonucleases typically trypsin owing to its satisfactory and magnificent characteristics (Matthiesen, 2013). Second, the mass spectroscopy analysis is performed to test the precise mass of the peptides, and this step gives the catalog of the peak inventory of such identified peptides. Third, this number of peak list associated with the abstract number of peaks obtained by *in-silico* digestion using various databases. Then the computer software identifies the protein by creating the leading match for it. One of the key advantages of the PMF is that it does not rely on protein sequencing for protein identification, and is very simple to do (Liebler, 2016).

Medicinal plants are commonly used for the treatment of bacterial and noninfectious diseases and disorders. Emerging infectious diseases present a relentless danger to human life (Aanouz et al., 2020; Shinwari et al., 2020). So, we selected a medicinal plant and a drug to check its activity against COVID-19. The medicinal plant, *Moringa oleifera* is extremely nutritional and serves as an antiretroviral lead molecule to improve the activity of immune-system with antipyretic, antiulcer, antispasmodic, antiulcer, antibacterial, antioxidant, antihypertensive, cardiac, and circulatory analeptic properties. *Moringa oleifera* is often known to be a "Miracle Tree" because of its strong immune-boosting and antimicrobial characteristics categorize in the family of *Moringacae* (Busani et al., 2011; Kini et al., 2017). By phytochemical screening and extraction of *M. oleifera* shows the presence of alkaloids, saponins, tannins, glycosides, carbohydrates, flavonoids, resins, acidic compounds and proteins in various parts of plant in specific, seeds, leaves, fruits, flowers, barks, and unripe pods (Kini et al., 2017; Shinwari et al., 2020). Flavonoids often have an inhibitory effect against viruses, especially a respiratory syncytial virus (Das et al., 2020). Many of the works reveals that *M. oleifera* derived gist inhibits initiation of the viral replication cycle (Shinwari et al., 2020). The presence of phytochemical Anthraquinone in *Moringa oleifera* extract serves as an antiviral and antifungal agent to rehabilitate minor diseases found in many plants (Kasolo et al., 2010). This compound exhibits the antiviral properties towards the human cytomegalovirus HCMV strain and displays the inhibitory effects against the polioviruses. It also indicate a strong degree of antiviral activity towards HIV-1 (Barnard et al., 1992; Schinazi et al., 1990; Semple et al., 2001). In *Moringa oleifera*, both the ethanolic and aqueous extracts are rich in flavonoids ($3.83 \pm 0.02$), ($3.56 \pm 0.03$), and anthraquinone ($10.86 \pm 0.06$), ($11.68 \pm 0.04$) respectively (Nkechinyere Onyekwere & Felix, 2014). *Moringa oleifera* is chosen due to these essential antiviral components which have demonstrated significant antiviral activity against viral diseases. Though the hydroxychloroquine HCQ a derivative of Chloroquine prescribed for the treatment of autoimmune diseases and anti-inflammatory agents is virulent than chloroquine. HCQ is also included in the triallist to treat COVID-19 (Liu et al., 2020; Wang et al., 2020). There is theoretical, experimental, preclinical, and clinical evidence of the effectiveness of hydroxychloroquine in patients affected with COVID-19. The hydroxychloroquine works by suppressing lysosomes and reducing various cell functions of immune cells that's why it is used in rheumatologic conditions and its anti-inflammatory effects. Many *in vitro* studies confirm that hydroxychloroquine is more potent in inhibiting SARS CoV-2 (Schrezenmeier & Dörner, 2020; Yao et al., 2020).

In computer-aided drug designing and structural molecular biology, a key tool in molecular docking. It aims to identify the major binding modes of a ligand. Docking is important for broader libraries of compounds for virtual screening (Barnard et al., 1992; Elmezayen et al., 2020; Muralidharan et al., 2020). AutoDock Vina is an effective,

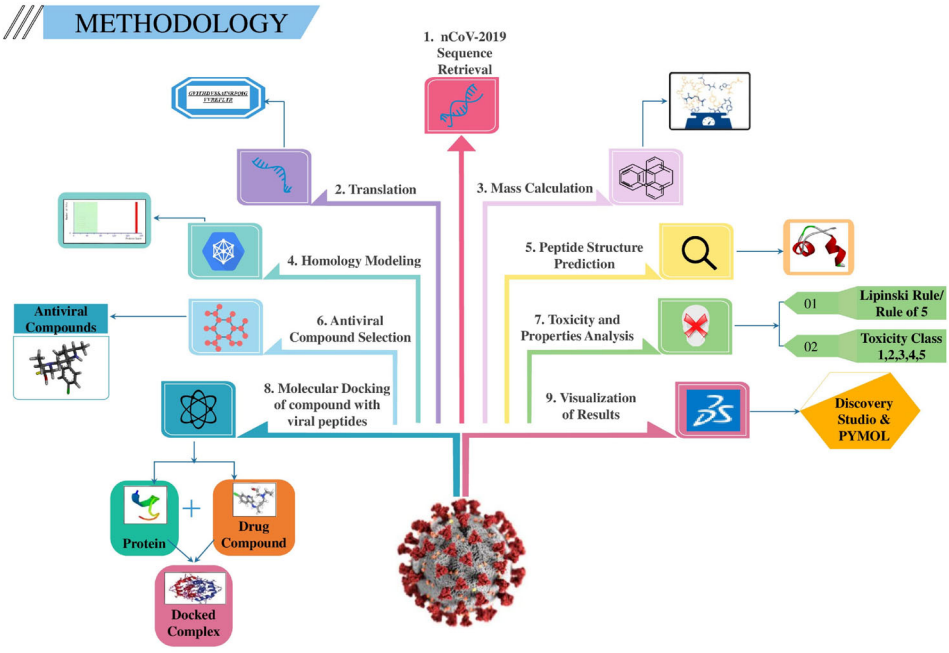



Figure 1. The schematic representation of nCOV-2019 peptide identification, characterization, and molecular docking analysis.

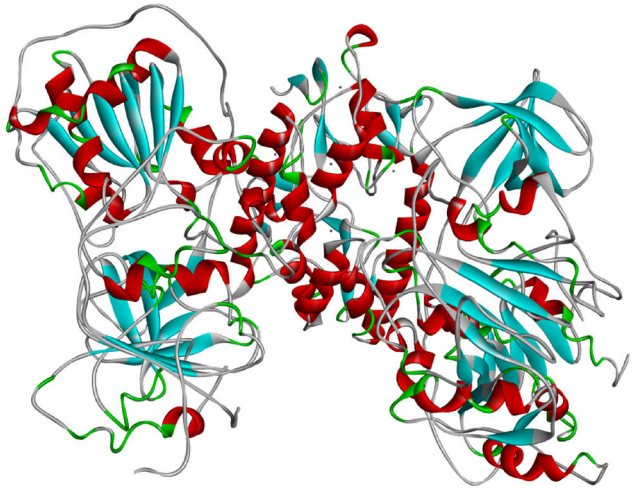


Figure 2. Structure of SARS-CoV-2 translated form nCOV-2019 whole-genome sequence.

readily available molecular docking technique that is recognized for its successful usage in research analysis and drug discovery (Forli et al., 2016). It is considered as a standardized system that uses a highly structured method from its local optimization from its local optimization mechanism with a single assessment (Trott and Olson, 2010). It acquires systematize files for ligand and receptor, then test and offers optimal dock conformities with well-tested default procedures. This is commonly used in various aspects, primarily virtual screening (Forli et al., 2016).

In our study, by using the mass fingerprinting technique, we use *Moringa oleifera* as an effective inhibitor against nCOV-2019. The extract of this medicinal plant will improve the immune system to produce antibodies against SARS-COV-2 (Figure 1).

## 2. Material and methods

### 2.1. Retrieval of nCoV-19 genome

The whole genome sequence of novel coronavirus was obtained through NCBI (https://www.ncbi.nlm.nih.gov/) with accession no: MN908947.3, 29,903bp in length and described as severe acute respiratory syndrome coronavirus 2 originated from Wuhan-Hu-1 on 18 March 2020 (Kruse, 2020).

### 2.2. Translation of nCOV-2019 sequence

The obtained sequence of SARS-COV-2 was translated into protein for the identification of peptides. The translation was done by EMBL EMBOSS TRANSEQ (http://www.ebi.ac.uk). It translates the nucleotide sequence into an amino acid sequence. It gives three forward and three reverse frames. This may also result in multiple outputs at once (Rice et al., 2000).

### 2.3. In-silico protein digestion and mass calculation

The protein sequence was digested by Protein Prospector (Baker et al., 2008), and the mass of peptide was calculated from Online Bioinformatics Tool PeptideMass (http://web.expasy.org/peptide_mass/). The purpose of PeptideMass tool is to help peptide-mapping experiments, culminating in the analysis of peptide-mass fingerprinting (PMF) and mass-spectrometry data (Wilkins et al., 1999). Trypsin was used to cut the protein sequence in order to estimate the number of peptide masses and mass to charge ratio of protein (Gundry et al., 2010). The peak list of peptides was generated by mass to charge ratio of protein from protein mass calculations.

Table 1. Peptide mass server calculation (average mass of protein/peptide, theoretical pI) total coverage of sequence, missed cleavages (MC), and selected enzyme to cleave the sequence.

| Protein mass | Peptide masses range | pI | Total coverage | Missed cleavages (MC) | Enzyme |
|---|---|---|---|---|---|
| 1078869.63 | 13822.9338 to 500.6616 | 9.00 | 99.7% | 1 | Trypsin |

Table 2. Identified matched protein from the MASCOT server along with their accession no, masses, description, *p*-value, and their respective scores.

| S No | Accession no. | Mass | Description | Threshold *p* | Score |
|---|---|---|---|---|---|
| 1 | NP_828870.1 | 66,868 | nsp13-pp1ab (ZD, NTPase/HEL) [Severe acute respiratory syndrome-related coronavirus] | <0.05 | 93 |
| 2 | APP13440.1 | 304,265 | nonstructural polyprotein, partial [SARS coronavirus Urbani] | <0.05 | 64 |
| 3 | AAQ13948.1 | 20,879 | small terminase subunit [Pseudomonas phage B3] | <0.05 | 22 |

Table 3. Identified peptides description from the MASCOT database, accession no, protein name, expected vs. calculated masses, calculated pI values of proteins/matched peptides.

| S No | Accession no | Protein name | Mr (expt) | Mr (calc) | pI (calc) | Matched peptides |
|---|---|---|---|---|---|---|
| 1 | NP_828870.1 | nsp13-pp1ab | 1392.5673 | 1392.5598 | 8.66 | CCYDHVISTSHK |
| 2 | NP_828870.1 | nsp13-pp1ab | 4011.3786 | 4011.3431 | 8.66 | NTCVGSDNVTDFNAIATCDWTNAGDYILANTCTERLK |
| 3 | NP_828870.1 | nsp13-pp1ab | 1698.9337 | 1698.9102 | 8.66 | LFAAETLKATEETFK |
| 4 | NP_828870.1 | nsp13-pp1ab | 3029.4540 | 3029.4119 | 8.66 | ELHLSWEVGKPRPPLNRNYVFTGYR |
| 5 | NP_828870.1 | nsp13-pp1ab | 2309.5172 | 2309.4856 | 8.66 | VQIGEYTFEKGDYGDAVVYR |
| 6 | NP_828870.1 | nsp13-pp1ab | 2853.2298 | 2853.1901 | 8.66 | YSTLQGPPGTGKSHFAIGLALYYPSAR |
| 7 | NP_828870.1 | nsp13-pp1ab | 3441.9370 | 3441.9306 | 8.66 | SHFAIGLALYYPSARIVYTACSHAAVDALCEK |
| 8 | NP_828870.1 | nsp13-pp1ab | 1794.0707 | 1794.0575 | 8.66 | IVYTACSHAAVDALCEK |
| 9 | NP_828870.1 | nsp13-pp1ab | 4754.3148 | 4754.2620 | 8.66 | VNSTLEQYVFCTVNALPETTADIVVFDEISMATNYDLSVVNAR |
| 10 | APP13440.1 | nonstructural polyprotein | 2253.6293 | 2253.5977 | 6.70 | HYVYIGDPAQLPAPRTLLTK |
| 11 | APP13440.1 | nonstructural polyprotein | 2765.1680 | 2765.1316 | 6.70 | GVITHDVSSAINRPQIGVVREFLTR |
| 12 | APP13440.1 | nonstructural polyprotein | 1289.4594 | 1289.4416 | 6.70 | EFLTRNPAWR |
| 13 | APP13440.1 | nonstructural polyprotein | 5654.1746 | 5654.1057 | 6.70 | AVFISPYNSQNAVASKILGLPTQTVDSSQGSEYDYVIFTQTTETAHSCNVNR |
| 14 | APP13440.1 | nonstructural polyprotein | 3976.2973 | 3976.2512 | 6.70 | ILGLPTQTVDSSQGSEYDYVIFTQTTETAHSCNVNR |
| 15 | APP13440.1 | nonstructural polyprotein | 1838.0905 | 1838.0652 | 6.70 | DLYDKLQFTSLEIPR |

### 2.4. Homology modeling of active peptides

The homology modeling of the identified peak list was conducted by the Mascot server (Tsugita et al., 2000). This server determines the active peptides by comparing the calculated peak lists with already existing masses of peptides in the database, resulting in the active peptides with additional information.

### 2.5. De novo peptide structure prediction

The peptides structures were predicted through the PEP-FOLD3 server, which is a *de novo* approach for peptide prediction. It predicts the structure of peptides having a sequence between 5 and 50 amino acids. PEP-FOLD3 is quicker and gives results in a few minutes (Lamiable et al., 2016).

### 2.6. Binding of active peptides with antiviral compounds

The selected active peptides of SARS-COV2 were docked with selected three antiviral activity containing compounds, to check their efficacy against SARS-CoV-2. Flavonoids and Anthraquinone are the phytochemical extracts of *Moringa oleifera,* which contain antiviral properties (Shinwari et al., 2020), and hydroxychloroquine which is the derivative of chloroquine and is used to treat autoimmune diseases (Liu et al., 2020). The compounds were tested for Lipinski rule of 5 by ACD/I Lab, while their toxicity was checked by the protox server (Drwal et al., 2014; Masunov, 2001).

### 2.7. Analysis and visualization of docked complexes

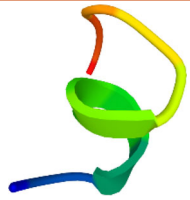
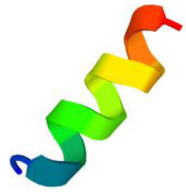
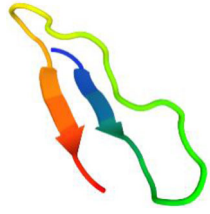
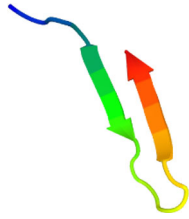
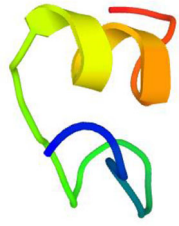
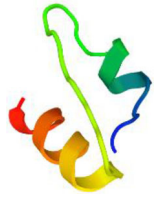
Resultant Docked Complexes were analyzed and visualized through Pymol and BIOVIA Discovery Studio 2016 to check and compare the accurate binding of different antiviral compounds that provide a three-dimensional (3D) platform for the visualization of the results. Such technologies are developed to facilitate the study and simulation of the docked complexes in drug designing phase (Gusman & Shoemake, 2017; Yuan et al., 2017).

## 3. Results and discussion

The complete genome of SARS-CoV-2 was downloaded up to 30 kb in size from NCBI. The length of the sequence was enough, so we do not need to split the sequence into fragments. As for peptide mass fingerprinting, this nucleotides sequence was then forwarded for further analysis. SARS-CoV-2 sequence was translated into an amino acid sequence to accomplish the targeted goal and calculate its peptide masses. We use the EMBOSS TRANSEQ server to translate the nCOV-2019 genome sequence. For every possible outcome, we selected the whole amino acid sequence to calculate the peptide masses. The protein structure of the translated amino acid sequence is shown in Figure 2.

The *in silico* based peptide mass calculation produced up to 4–5 decimals masses of peptides for all amino acids sequences based on enzyme digestion. Average monoisotopic and isotopic mass values were also given for these modifications. Peptide Mass online server is used to identify peptide masses. We have digested the amino acid sequence of SARS-CoV2 *in silico* with trypsin and selected some online

Table 4. PEP-FOLD 3 server structures prediction of peptides with protein name, missed cleavages, and amino acid sequence of the respective structures.

| S. No | Protein name | Missed cleavage | Peptides | Peptide structures |
|---|---|---|---|---|
| 1 | nsp13-pp1ab | 0 | CCYDHVISTSHK |  |
| 2 | nsp13-pp1ab | 1 | NTCVGSDNVTDFNAIATCDWTNAGDYILANTCTERLK |  |
| 3 | nsp13-pp1ab | 1 | LFAAETLKATEETFK |  |
| 4 | nsp13-pp1ab | 1 | ELHLSWEVGKPRPPLNRNYVFTGYR |  |
| 5 | nsp13-pp1ab | 1 | VQIGEYTFEKGDYGDAVVYR |  |
| 6 | nsp13-pp1ab | 1 | YSTLQGPPGTGKSHFAIGLALYYPSAR |  |
| 7 | nsp13-pp1ab | 1 | SHFAIGLALYYPSARIVYTACSHAAVDALCEK |  |
| 8 | nsp13-pp1ab | 0 | IVYTACSHAAVDALCEK | N/A |

(*continued*)

parameters, a threshold value as the resultant peptide must be greater than 500 Daltons, peptides below this threshold may be too small to visualize in a mass spectrum, and we also allow 1 missed cleavage and then generated the masses of peptides. Peptide Mass also results in masses values of concerned protein and theoretical points. Protein Prospector was also used for cross validation. In the results, 99.7% of the sequence was covered. The estimated masses of SARS-

Table 4. Continued.

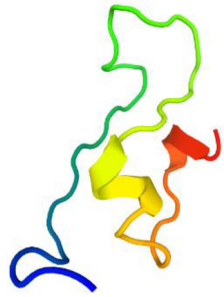
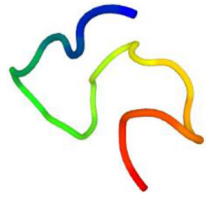
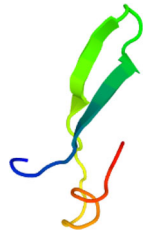
| S. No | Protein name | Missed cleavage | Peptides | Peptide structures |
|---|---|---|---|---|
| **9** | nsp13-pp1ab | 0 | VNSTLEQYVFCTVNALPETTADIVVFDEISMATNYDLSVVNAR |  |
| **10** | nonstructural polyprotein | 1 | HYVYIGDPAQLPAPRTLLTK |  |
| **11** | nonstructural polyprotein | 1 | GVITHDVSSAINRPQIGVVREFLTR |  |
| **12** | nonstructural polyprotein | 1 | EFLTRNPAWR |  |
| **13** | nonstructural polyprotein | 1 | AVFISPYNSQNAVASKILGLPTQTVDSSQGSEYDYVIFTQTTETAHSCNVNR | N/A |
| **14** | nonstructural polyprotein | 0 | ILGLPTQTVDSSQGSEYDYVIFTQTTETAHSCNVNR |  |
| **15** | nonstructural polyprotein | 1 | DLYDKLQFTSLEIPR | N/A |

Table 5. Lipinski rule and toxicity values for flavonoids, anthraquinone, and hydroxychloroquine.

| Name | Mol weight | HBA | HBD | TPSA | logP | Toxicity |
|---|---|---|---|---|---|---|
| Hydroxychoroquine | 335.87 | 4 | 2 | 48.39 | 3.01 | 4 |
| Flavonoids | 286.24 | 6 | 4 | 107.22 | 2.30 | 5 |
| Anthraquinone | 208.21 | 2 | 0 | 34.14 | 2.46 | 5 |

CoV-2 peptides and overall, *in silico* fragmentation of the protein sequence are shown in Table 1.

We searched the MASCOT database against the peptides obtained from digestion.

The peak list is needed for Peptide Mass Fingerprint. The peak list was created by translating the raw file to peak list through peaking or peak detection. The MASCOT server is used for peptide mass fingerprinting. It is an important tool

to use mass spectrometry data for peptide identification. Several other programs are similar to MASCOT, but it is unique since it integrates all the validated searching methods (Damodaran et al., 2007). As nCOV-2019 is a viral disease, we selected the whole taxonomy of viruses to be looked for in MASCOT with cleavage enzyme trypsin and missed cleavage 1, which implies that it will allow up to 1 mutation in related peptides. The peak list was entered as a peptide mass fingerprinting data file. We picked Swissprot for the search database because it is nonredundant and fairly small, helpful for statistically meaningful matches (Bairoch and Apweiler, 2000). In the homology search results, we found three most substantial matches by a significant threshold *p*-value of less than 0.05, with the highest score of 93. Two of them match with the SARS coronavirus (Maier et al., 2015). While the other one belongs to Pseudomonas phage B3. A brief description is shown in Table 2.

MASCOT database identifies the viruses and proteins which contain the matched peptides sequences along with complete annotation. We screened the highest scored matched peptides, as shown in Table 3.

As we have to predict the structures of identified peptides because the 3D structure determines their function (Stryer, n.d.). So we predicted the peptide structures for each matched peptide sequence from the *de novo* peptide predicting approach. PEP-FOLD 3 online server was used for this purpose (Lamiable et al., 2016). The 3D structures of identified peptides are given in Table 4 with the peptide sequence and missed cleavages.

The identified peptide structures of SARS-COV-2 peptides were bound with a drug and a medicinal compound. Before binding, it was important to test if the compounds are valid or not. So, for this purpose, the properties of the compounds were checked by ACD/I labs and ProTox Server. Lipinski rules were identified by ACD/I labs for either less or no adverse effects (Gupta et al., 2020; Hamza et al., 2019), and it consists of 5 properties, molecular weight should be less than 500 Da, Hydrogen bond acceptor (HBA) $<=10$, Hydrogen bond donor $\leq 5$, topological polar surface area (TPSA) $< 140$ and logP $< 5$ while the toxicity was predicted by the ProTox web server. This includes the details of both chemical and molecular targets. According to the Globally Harmonized System (GHS), toxicity has been classified into six classes. On the scale class VI is non-toxic, class V is moderate, class IV is

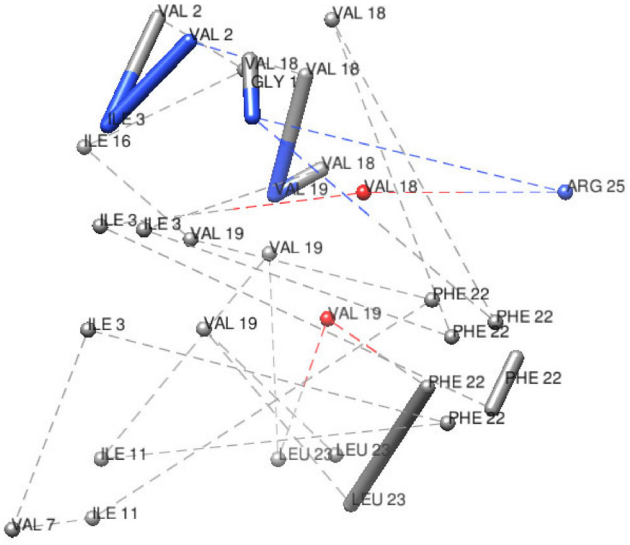



**Figure 3.** Druggability pocket identified for selected peptide through the PockDrug server and the interacting amino acids of the peptides are ARG25, PHE22, VAL2, VAL18, VAL19, GLY1, ILE3, and GLU21. Molecular docking is now extensively used to study the interaction between large molecule and small ligand for drug development or drug discovery. Docking is performed to determine the binding energy and the bound conformation of small molecules to the protein target (Pant et al., 2020; Seeliger & De Groot, 2010). There are actually different methods available for the ligand docking. AutoDock 4 and AutoDock Vina are effective and manual among them. After the data was organized and interpreted, both of the above methods are freely accessible, but AutoDock Vina, due to its extra default features considered to be credible and quick (Forli et al., 2016). Furthermore, AutoDock 4 has some limitations, AutoDock Vina helps to improve all those limitations specifically limited grid map size, rotatable bonds, and many atom numbers, it computes it by itself. As a consequence, for binding mode prediction, consequently, AutoDock Vina is more satisfactory and preferable (Trott & Olson, 2009).

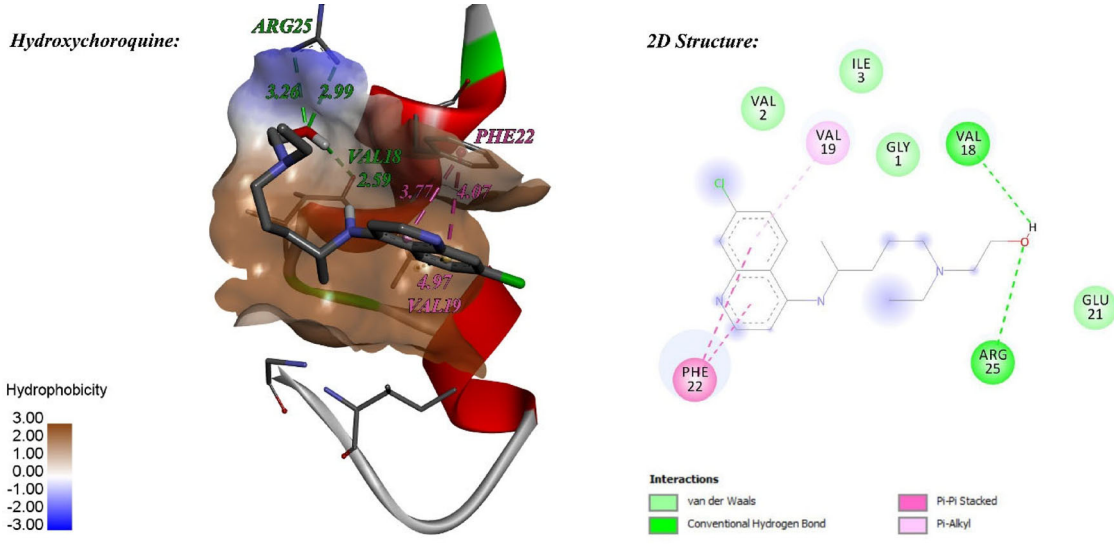



**Figure 4.** (A) Docked complex of predicted peptide (GVITHDVSSAINRPQIGVVREFLTR) with Hydroxychloroquine. Showing the interacting amino acid residues along with positions and distances. Ligand completely fits into the hydrophobic surface with the energy of −5.1 kcal/mol. (B) 2D structure shows the interacting amino acids of the peptide and type of interactions.

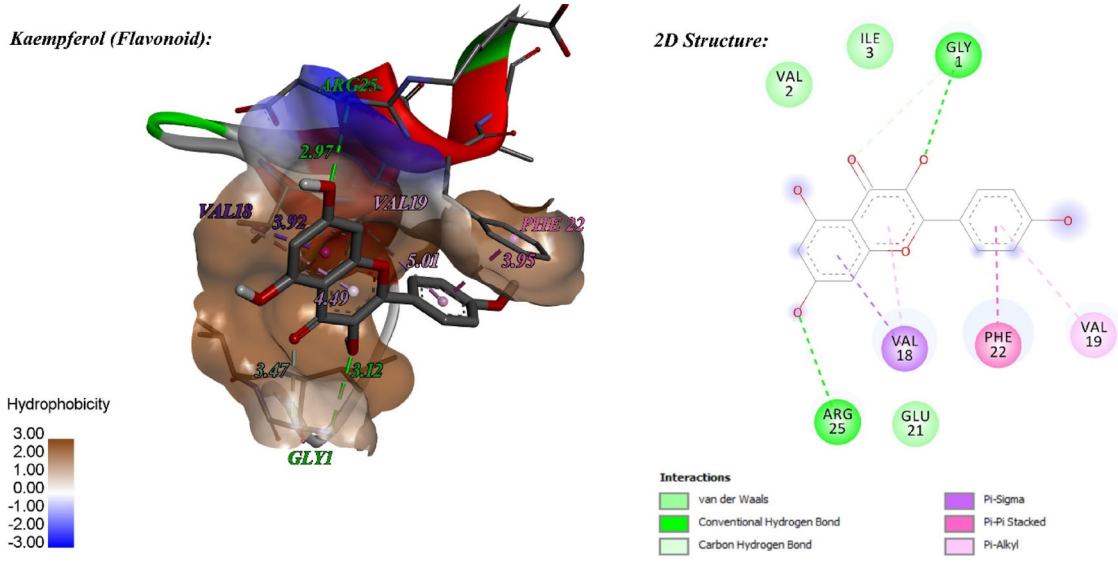



**Figure 5.** (A) Docked complex interacting amino acid residues analysis of predicted peptide (GVITHDVSSAINRPQIGVVREFLTR) with Kaempferol (flavonoids). Shows that ligand fits into the hydrophobic surface with the energy of –6.2 kcal/mol. (B) 2D interacted structure of peptide and Kaempferol.

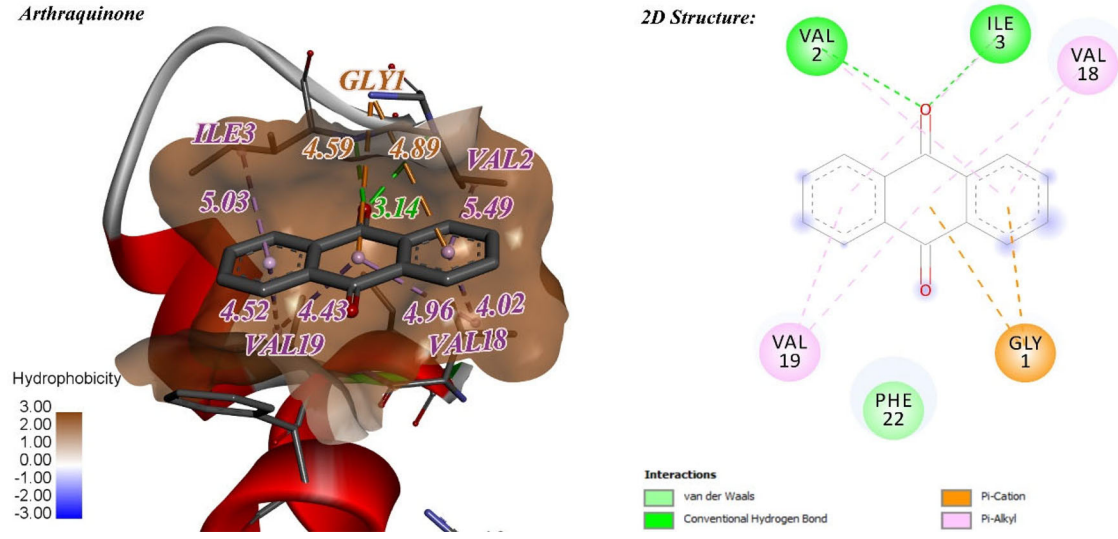



**Figure 6.** (A) Docked complex interacting amino acid residues analysis of predicted peptide (GVITHDVSSAINRPQIGVVREFLTR) with Anthraquinone. Shows that ligand fits into the hydrophobic surface with the energy of –6.0 kcal/mol. (B) 2D interacted structure of peptide and Anthraquinone. As described above that the druggability pocket contains several amino acids (ARG25, PHE22, VAL2, VAL18, VAL19, GLY1, ILE3, and GLU21) and these interacting amino acids are correlated with the three selected compounds for docking.

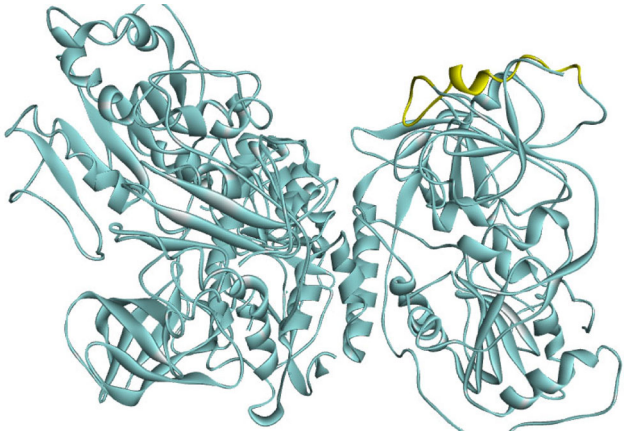


**Figure 7.** Surface exposed selected peptide from full-length protein. In Figure 7, the blue color shows the whole protein while yellow is the selected peptide that lies on the side/front of the protein. The molecule can easily access the peptide.

less toxic, class III is toxic, while class II and I are fatal (Hamza et al., 2019).

All the three selected compounds fulfill the Lipinski rules and lie in the safe zone of toxicity, as shown in Table 5.

The ability of the protein to bind a drug-like molecule with high affinity is considered as the druggability of protein. The pocket druggability of the selected peptide was predicted through the PockDrug server, providing consistent druggability results with several estimation methods (Hussein et al., 2015). The selected peptide (GVITHDVSSAINRPQIGVVREFLTR) for docking was chosen and the pocket of peptide is shown in Figure 3.

AutoDock Vina was used to bind the SARS-CoV-2 peptides to our chosen antiviral compounds. AutoDock Vina is an open-source and free for its users (Chang et al., 2010; Jaghoori et al., 2016; Yang & Sharp, 2006). AutoDock Vina input requires

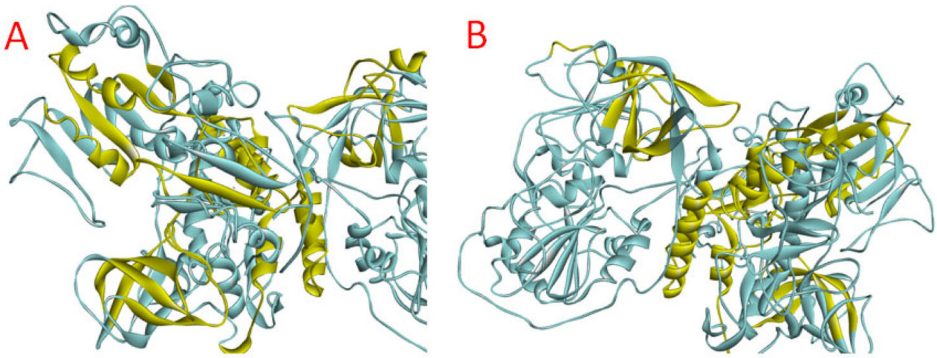



Figure 8. Full-length protein with highlight peptides, (A) one side view (B) another side view. In Figure 8, the identified peptides are mostly surface exposed and can be easily accessible by the molecule. The highlighted yellow colors are the identified peptides.

PDBQT files of both ligand and receptor while it outputs a poses list ranking by $\Delta G$ in kcal/mol, the binding energy. For the maximum number of poses output, the num_modes were set to 20 while the energy range to 10. The best poses were screened using RMSD values: ranging from $0 < 1.5$. First of all, we prepared our peptide (GVITHDVSSAINRPQIGVVREFLTR) for docking, receptor was saved as macromolecule in the PDBQT file. Then by default, the grid options (*x*,*y*,*z* dimensions) to 26 and Spacing (angstrom) = 1 to cover the maximum area of a peptide were set. Second, ligand compounds were prepared by adding torsions = 6 and saved in the PDBQT file. In the configuration file, we selected the exhaustiveness = 8 and performed further simulations of docking. The best poses obtained from molecular docking are shown in Figures 2–4, along with interacting residues with peptides position. The energies obtained from AutoDock Vina for hydroxychloroquine was −5.1 kcal/mol, kaempferol (flavonoid) −6.2 kcal/mol, and anthraquinone −6.0 kcal/mol.

Figures 2–4 shows the visualization of the drug complex, visualized through pymol and discovery studio. The drug compounds accurately fit into the hydrophobic surface of peptides that ensure the optimal attachment of drug compounds to the target peptides. In the figures, 2D structures of drug compounds shows different types of bonding with receptor molecules. It does not produce any unfavorable bumps that can affect the nature of bonding. So, from the aforementioned findings, it is confirmed that the chosen antiviral compounds are effective inhibitors against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and can help the immune system to fight against COVID-19 (Figures 5 and 6).

Since peptides are shorter in length and could be embedded in the full-length protein a question arises that whether the ligand compound can meet the target peptide for binding mechanism? But the surface was exposed to the chosen binding and can easily be accessed by a ligand compound successfully results in a docked complex. The selected peptide is modeled as yellow color in Figures 7 and 8.

## 4. Conclusions

In this research work, the viral peptides of SARS-CoV-2 were identified through peptide mass fingerprinting technique. This approach involves the mass calculation of protein translated from the whole genome of SARS-CoV-2, these masses were searched against databases for the identification of viral peptides. Fifteen viral peptides were identified for which the 3D structures were predicted. In this study, three compounds, Hydroxychloroquine, Kaempferol, and Anthraquinone, were chosen on the basis of their antiviral properties which could bind to the target and were nontoxic. For molecular docking of viral peptides and antiviral compounds, AutoDock Vina was used for molecular docking of viral peptides and anti-viral compounds because it is more accurate and reliable than other online servers or techniques. AutoDock Vina conforms to the ligand rotational and conformational shifts and results in the optimum poses of the compounds. The chosen compounds provided strongest interaction with peptides. In future, it is suggested that these compounds should be considered and clinically tested for new effective antiviral compounds against COVID-19.

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