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Identification of natural peptides from "PlantPepDB" database as anti-SARS-CoV-2 agents: A protein-protein docking approach

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ABSTRACT

Background: A global pandemic owing to COVID-19 infection has created havoc in the entire world. The etiological agent responsible for this viral outbreak is classified as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Still, there's no specific drug or preventive medication to treat SARS-CoV-2. This study was designed to demonstrate the efficacy of some anti-viral peptides obtained from a plant database i.e., PlantPepDB as potential ACE-2-Spike (S) protein complex neutralizers using a structure-based drug designing approach.

Method: A total of 83 anti-viral plant peptides were screened from a peptide database i.e. PlantPepDB based on their reported anti-viral activities against various viral strains. In order to screen peptides that may potentially interfere with ACE-2 and S complex formation, molecular docking studies were conducted using the flare module of Cresset software and subsequently, analysed the crucial interactions between the peptides and S complexes and ACE-2/S complex. Herein, the interactions and docking scores obtained for ACE-2/S complex were considered as references. The S-peptides complexes which displayed superior interactions and docking scores than reference complex i.e., ACE2-S were considered as final hits. The Molecular dynamics studies were conducted for a period of 30 ns for each of the final hit/S complex to understand the interaction stability and binding mechanism of designed peptides.

Results: The molecular docking results revealed that five peptides including Cycloviolacin Y3, Cycloviolacin Y1, White cloud bean defensin, Putative defensin 3.1, and Defensin D1 showed superior docking scores (i.e. -1372.5 kJ/mol to -1232.6 kJ/mol) when docked at the ACE2 binding site of S-protein than score obtained for the complex of ACE-2 and S protein i.e. -1183.4 kJ/mol. Moreover, these top five peptides manifested key interactions required to prevent the binding of S protein with ACE2. The molecular dynamics simulation study revealed that two of these five peptides i.e. Cycloviolacin Y3 and Cycloviolacin Y1 displayed minimal RMSD fluctuations.

Conclusions: The current structure-based drug-designing approach shows the possible role of anti-viral plant peptides as potential molecules to be explored at the initial stage of viral pathogenesis.

Introduction

COVID-19 disease shows mild as well as severe symptoms such as respiratory tract illness, progressive pneumonia, and multi-organ failure (Sohrabi et al., 2020). These associated symptoms are responsible for its high mortality rate. There are three strategies to control the disease progression i.e. interrupting the interactions between viral spike (S) protein and human angiotensin-converting enzyme (hACE2) by targeting interface residues, targeting the enzymes involved in viral replication and translation, and managing the symptoms associated with this disease *via* anti-inflammatory, immunomodulatory therapy, etc. Currently, scientists are working on these strategies by drug repurposing, exploring herbal formulations, or conducting trials for appropriate vaccines (Shetty et al., 2020). Till now, more than 60 peptide drugs have been approved for different diseases, over 200 peptide drugs are in different stages of clinical trials and around 600 are being evaluated in pre-clinical studies (Singh et al., 2016). Currently, a recombinant hACE2 peptide i.e., RhACE2-APN01 has already entered in phase II clinical

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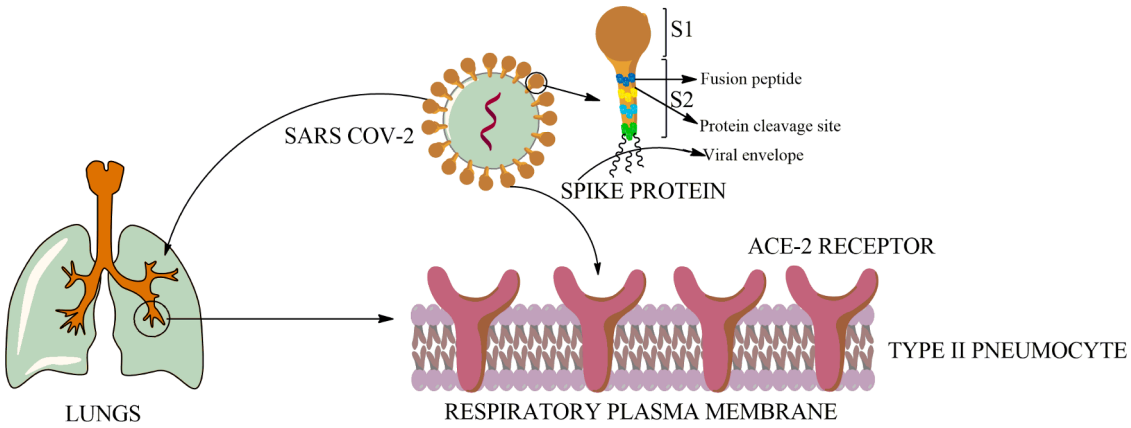

Fig. 1. Spike protein architecture and molecular mechanism by which SARS-CoV2 gains entry to host cell.

trials (NCT04335136) for treating Covid-19 (Shah et al., 2022). Bioactive plant peptides possess diverse therapeutic activities including antimicrobial, anticancer, immunomodulatory, antihypertensive, etc. (Maestri et al., 2016; Sharma et al., 2021). In the current study, we explored the amino acid residues present at the interface of S protein which is responsible for interacting with angiotensin I converting enzyme 2 (ACE-2). ACE-2 is the receptor present on the surface of pneumocytes through which the virus gains entry to the host cell (Bhuiyan et al., 2020). The S protein is a membrane-bound trimetric protein of SARS-CoV-2, its receptor-binding domain (RBD) couples with the ACE2 receptor on human cells and further mediates cell attachment and membrane fusion (Wang et al., 2020). The architecture of the S protein and its main site of binding to get access to the host cell have been illustrated in Fig. 1. This makes S protein the target of interest for scientists to neutralize SARS-CoV-2 infection (Du et al., 2009).

Various researchers have explored different synthetic peptides, and antibodies as potential therapeutic agents for COVID-19 therapy (Mahendran et al., 2020; VanPatten et al., 2020). In this context, Fakih et al. 2020 explored dermaseptin as a potential ACE-2-S protein complex neutralizer as a peptide identified from frogs of the genus Phyllomedusa. In another study, Huang et al., 2020 designed novel peptides to block the association of the SARS-COV-2 'S' protein with human ACE-2 by linking two fragments grafted from the interface of ACE-2 protein (a.a. 22–44 and 351–357) with a linker glycine. Likewise, Larue et al., 2020 rationally designed a panel of ACE2-derived peptides based on the RBD-ACE2 binding interfaces of SARS-CoV-2 and SARS-CoV to inhibit SARS-CoV-2. The immunogenic peptides and protective monoclonal antibodies were also identified as COVID-19 therapeutics by exploring different phage libraries (Li et al., 2021). In the search for peptides-based vaccines Kalita et al., 2020 designed a multi-peptide subunit-based epitope vaccine against COVID-19 comprising of an adjuvant, cytotoxic T-lymphocyte (CTL), helper T-lymphocyte (HTL), and B-cell epitopes joined by linkers. Despite these numerous efforts, herein an attempt has been made to perform an *in-silico-based* assessment of some plant peptides which have been reported to possess anti-viral activity against other virus strains but not for SARS-CoV-2 by binding with S protein with superior affinity than that of ACE-2/S complex. This study is of great importance, as plant peptides are advantageous over synthetic peptides because they exhibit high target selectivity and specificity with limited toxicity (Das et al., 2020; Parvathaneni and Gupta, 2020). To mimic the interactions between RBD of ACE-2 and S, herein we screened plant peptides from the peptide database. The 'PlantPepDB' is developed recently in 2020 and it is the first initiative to filter the peptides and obtain the 3D structure of the collected peptides from the same database to subject the latter to structure-based drug design approaches. Then the obtained peptides were docked with the S protein of SARS-CoV-2 based on the protein-protein docking approach. As a result, some of the plant peptides were found to display better binding scores in comparison to the ACE2-S protein complex and mimic the interactions between the interfacial residues of ACE-2 and S. Later each peptide-S complex was analysed for its stability and retention of the initial docked interactions.

Material and methods

Retrieval of plant peptides

Plant peptides with anti-viral activity were obtained from the PlantPepDatabase. It is a manually curated database and is freely available at http://www.nipgr.ac.in/PlantPepDB (Das et al., 2020). This database provides in-depth information about most of the bioactive and therapeutic plant peptides published so far.

3D structures of plant peptides

The 3D structures of the screened peptides with anti-viral potential were obtained from the above-mentioned database. This database constitutes about 6172 peptide entries, out of which 3199 peptides have been modeled using MODELLER v9.21, and the final model with the lowest DOPE (Discrete optimized protein energy) score can be downloaded from this database in .pdb format.

Protein-protein docking

The RCSB PDB is the single worldwide archive of structural data of biological macromolecules (http://www.wwpdb.org), which was used to obtain the 3D structure of the SARS-CoV-RBD in complex with the ACE2 receptor (PDB ID: 6LZG) (Wang et al., 2020). The molecular docking experiments were performed with the aid of the ClusPro 2.0 server (https://cluspro.org). Cluspro 2.0 is a fully automated, web-based server for filtering, clustering, and ranking the protein-peptide complex. Cluspro uses the Fast-Fourier correlation approach and ranks the models *via* the cluster population. The docking grid was generated around the interface amino acids of S protein (site-specific), which have been reported to interact with ACE-2. PIPER represents the interaction energy between two proteins using an expression of form *E*.

$$E = w_1 E_{rep} + w_2 E_{attr} + w_3 E_{elec} + w_4 E_{DARS}$$

Where,

E_{rep} and E_{attr} are the attractive and repulsive contributions to the van der Waals interaction energy, and

E_{elec} denotes electrostatic energy.

E_{DARS} is a pairwise structure-based potential; it primarily represents desolvation contributions, i.e., free energy change by removal of the water molecules from the interface.

The coefficients w_1, w_2, w_3, and w_4 define the weights of the corresponding residues.

Table 1
Selected plant-peptides from the PlantPep database comprising antiviral properties.

Plant pep ID	Peptide name	Biological source	Class	Family	Biological activities
PPepDB_1491	Griffithsin	*Gly-griffithsia*	Florideophyceae	Wrangeliaceae	Antiviral
PPepDB_1536	Ginkbilobin	*Ginkgo biloba*	Ginkgoopsida	Ginkgoaceae	Antibacterial, Antifungal, Antiviral, Anti-HIV
PPepDB_1538	Antifungal protein from coconut	*Cocos nucifera*	Commelinids	Arecaceae	Antifungal, Antiviral, Anti-HIV
PPepDB_1539	Alpha-basrubrin	*Basella alba*	Eudicots	Basellaceae	Antifungal, Antiviral
PPepDB_1545	Circulin D	*Chassalia parvifolia*	Magnoliopsida	Rubiaceae	Antiviral, Anti-HIV
PPepDB_1549		*Chassalia parvifolia*	Magnoliopsida	Rubiaceae	Antiviral, Anti-HIV
PPepDB_1566	Cycloviolacin O16	*Viola odorata*	Rosids	Violaceae	Antibacterial, Antifungal, Antiviral, Antiparasitic
PPepDB_1569	Cycloviolacin Y2	*Viola philippica, Viola yedoensis*	Rosids	Violaceae	Antiviral, Insecticidal, Anti-HIV
PPepDB_1582	Cycloviolacin O19	*Viola odorata*	Rosids	Violaceae	Antibacterial, Antifungal, Antiviral, Insecticidal, Enzymatic-degradation
PPepDB_1601	Cycloviolacin H2	*Viola hederacea*	Rosids	Violaceae	Antimicrobial, Anti-HIV, Antiviral
PPepDB_1602	Cycloviolacin H3	*Viola hederacea*	Rosids	Violaceae	Antimicrobial, Nematocide, Antiviral
PPepDB_1603	Vhl-2	*Viola hederacea*	Rosids	Violaceae	Antiviral, Anti-HIV, Anticancer
PPepDB_1612	Vhl-1	*Viola hederacea*	Rosids	Violaceae	Antiviral, Antimicrobial
PPepDB_1925	Cyclotoviolacin O15	*Viola odorata*	Rosids	Violaceae	Nematocide, Hemolytic, Antibacterial, Antifungal, Antiviral, Antiparasitic
PPepDB_1926	Cycloviolacin O14	*Viola odorata*	Rosids	Violaceae	Nematocide, Anti-HIV, Hemolytic, Enzymatic-degradation, Antibacterial, Antifungal, Antiviral, Anti-HIV, Antiparasitic
PPepDB_1931	Cycloviolacin Y3	*Viola yedoensis*	Rosids	Violaceae	Insecticidal, Anti-HIV, Antiviral
PPepDB_1937	Cycloviolacin Y1	*Viola yedoensis*	Rosids	Violaceae	Nematocide, Hemolytic, Anti-HIV, Antiviral
PPepDB_1939	Kalata B8	*Oldenlandia affinis*	Eudicots	Rubiaceae	Molluscicidal, Anti-HIV, Cytotoxic, Antiviral, Antibacterial
PPepDB_1965	Cycloviolacin O22	*Viola odorata, Viola tricolor, Palicourea tetragona*	Rosids	Violaceae	Insecticidal, Enzymatic-degradation, Antibacterial, Antifungal, Antiviral
PPepDB_1966	Cycloviolacin O23	*Viola odorata*	Rosids	Violaceae	Insecticidal, Enzymatic-degradation, Antibacterial, Antifungal, Antiviral
PPepDB_2024	Leaf cyclotide, Vhl-1	*Viola hederacea*	Rosids	Violaceae	Antibacterial, Antiviral, Antifungal
PPepDB_2030	White cloud bean defensin	*Phaseolus vulgaris*	Rosids	Fabaceae	Antibacterial, Antiviral, Anticancer, Antifungal
PPepDB_2031	AB2, red bean antifungal peptide, Putative defensin 3.1	*Adzuckia angularia, Medicago sativa*	Rosids	Fabaceae	Antiviral, Antifungal
PPepDB_2032	PTA2c, pinto bean antifungal peptide, Defensin D1	*Phaseolus vulgaris*	Rosids	Fabaceae	Antiviral, Antifungal
PPepDB_2082	Gymnin	*Gymnocladus chinensis*	Rosids	Fabaceae	Antiviral, Anticancer, Antifungal
PPepDB_2084	Antifungal lectin PVAP	*Phaseolus vulgaris*	Rosids	Fabaceae	Antiviral, Anticancer, Antifungal
PPepDB_2101	Sesquin	*Vigna unguiculata*	Rosids	Fabaceae	Antibacterial, Antifungal, Antiviral, Anti-HIV, Anticancer
PPepDB_2104	Coccinin	*Phaseolus coccineus*	Rosids	Fabaceae	Antiviral, Anticancer, Antifungal, Hemolytic, Antiproliferative, HIV-1-reverse-transcriptase-inhibition
PPepDB_2170	Tricyclon-A	*Viola arvensis, Viola tricolor*	Rosids	Violaceae	Antibacterial, Anticancer, Antifungal, Antiviral, Hemolytic, Antimicrobial
PPepDB_2189	Palicourein	*Palicourea condensate*	Eudicods	Rubiaceae	Antiviral, Anti-HIV
PPepDB_2190	Circulin-E	*Chassalia parvifolia*	Eudicods	Rubiaceae	Antiviral, Anti-HIV
PPepDB_2207	Kalata B2	*Oldenlandia affinis*	Eudicods	Rubiaceae	Antibacterial, Anticancer, Antifungal, Nematocide, Molluscicidal, Insecticidal, Hemolytic, Antiviral, Antiparasitic
PPepDB_2212	Circulin-D	*Chassalia parvifolia*	Eudicods	Rubiaceae	Antimicrobial, Antiviral, Anti-HIV
PPepDB_2213	Varv peptide E (Cycloviolacin-O12)	*Viola tricolor, Viola arvensis, Viola baoshanensis, Viola yedoensis, Viola tianshanica, Viola abyssinica, Viola philippica*	Rosids	Violaceae	Antiviral, Anticancer, Nematocide, Anti-HIV
PPepDB_2214	Kalata-B1	*Oldenlandia affinis, Viola yedoensis*	Eudicods	Rubiaceae	Antibacterial, Antifungal, Antiviral, Anticancer, Hemolytic, Cytotoxic, Nematocide, Molluscicidal, Insecticidal, Enzymatic-degradation, Anti-HIV, Enzyme-inhibitor
PPepDB_2215	Circulin-B, CIRB	*Chassalia parvifolia*	Eudicods	Rubiaceae	Antibacterial, Antifungal, Hemolytic, Cytotoxic, Antiviral, Insecticidal, Anti-HIV
PPepDB_2333	Cycloviolacin H2	*Viola hederacea*	Rosids	Violaceae	Antimicrobial, Antiviral
PPepDB_2335	Cycloviolacin H3	*Viola hederacea*	Dicotyledons	Violaceae	Antimicrobial, Antiviral
PPepDB_2336	Cyclotide vhl2	*Viola hederacea*	Dicotyledons	Violaceae	Antimicrobial, Antiviral
PPepDB_2395	Anti-HIV peptide, Contrajervin	*Dorstenia contrajerva*	Dicotyledons	Moraceae	Antiviral
PPepDB_244	Cycloviolacin VY1	*Viola yedoensis*	Equisetopsida	Violaceae	Antiviral
PPepDB_2578	Circulin E	*Chassalia parvifolia*	cyclotides	Rubiaceae	Antimicrobial, Antiviral
PPepDB_2718	Circulin C	*Chassalia parvifolia*	cyclotides	Rubiaceae	Antimicrobial, Antiviral
PPepDB_2728	Anti-HIV peptide, Treculavirin	*Treculia obovoidea*	Arachnida	*Moraceae*	Antiviral
PPepDB_274	Cycloviolacin Y4	*Viola yedoensis*	Equisetopsida	Violaceae	Antiviral, Anti-HIV, Nematocide, Hemolytic, Antimicrobial

(*continued on next page*)

Table 1 (*continued*)

Plant pep ID	Peptide name	Biological source	Class	Family	Biological activities
PPepDB_2791	Palicourein	*Palicourea condensate*	Magnoliopsida	Rubiaceae	Antimicrobial, Antiviral
PPepDB_2824	Cycloviolin D	*Leonia cymose*	Magnoliopsida	Violaceae	Antimicrobial, Antiviral
PPepDB_2825	Cycloviolin A	*Leonia cymose*	Magnoliopsida	Violaceae	Antimicrobial, Antiviral
PPepDB_2826	Cycloviolin C	*Leonia cymose*	Magnoliopsida	Violaceae	Antimicrobial, Antiviral
PPepDB_2827	Cycloviolin B	*Leonia cymose*	Magnoliopsida	Violaceae	Antimicrobial, Antiviral
PPepDB_3027	Cycloviolacin O12	*Viola odorata*	Dicotyledons	Violaceae	Antibacterial, Antifungal, Antiviral
PPepDB_3047	Cycloviolacin O1	*Viola odorata*	Dicotyledons	Violaceae	Antibacterial, Antifungal, Antiviral
PPepDB_3048	Cycloviolacin O8	*Viola odorata*	Dicotyledons	Violaceae	Antibacterial, Antifungal, Antiviral
PPepDB_3049	Cycloviolacin O6	*Viola odorata*	Dicotyledons	Violaceae	Antibacterial, Antifungal, Antiviral
PPepDB_3050	Cycloviolacin O11	*Viola odorata*	Dicotyledons	Violaceae	Antibacterial, Antifungal, Antiviral
PPepDB_3051	Cycloviolacin O4	*Viola odorata*	Dicotyledons	Violaceae	Antibacterial, Antifungal, Antiviral
PPepDB_3052	Cycloviolacin O2	*Viola odorata*	Dicotyledons	Violaceae	Antibacterial, Antifungal, Antiviral, Anticancer
PPepDB_3053	Cycloviolacin O5	*Viola odorata*	Dicotyledons	Violaceae	Antibacterial, Antifungal, Antiviral
PPepDB_3054	Cycloviolacin O3	*Viola odorata*	Dicotyledons	Violaceae	Antibacterial, Antifungal, Antiviral
PPepDB_3055	Cycloviolacin O9	*Viola odorata*	Dicotyledons	Violaceae	Antibacterial, Antifungal, Antiviral
PPepDB_3056	Cycloviolacin O7	*Viola odorata*	Dicotyledons	Violaceae	Antibacterial, Antifungal, Antiviral
PPepDB_3057	Cycloviolacin O10	*Viola odorata*	Dicotyledons	Violaceae	Antibacterial, Antifungal, Antiviral
PPepDB_3058	Cycloviolacin O20	*Viola odorata*	Dicotyledons	Violaceae	Antibacterial, Antifungal, Antiviral
PPepDB_3059	Cycloviolacin O17	*Viola odorata*	Dicotyledons	Violaceae	Antibacterial, Antifungal, Antiviral
PPepDB_3060	Cycloviolacin O18	*Viola odorata*	Dicotyledons	Violaceae	Antibacterial, Antifungal, Antiviral
PPepDB_3388	Vulgarinin	*Phaseolus vulgaris*	Dicotyledons	Fabaceae	Antibacterial, Antifungal, Antiviral
PPepDB_3804	Lunatusin	*Phaseolus lunatus*	Dicotyledons	Faboideae	Antibacterial, Antifungal, Antiviral, Anticancer, Anti-HIV
PPepDB_3805	Cycloviolacin Y5	*Viola odorata*	Dicotyledons	Violaceae	Antimicrobial, Nematocide, Hemolytic, Anti-HIV, Antiviral
PPepDB_3860	Cyclovialacin O24	*Viola odorata*	Dicotyledons	Violaceae	Antibacterial, Antifungal, Antiviral, Nematocide, Anti-HIV, Hemolytic, Enzymatic-degradation
PPepDB_3964	Circulin-C (CIRC)	*Chassalia parvifolia*	cyclotides	Rubiaceae	Antiviral, Anti-HIV
PPepDB_3966	Leaf cyclotide 1 (Vhl-1)	*Viola hederacea*	Dicotyledons	Violaceae	Antibacterial, Antiviral, Antifungal, Nematocide, Anti-HIV
PPepDB_3970	Circulin-F (CIRF)	*Chassalia parvifolia*	cyclotides	Rubiaceae	Antimicrobial, Antiviral, Anti-HIV
PPepDB_3983	Cycloviolin-D	*Leonia cymose*	Magnoliopsida	Violaceae	Antimicrobial, Antiviral, Anti-HIV
PPepDB_3984	Cycloviolin-C	*Leonia cymose*	Magnoliopsida	Violaceae	Antimicrobial, Antiviral, Anti-HIV
PPepDB_3985	Cycloviolin-B	*Leonia cymose*	Magnoliopsida	Violaceae	Antimicrobial, Antiviral, Anti-HIV
PPepDB_3986	Cycloviolin-A	*Leonia cymose*	Magnoliopsida	Violaceae	Antimicrobial, Antiviral, Anti-HIV
PPepDB_3992	Cycloviolacin-O13 (Cyclotide c3)	*Viola odorata*	Dicotyledons	Violaceae	Nematocide, Anti-HIV, Hemolytic, Enzymatic-degradation, Antibacterial, Antifungal, Antiviral, Antiparasitic
PPepDB_3994	Cycloviolacin-O21	*Viola odorata*	Dicotyledons	Violaceae	Antibacterial, Antifungal, Antiviral
PPepDB_607	Thaumatin-like protein	*Castanopsis chinensis*	Dicotyledons	Fagaceae	Antifungal, Antiviral
PPepDB_633	Thaumatin-like protein, Actc2	*Actinidia chinensis*	Magnoliopsida	Actinidiaceae	Antifungal, Antiviral
PPepDB_661	Circulin-C	*Chassalia parvifolia*	cyclotides	Rubiaceae	Antiviral
PPepDB_716	Cycloviolacin Y5	*Viola philippica*	Magnoliopsida	Violaceae	Antiviral
PPepDB_726	Beta-basrubin	*Basella alba*	Magnoliopsida	Basellaceae	Antifungal, Antiviral

Molecular dynamics

The top screened peptides were further subjected to molecular dynamics simulations (MD) using commercially available software i.e. Cresset (https://www.cresset-group.com). This tool utilizes AMBER/GAFF2 force fields and AM1-BCC as the charge method. Each of the top-screened RBD–plant peptide complexes was initially submitted for system building using the explicit solvent water model. Herein, the shape of the box was kept as orthorhombic with dimensions 10 × 10 × 10 Å. Later, each complex was minimized to 0.25 kcal/mol and equilibrated for 200 ps before initiating the MD run. The simulations were performed at a time step of 1 fs and 30 ns as recording time.

Results

Screening of plant peptides

As a result of the screen from the PlantpepDB, about 83 plant peptides were obtained which have been displayed in Table 1.

Protein-protein docking

As *per* reports, amino acid residues i.e. A475, N487, E484, and Y453 present within the C-terminal domain (CTD) of SARS-CoV-2 S protein shows polar contacts with S19, Q24, K31, and H34 of hACE2, respectively. The residue K417 located in the helix α3 CTD core is reported to show ionic interactions with the D30 residue of hACE2. Several residues including G446, Y449, G496, Q498, T500, and G502 located in the bulged loops of SARS-CoV-2-CTD form H-bonds near interface amino acids (D38, Y41, Q42, K353, and D355) of hACE2. A small patch of hydrophobic interactions was observed between amino acids Y489 and F486 of SARS-CoV-2-CTD and residues F28, L79, M82, and Y83 of hACE2. Among all type of interactions, polar contacts mediated by the hydrophilic residues were found to be the most dominating one (Wang et al., 2020). The polar contacts are very crucial for establishing a strong contact between SARS-CoV-2-CTD and hACE2. In the current study, host protein ACE2 was not considered for inhibition, as it is a major component of the human renin-angiotensin system (RAS) that is important for maintaining blood pressure and another important homeostatic mechanism. This important physiological functioning of ACE2 marks the basis for considering the RBD of the S protein in SARS-CoV-2 as a crucial target. Inhibiting the CTD of S protein can inhibit interactions between virus and receptor.

The peptides screened from the database were docked at the interface of S protein and were analyzed for their ability to inhibit the binding of CTD of S protein with hACE2. For carrying docking, the prepared protein 6LZG was utilized and imported into ClusPro for a protein-protein docking study. In PDB 6LZG, chain A indicates hACE2,

Table 2
The lowest binding energy scores correspond to each plant peptide.

Serial no	Plant pep ID	Lowest Binding Energy from ClusPro (kJ/mol)
1.	PPepDB_1491	−1126.3
2.	PPepDB_1536	−1136.1
3.	PPepDB_1538	−922.4
4.	PPepDB_1539	−950.3
5.	PPepDB_1545	−1137.4
6.	PPepDB_1549	−1097.3
7.	PPepDB_1566	−775.2
8.	PPepDB_1569	−1165.2
9.	PPepDB_1582	−987.7
10.	PPepDB_1601	−954.6
11.	PPepDB_1602	−989.9
12.	PPepDB_1603	−971.3
13.	PPepDB_1612	−975.1
14.	PPepDB_1925	−942.9
15.	PPepDB_1926	−798.1
16.	PPepDB_1931	−1287.8
17.	PPepDB_1937	−1237.3
18.	PPepDB_1939	−981.2
19.	PPepDB_1965	−593.2
20.	PPepDB_1966	−999.3
21.	PPepDB_2024	−921
22.	PPepDB_2030	−1372.5
23.	PPepDB_2031	−1232.6
24.	PPepDB_2032	−1307
25.	PPepDB_2082	−762.1
26.	PPepDB_2084	−1033.1
27.	PPepDB_2101	−872.2
28.	PPepDB_2104	−873.4
29.	PPepDB_2170	−1078.9
30.	PPepDB_2189	−969.8
31.	PPepDB_2190	−1047.2
32.	PPepDB_2207	−904.2
33.	PPepDB_2212	−1098.1
34.	PPepDB_2213	−807.5
35.	PPepDB_2214	−943
36.	PPepDB_2215	−1009.8
37.	PPepDB_2333	−1107.6
38.	PPepDB_2335	−999.4
39.	PPepDB_2336	−970.8
40.	PPepDB_2395	−1068.4
41.	PPepDB_244	−965.1
42.	PPepDB_2578	−1120.2
43.	PPepDB_2718	−1019
44.	PPepDB_2728	−984
45.	PPepDB_274	−1022.8
46.	PPepDB_2791	−1143.9
47.	PPepDB_2824	−1107.9
48.	PPepDB_2825	−1130.2
49.	PPepDB_2826	−1019.7
50.	PPepDB_2827	−1162.2
51.	PPepDB_3027	−906.9
52.	PPepDB_3047	−969.1
53.	PPepDB_3048	−1040.9
54.	PPepDB_3049	−958.9
55.	PPepDB_3050	−990.6
56.	PPepDB_3051	−1146.5
57.	PPepDB_3052	−1083.8
58.	PPepDB_3053	−1057.6
59.	PPepDB_3054	−1086
60.	PPepDB_3055	−1144
61.	PPepDB_3056	−973.9
62.	PPepDB_3057	−975.9
63.	PPepDB_3058	−922.4
64.	PPepDB_3059	−927
65.	PPepDB_3060	−881
66.	PPepDB_3388	−955.5
67.	PPepDB_3804	−1008.9
68.	PPepDB_3805	−1091.1
69.	PPepDB_3860	−950.9
70.	PPepDB_3964	−1037.2
71.	PPepDB_3966	−1068
72.	PPepDB_3970	−907.2
73.	PPepDB_3983	−1055.2
74.	PPepDB_3984	−859.8
75.	PPepDB_3985	−974.9
76.	PPepDB_3986	−1025.9
77.	PPepDB_3992	−970.2
78.	PPepDB_3994	−945.9
79.	PPepDB_607	−1042.1
80.	PPepDB_633	−1162
81.	PPepDB_661	−975.7
82.	PPepDB_716	−1007.3
83.	PPepDB_726	−1000.5

Table 3
Plant peptides show better interaction with spike protein than hACE2 with the lowest binding energy.

Plant pep ID	Peptide name	Lowest binding energy (kJ/mol)
PPepDB_1931	Cycloviolacin Y3	−1287.8
PPepDB_1937	Cycloviolacin Y1	−1237.3
PPepDB_2030	White cloud bean defensin	−1372.5
PPepDB_2031	Putative defensin 3.1	−1232.6
PPepDB_2032	Defensin D1	−1307

Table 4
Interaction with spike protein than hACE2 with lowest binding energy and interacting residues.

Peptide name	Interacting residues
Cycloviolacin Y3	N501, Y17, Y505, Y17, Y505, T15, Y453, E9, Q493, C7, Q493, G8
Cycloviolacin Y1	K417, Y26, Y453, T10, Y505, T15, G502, Y17, Q498, E9, Y449, T10, S494, E9, Q493, G27, Q493, G8, Q493, E9
White cloud bean defensin	E484, V15, Y453, C10, G496, G8, Q498, E2, Y449, F6, E484, V15, N501, G8, G496, G8
Putative defensin 3.1	Y505, F17, Q493, F13, Q493, F11, N487, K4
Defensin D1	T500, E21, N501, E21, N501, A19, Q498, Q21, Q493, V11

while chain B indicates S protein. Initially, to obtain a reference cut-off that can be utilized for comparing other peptide-S complexes, hACE2 (chain A of hACE2) was docked with S protein (chain B of S protein). The docking score corresponding to S-hACE2 was found to be −1183.4 kJ/mol. Docking scores in terms of the lowest binding energy corresponding to each plant peptide are displayed in Table 2. It was observed that five plant peptides showed better docking scores in the range of i.e. -1200 to -1300 kJ/mol than the former S-ACE2 complex i.e. -1183 kJ/mol. From Table 3, it can be seen that Cycloviolacin Y3, Cycloviolacin Y1, white cloud bean defensin, Putative defensin 3.1, and Defensin D1, each form a complex with the binding domain of S protein with the lowest energy weighed score-1287.8 kJ/mol, -1237.5 kJ/mol, −1372.5 kJ/mol, -1232.6 kJ/mol, and -1307 kJ/mol respectively. Furthermore, these complexes were analyzed for their key interactions at the interface of the S protein. As discussed initially that K455, F486, Q493, S494, N501, and Y505 in the RBD are extensively responsible for the efficient binding with hACE2. Apart from this, some additional amino acids such as K417, G446, Y449, Y453, A475, E484, N487, Y489, G496, E498, T500, and G502 also play an important role in hACE2 binding with RBD of S protein. The top two peptides discussed before showed interactions with residues of S protein that are important for binding with ACE-2 (Table 4) *via* hydrogen bonding, π-π stacking, and π-cationic interactions (Figs. 2 and 3). From these figures, it can be observed that these five peptides mostly show polar contacts, which are crucial for preventing the interactions between RBD of S protein and hACE2. Other three peptides including PPepDB_1931, PPepDB_1937, and PPepDB_2031 showed important key interactions with the interfacial amino acids of S-protein (Fig. S1-S3, supplementary data). The 3D interaction diagram corresponding to the top two peptides-ACE2 complexes is displayed in Figs. 4 and 5. On the other hand, 3D interactions for the rest of the three

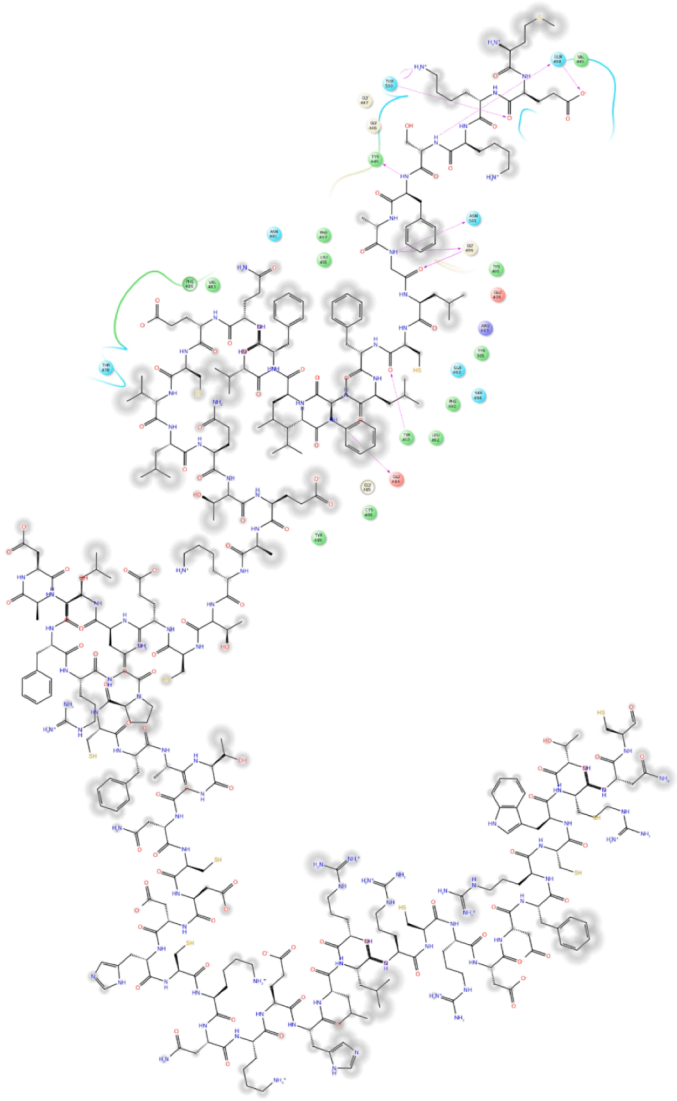

Fig. 2. 2D interaction diagram between the peptide PPepDB_2030 and interface residues of spike protein of SARS-CoV2.

complexes are illustrated in **Fig. S4-S6**. It is clear from these figures that these peptides have the potential to prevent SARs-CoV-2 infection at the initial phase.

Molecular dynamics

Based on docking results, it was found that five plant peptides Cycloviolacin Y3 (PPepDB_1931), Cycloviolacin Y1 (PPepDB_1937), White cloud bean defensin (PPepDB_2030), Putative defensin 3.1 (PPepDB_2031), and Defensin D1 (PPepDB_2032) mentioned in Table 3 can circumvent the binding of S protein with ACE-2. To analyze the stability of each complex of plant peptides and S protein, MD simulations were performed for a period of 30 ns. The PPepDB_1931-S protein complex showed limited deviation during the simulation and attained equilibrium by exhibiting a stable confirmation. The level of RMSD snapshots was found between 1.7 to 2.7 Å. This limited range indicates that this peptide adapts well in the binding domain of S protein throughout the simulation. The second complex between PPepDB_1937-S proteins exhibited slight deviations between 1.6 to 3.6 Å in the 30 ns of the time scale shown in Fig. 6.

Discussion

Since the current outbreak of a viral epidemic, scientists around the world are making enormous efforts to understand the pathophysiology of SARS-CoV-2. Drug repurposing of available antihypertensive, antifungal, antibacterial, and anticoagulant drugs has been one way of addressing this health issue (Yang et al., 2020). Herein, we have utilized the co-crystal structure of the best-categorized structure of S protein and ACE2 complex (6LZG) to search for protein-protein interaction blockers from anti-viral plant peptides. Since it is reported that the amide linkage of peptides provides flexibility to fit comfortably in the active site of the target (Pant et al., 2020), it was worthwhile to screen the plant peptides which may effectively bind with S protein and prevent the interaction of S protein and ACE2 at the early stage of viral pathophysiology. Later, the best S-peptide complexes were studied to analyze the molecular interactions at their interface.

The preliminary *in-silico* study revealed that five plant peptides showed important interactions with key amino acid residues present at the interface of S protein that was important for binding with ACE-2 (an important receptor by which the virus accesses the host cell). From these results, it can be assumed that these peptides may prevent the virus prognosis at the early stage. Further, to analyze the stability of each peptide-S complex, MD simulations were performed. It signifies that PPepDB_1931 or Cycloviolacin Y3 could inhibit the interaction of S protein with hACE2, as its complex with S was found to be stable throughout the MD simulations for a period of 30 ns. Additionally, the best peptides in the present study are already reported as potent inhibitors of HIV-1 reverse transcriptase (Vilas Boas et al., 2019; Wong et al., 2012), these peptides hold the potential to be explored against the novel coronavirus infection. Since it is reported in the literature that the bioactive proteins and peptides are sensitive to environmental factors such as temperature, humidity, pH, and proteolytic environment in the body. It is very crucial to maintain the physicochemical properties, stability, and therapeutic potential of the peptides (McClements, 2018). To solve the stability issues and poor bioavailability of peptides due to pre-systemic enzymatic degradation, these peptides can be formulated by spray-drying (Faheem and Haggag, 2015; Renukuntla et al., 2013), which is a stable, economical, effective, and efficient means of producing peptide-loaded powders suitable for pulmonary delivery (Sarabandi et al., 2020). If the correct formulation and spray-drying conditions can be identified, then a product with a high yield and a large fine-particle dose can be obtained (Niv, 2020). To address the stability issues of these peptides while preparing spray-dried solid dispersion formulation, cryoprotectants such as sorbitol, mannitol and trehalose can be utilized, as reported by various researchers (Dalvi et al., 2021; Eedara et al., 2021; Kaur et al., 2015). The solution of formic acid and tween-80 has been explored in the preparation of spray-dried products (Kaur et al., 2015). Aerosols containing the best peptide according to the present study can be used as preventive aid in immunocompromised individuals who are at high-risk to get infected with this devastating infection.

Conclusion

The current study was focused on identifying plant peptides that can prevent the interactions between the receptor binding site of the S protein and the human ACE2 receptors. The screened plant peptides with reported anti-viral properties were analyzed for the interactions and binding affinity towards the RBD domain of S by docking and dynamic simulations. Overall, five plant peptides were found to possess good docking scores, while only two of the complexes showed small RMSD fluctuations after molecular dynamic simulations i.e. Cycloviolacin Y3 and Cycloviolacin Y1. It is concluded that these two plant peptides not only showed good docking scores in comparison to that for S and ACE-2 but also retained their stability for a period of 30 ns during MD simulation. Owing to strong interactions between the S protein and natural peptides in comparison to that of the S-ACE-2 complex, it can be

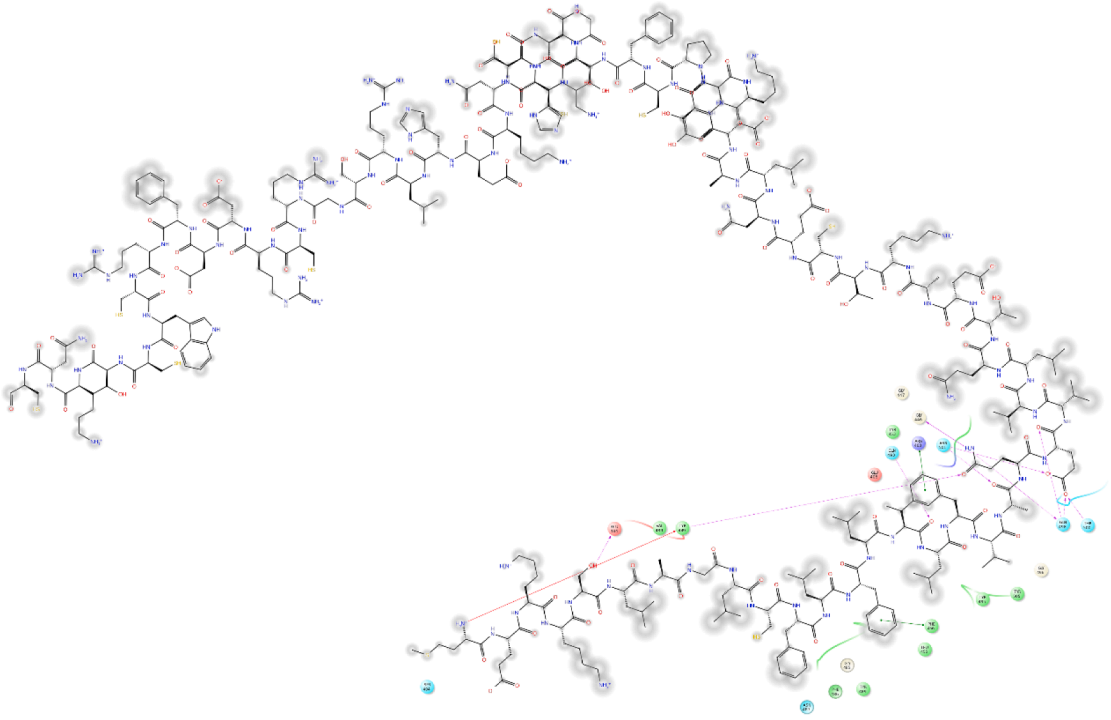

Fig. 3. 2D interaction diagram between the peptide PPepDB_2032 and interface residues of spike protein of SARS-CoV2.

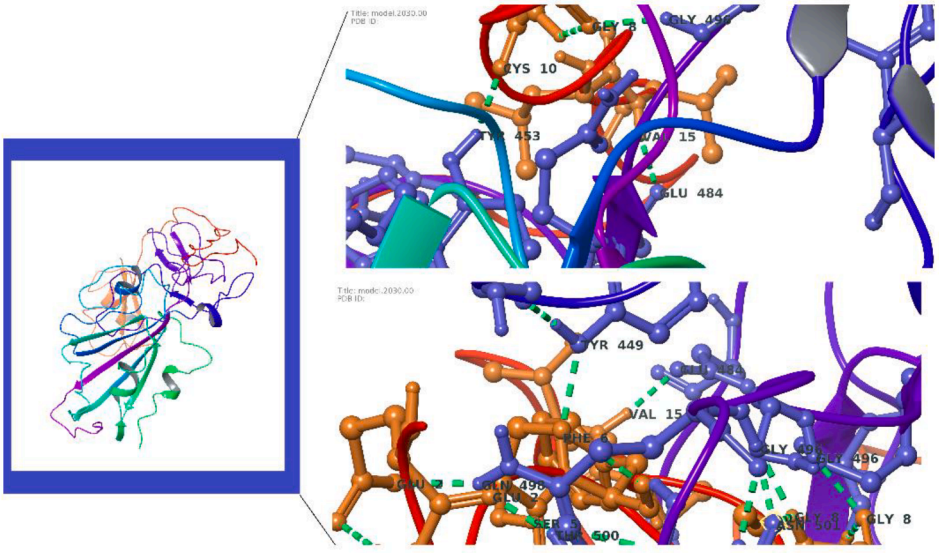

Fig. 4. 3D non-covalent interactions between key residues of RBD of spike protein and PPepDB_2030.

concluded that these peptides may prove to be probable candidates to overcome the COVID-19 infection at an initial stage. This is a preliminary *in-silico* study and the work associated with this study is further open to researchers for extraction of peptides, purification, and *in-vitro* analysis.

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CRediT authorship contribution statement

Priyanka Bhandu: Conceptualization, Data curation, Writing – original draft. **Himanshu Verma:** Writing – review & editing. **Baddipadige Raju:** Writing – review & editing. **Gera Narendra:** Formal analysis. **Shalki Choudhary:** Writing – review & editing. **Manmeet Singh:** Formal analysis. **Pankaj Kumar Singh:** Writing – review & editing. **Om Silakari:** Conceptualization, Formal analysis, Supervision, Writing – original draft.

Declaration of Competing Interest

The author (s) declares no conflict of interest.

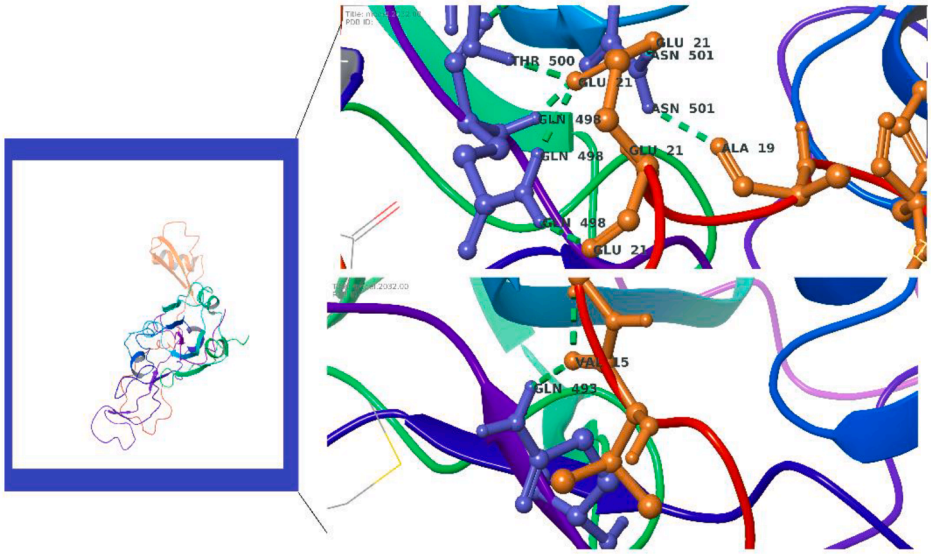

Fig. 5. 3D non-covalent interactions between key residues of RBD of spike protein and PPepDB_2032.

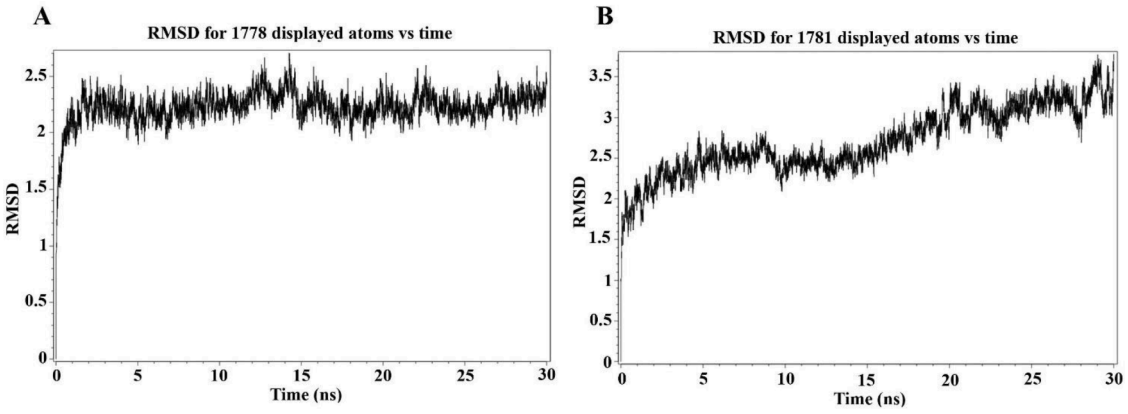

Fig. 6. RMSD values of spike protein complexed with A) Complex PPepDB_1931. B) PPepDB_1937.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.phyplu.2023.100446.

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