Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.
Virtual screening and molecular dynamics simulation study of plant protease inhibitors against SARS-CoV-2 envelope protein

Manisha Kirar, Hitesh Singh, Neelam Sehrawat

Department of Genetics, Maharshi Dayanand University, Rohtak, Haryana, India

ABSTRACT

Due to the outbreak of a new strain of pandemic coronavirus, there is a huge loss of economy and health. In 2021, some vaccines are recommended as emergency licensed vaccines to protect against the virus, and efforts are continuously ongoing to evaluate the vaccine safety measures for licensed vaccines. Recently, there was an increase in the cases of a new variant of coronavirus (omicron). Envelope protein plays an important role in virus packaging and assembly. If viral assembly is blocked, there is less chance of spreading the infection to another cell. In the present study, the plant protease inhibitors (PPIs) were screened against the envelope protein of SARS-CoV-2. The structures were downloaded from the protein data bank. The plant protease inhibitors cystatin-I, Eravatmin, squash, Kunitz, Bowman-Birk, Alpha-amylase inhibitors, and potato serine protease inhibitors were screened and out of them Kunitz, alpha-amylase, and squash protease inhibitors have shown maximum binding energy. The molecular dynamics simulation was performed for docked complexes showing the lowest binding energy by NMA (normal mode analysis) to visualize the motion and stability of complexes. These plant-based protease inhibitors are a good target to fight against the new emerging strain of coronavirus because plant extracted compounds are natural and there is fewer side effect than synthetic compounds.

ARTICLE INFO

Keywords:
Plant protease inhibitors
Envelope protein
SARS-CoV-2
Molecular docking
Molecular dynamics simulation etc

1. Introduction

In recent times, the whole world faced the threat of deadly communicable pathogens. Around 2 million people lost their lives due to no permanent cure for this dangerous virus [1]. Several types of research are still ongoing all over the world to eliminate this disease. SARS-CoV-2 (Severe Acute Respiratory Syndrome) is a highly transmissible human causative virus. The first outbreak of SARS CoV was identified in bats in 2003 in Guangdong province, China [2]. Human SARS-CoV-2 was observed as a new disease in 2019 which is very severe and quickly transmissible as compared to previous reported SARS-CoV [3]. In Wuhan city of China, this virus was first identified in the respiratory tract of a pneumonia patient. The newly identified β-coronavirus (n-CoV) was reported (n-CoV) in December 2019 [4,5]. It is a single-stranded (ss RNA) virus. It has four main structural proteins—Spike glycoprotein, small envelope glycoprotein, Membrane glycoprotein, nucleocapsid protein, and many accessory proteins. The entry of SARS-CoV-2 in a human cell is through the attachment of Spike glycoprotein to ACE2 (Angiotensin Converting Enzyme) receptor of the host cells [6]. Firstly, it infects the ciliated bronchial epithelial cells and types II pneumocytes of the host cell [7]. The Pfizer/BioNTech Comirnaty vaccine was listed for WHO Emergency Use Listing (EUL) on 31 December 2020. Vaccine safety and its efficacy against infection is ongoing and checked by WHO experts in collaboration with the research institute [8]. Globally, there were 402,044,502 confirmed cases of COVID-19 and 5,770,023 deaths were reported in the last week across the world [9]. As of 6th February 2022, a total of 10,095,615,243 vaccine doses have been administered [10]. On 26 November 2021, WHO designated the variant B.1.1.529 a variant of concern, named Omicron, on the advice of WHO’s Technical Advisory Group on Virus Evolution (TAG-VE). The TAG-VE that Omicron has several mutations that it is not yet clear how it spread or how it is severe [11]. The Omicron variant is the most heavily mutated variant which paves the way for enhanced transmissibility and partial resistance to immunity induced by COVID-19 vaccines [12]. Omicron will not be the last variant and the next variant of concern is likely to be more transmissible and there will be more immune escape making existing vaccines less effective against new variants [13].

The plant contains various compounds which act in its defense mechanism against various pests. Plants are a good source for drug...
discovery against various diseases. Plant extraction is the field of investigation which acts as herbal medicines to cure human disease from a long time ago. There are many plant-based drugs like quinine (antimalarial drug), taxol (anticancer drug), and reserpine (antihypertensive drug) were extracted from different plants [14,15]. Plant protease inhibitors have targeted some proteases and shown antiviral activities [16–19]. The envelope protein of SARS-CoV 2 is the most mysterious and smallest protein of 8.4–12 kDa among all the structural proteins. It is integral membrane protein and consists of 76–109 amino acids [20,21]. It undergoes a post-translational modification. It generates a viroporin (ion channel protein) by homotypic interaction [22,23]. The SARS-CoV E protein has recently been found to contain a binding motif known as the postsynaptic density protein 95 (PSD95)/Drosophila disc large tumour suppressor (Dlp1)/zonula occludens-1 protein (zo-1) (PDZ)-binding motif (PBM), located in the last four amino acids of the C terminus [24]. The PDZ domain is a protein-protein interaction module that can bind to the C-terminus of target proteins such as the cellular adapter proteins involved in host-cell processes important for viral infection [25–27]. The PBM-based attenuated and live vaccine would be designed or also enough to abolish and non-functional the pathogenicity of the virus. The Co-expression of M and E proteins led to the formation of virus-like particles (VLP), but not an expression of M protein alone [28,29]. It is abundantly expressed inside the infected cell during replication of the cell cycle but a small portion is incorporated into the virion envelope [30]. Envelope protein has played main three roles, in viral assembly, the release of virions, and pathogenesis of virus [31–33]. Presently, Bowman-Brik Inhibitors, Oryza cystatin, Alpha-amylase, squash, Kunitz, vitamin and Potato Serine Protease Inhibitors were selected according to their inhibitory activity reported against different proteins. The present study aimed to estimate the inhibitory interaction of coronavirus E (Envelope) protein with various types of plant proteases inhibitors by use of bioinformatics tools.

2. Materials and methods

2.1. Preparation of protein and ligands

3-D structure of coronavirus envelop protein (SARS-CoV-2) was downloaded from Protein Data Bank (PDB) https://doi.org/10.2210/pdb2mm4/pdb by using PDB ID 2mm4 and the accession number is M. Kirar et al.
UNIPROT id P59637 as shown in Fig. 1. Plant protease inhibitors [cystatin-I (UNIPROT id P09229), Eravatmin (UNIPROT id P83654), squash(UNIPROT id P01074), Kunitz (UNIPROT id P83667), Bowman-Brik (UNIPROT id P00761) and Alpha-amylase inhibitors (UNIPROT id V5W9K8) and potato serine protease inhibitors (UNIPROT id Q8S380)] were selected based on inhibitory activity against various proteins. The 3-D structures of selected PPIs inhibitor proteins were downloaded from RCSB online software and visualized using the Autodock tool and shown in Fig. 2. Hydrogen atoms were removed from the PDB file by BIOVIA Discovery Studio Visualizer and a modified file was used for docking study.

### 2.2. Active pocket sites prediction for interaction

The Active site of SARS-CoV-2 envelope protein was recognized by CASTp server (Computer Atlas of Surface Topology of protein) [34]. The functionality of CASTp, for measuring protein pockets and cavities is based on accurate computational geometry methods. Active site of envelope protein of coronavirus was displayed in Fig. 3.

### 2.3. Molecular docking

Patchdock (https://bioinfo3d.cs.tau.ac.il/PatchDock) online software was used to dock ligands into protein binding pockets and analyzed the binding affinity of docked ligands. The complex files were submitted to the Firedock (https://bioinfo3d.cs.tau.ac.il/FireDock) to analyze the binding affinity of protein with ligand and verification of docking results.

### 2.4. Molecular dynamic simulation of the complexed structure of receptor-ligand

The docking structures showing the lowest docking energy were

### Table 1
Calculated area, volume and active sites residue of predicted active sites.

| Pocket Id | Area (SA) | Volume (SA) | Active site residue |
|-----------|-----------|-------------|---------------------|
| 1         | 6.677     | 24.593      | (24) Leu, (45) Val, (52) Tyr |
| 2         | 34.086    | 8.634       | (28) Tyr, (31) Arg, (32) Leu, (35) Tyr, (43) Ser, (44) Leu, (48) Thr, (51) Val |
| 3         | 5.593     | 1.010       | (13) Phe, (16) Phe, (17) Val, (20) Leu |
| 4         | 2.182     | 0.401       | (12) Leu, (15) Ala, (16) Phe, (19) Ala |
| 5         | 0.174     | 0.060       | (28) Thr, (32) Leu, (43) Ser, (44) Leu |
| 6         | 0.464     | 0.007       | (35) Ile, (39) Ile, (40) Val, (42) Val |

### Table 2
The binding affinity of ligands with envelope protein.

| Proteases                        | Global Energy (k/mol) | Attractive VdW | Repulsive VdW | ACE | Hydrogen bond | Area of contact |
|----------------------------------|-----------------------|----------------|---------------|-----|---------------|----------------|
| Alpha-Amylase Inhibitor Wrightide R1 | 2.82                  | 0.00           | -0.10         | 0.00| 1198.80       |
| Trypsin:IbI Complex             | 678.05                | 57.03          | 953.37        | -4.45| -3.33        | 2581.30        |
| Potato Serine Protease Inhibitor | 11566.29              | 101.11         | 14783.22      | -43.47| -12.91       | 3177.50        |
| Cysteine Protease Ervatamin     | 1904.89               | 51.98          | 2482.03       | -12.33| -4.65        | 2648.80        |
| Kunitz (Sti) Type Inhibitor     | -33.67                | -25.00         | 12.30         | -5.88| -3.68        | 1760.90        |
| Oryzacystatin-I, A Cysteine     | 561.93                | -16.48         | 779.29        | -14.49| 0.00         | 1811.70        |
| Squash                          | 0.04                  | -1.76          | 0.00          | 0.06| 157.40        |

Fig. 3. Predicted active pocket site from CASTp software for SARS-CoV2 envelope protein.
evaluated for protein stability and motion of complex by molecular dynamic simulation. The molecular dynamics was performed by the mods server (http://imods.chaconlab.org). iMODS facilitates the exploration of such modes and generates feasible transition pathways between two homologous structures [35]. TheiMOD server evaluates the protein stability by computing its internal coordinates through normal mode analysis (NMA). The stability of the protein is represented in terms of its main-chain deformability plot, B-factor values, eigen value, covariance matrix, and elastic network model.

2.5. Structure analysis and visualization

Based on the binding affinity of protease-inhibitors for SARS-CoV-2 envelope protein, the complex structure was analyzed in Discovery

![Molecular docking and its binding pattern, in figure grey colour was used for ligand and red and green was used for envelope protein. In figures ligand alpha-amylase inhibitor wrightidine r1 (Fig. 4a), kunitz (sti) type inhibitor (Fig. 4b) and Squash (Fig. 4c) are shown respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)](image-url)
Fig. 5. Molecular dynamics simulation of squash, kunitz and alpha amylase proteases inhibitors docked with E protein of SARS CO-V. 3 D structure of interaction (a, b), deformability (c, d), B- factor (e, f), eigen value (g, h), variance map (i, j), correlation matrix (k, l), elastic network model (m, n). In the correlation matrix, Colored bars showed the individual (red) and cumulative (green) variances. In the elastic network graph, dots are colored according to their stiffness, the darker greys indicate stiffer springs and vice versa. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)
Fig. 5. (continued).
C. annuum inhibitory activity for MARS-CoV [36]. Stability of complex squash-E. The covariance matrix between the pairs closely related to the energy required to deform the structure. The lower atomic displacements around the equilibrium conformation. The eigen energy is best to deform the structure. The variance map was shown in Fig. 5 (p, q, r), indicating their correlations (red: correlated, white: uncorrelated, blue: anti-correlated). Fig. 5 (s, t, u) shows the elastic network model of amino acids residues. The reference ligands as shown in Fig. 2 were docked into the pocket site of the envelope protein; the binding affinity of the protein with ligand was calculated and shown in Table 2. Proteins with the higher binding affinity were shown in Fig. 4. The criteria of selection were based on its global energy score, lower the global energy higher the binding affinity between protein and ligands. From all proteases alpha-amylase inhibitor wrightideR1 (Fig. 4a) is showing global energy (2.82 k/mol), kunitz (stj) type inhibitor (Fig. 4b) is with global energy (−33.67 k/mol), and Squash (Fig 4c) showing the global energy (0.04 k/mol). From these three, kunitz was the best target inhibitor protease to fight against coronavirus envelope protein because of the highest binding affinity for envelope protein of coronavirus.

3.2. Molecular dynamics simulation of docked receptor-ligand complexes

The 3 D interaction of receptor and ligand complexes of squash, kunitz and alpha amylase proteases inhibitors with envelope protein of coronavirus were shown in Fig. 5 (a, b, c). The arrow indicates the direction of motion of amino acids residues. Fig. 5 (d, e, f) represents the deformability graph which showed that the higher deformability is present at mainly hinge regions that is flexible. The NMA B-factor represents Fig. 5 (g, h, i) the profile of mobility and relative amplitude of the atomic displacements around the equilibrium configuration. The eigen values are shown in Fig. 5 (j, k, l) represent the motion stiffness which is closely related to the energy required to deform the structure. The lower energy is best to deform the structure. The variance map was shown in Fig. 5 (m, n, o) that is inversely proportional to the eigen value. The eigen value for squash, kunitz and alpha amylase complexed with E protein is 4.549e-05, 8.836e-05, 7.540e-05 that indicating the greater stability of complex squash-E. The covariance matrix between the pairs of residues is shown in Fig. 5 (p, q, r), indicating their correlations (red: correlated, white: uncorrelated, blue: anti-correlated). Fig. 5 (s, t, u) shows the elastic network model of amino acids residues.

4. Discussion

In 2020, Coronavirus has become a big challenge for world. Recently, the virus spread worldwide and causing numerous deaths. There is no specific drug available for the disease but some vaccines are available to activate immunity against virus for future infection. It becomes a controversy that these vaccines are effective for new strain of virus. There is no surety of vaccines that is completely effective against coronavirus so we focused on some natural proteases inhibitors which can be helpful to control the spreading of Coronavirus. The study is based on specific targeting of SARS CoV-E protein to find novel compounds that can be used as a new agent against Coronavirus. Plant extracts are a good source to fight against HIV, Hepatitis C virus, and also for MARS-CoV [36–38]. Two protease inhibitors were purified from C. annuum leaves (i.e., CapA1 and CapA2) with in vitro and in vivo inhibitory activity for Helicoverpa armigera proteinases. Trypsin inhibitors from Capsicum baccatum var. pendulum leaves show inhibitory activity against Pepper yellow mosaic virus [39]. The Plant Proteases inhibitors (PPIs) are small proteins that are accumulated in storage tissues e.g. seeds and tubers and also found in plant aerial parts [40]. The protease inhibitors have been found in legumes and cereals [41–43]. The widespread distribution of protease inhibitors throughout the plant kingdom is well known since 1938 [44]. The plant contains various kinds of proteases inhibitors belonging to the family such as Kunitz, Bowman-Birk, Potatol, Potato II, serpine, squash, rapeseed, mustard, cereal [45,46]. These inhibitors have been used as therapeutic agents [47–49]. Ervatamia C, a cysteine protease was isolated from the Ervatamia coronaria was a potent inhibitor of Ervatamin. It can hydrolyze (BAPA) benzoylarginine-p-nitroanilide, a potent substrate of papain and very highly specific activity cleaves denatured natural proteins [50]. From Delonix Regia seeds (DrTI), a kunitz (STI) inhibitor was isolated and it is an effective inhibitor of HPK (Human Plasmat Kallikrein) and trypsin [51]. Alpha-amylase inhibitors are Wr-AI1 and Wr-AI2 isolated from W. religiosa and both of them have antibacterial and hemolytic activity and inhibition against TMA (Tenebrio Mollit α-amylase) [52]. Bowan- Brik Inhibitors are isolated from barley seeds that inhibit two trypsin molecules [53]. Oryza cystatin has potent inhibitory activity against papain and cysteine proteases and is isolated from Oryza sativa L. Japonica [54]. Potato tuber’s protein inhibits the serine protease [55]. These plant proteases inhibitors inhibit the target protein and may be useful for natural drugs formation.

New Kunitz TI from seeds of B. variegata var. Variegata Bauhinia trypsin inhibitor with the abilities to induce the production of multiple cytokines and selectively inhibit the proliferation of nasopharyngeal carcinoma CNE-1 cells [56]. We perform molecular docking of the seven PPIs (Bowan Brik Inhibitors, oryzacystatin, alpha amylase, squash, kunitz, ervatamin and potato serine protease inhibitors) which already have potential efficacy against TMA (Tobacco Mosaic Virus), HIV virus [57]. Envelope protein of virus has main role in assembly, packaging and pathogenesis. The protein is also a role in the formation of VLP (Virus-Like Particle) by co-associated with the M protein. Targeting the envelope protein of coronavirus is efficient to stop viral assembly and pathogenesis of the virus. Among seven PPIs only 3 (alpha-amylase, squash, kunitz) have the lowest docked binding energy with the target. The alpha-amylase, squash, and kunitz have binding energy 2.82, 0.04, −33.67. The kunitz was having the lowest binding energy in comparison to squash and alpha-amylase inhibitors. We have also done molecular dynamics simulation of these three docked complexes to check the stability and motion of the structure. The eigen value for squash, kunitz, and alpha-amylase complexed with E protein is 4.549e-05, 8.836e-05, 7.540e-05 indicating the greater stability of complexes. The results reveal that the complex structure is stable and can be used as a drug against coronavirus. Our study may be helpful to the formation of a new drug against coronavirus.

5. Conclusion

From the current effort, we have screened PPIs (Plant Protease inhibitors) against SARS CoV envelope protein by molecular docking and molecular dynamic simulation. Among these screened plant proteases inhibitors kunitz inhibitor, alpha amylase inhibitors and squash inhibitors have showed good binding energy. These inhibitors are the fine target for drug development against coronavirus strain. The binding energy of kunitz inhibitor was the best among these three inhibitors. Kunitz inhibitors are the superior protease inhibitors to fight against new strain of coronavirus.

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.
Acknowledgement

Authors are thankful to Radha Krishnan Fund, Maharshi Dayanand University, Rohtak for providing computational facility.

References

[1] WHO. Weekly epidemiological update on covid. https://www.who.int/publications/m/item/weekly-epidemiological-update-on-covid-19. Accessed date: 6-July-2021. 2021.

[2] Jie C, Li F, Shi ZL. Origin and evolution of pathogenic coronaviruses. Nat Rev Microbiol 2019;17(1):1-22.

[3] Hu B, Guo H, Zhou F, Shi ZL. Characteristics of SARS-CoV-2 and COVID-19. Nat Rev Microbiol 2021;19:141-54.

[4] Guo YR, Cao QD, Hong ZS, Tan YY, Chen SD, et al. The origin, transmission and clinical therapies on coronavirus disease 2019 (COVID-19) outbreak–an update on the status. Mil Med Rev 2020;7(1):1-10.

[5] Tian X, Li HU, Huang A, Xia S, Lu S, Li Z, et al. Potent binding of 2019 novel coronavirus spike protein by a SARS coronavirus-specific human monoclonal antibody. Emerg Microbes Infect 2020;9(1):325-5.

[6] Rabi FA, Al Zoubi MS, Kanabehe GA, Salehme DM, Al-Nasser AD. SARS-CoV-2 and Coronavirus Disease 2019: what we know so far. Pathogens 2020;9:231.

[7] Qian Z, et al. Innate immune response of human alveolar type II cells infected with severe acute respiratory syndrome coronavirus. Am J Resp Cell Mol Biol 2013;48:742-7.

[8] Fang EF, Wong JH, Ng TB. Thermostable Kunitz trypsin inhibitor with cytokine inducing, antitumor and HIV-1 reverse transcriptase inhibitory activities from L. japonica. Biochemistry 2000;39:14753-60.

[9] Ye Y, Hogue BG. Role of the coronavirus E viroporin protein transmembrane domain in virus assembly. J Virol 2007;81(7):3597-607.

[10] Jenner-Guadarmo NJ, Mieto-Torres JL, DeDiego ML, Regla-Nava JA, Fernandez-Lozano JM, Nieto-Torres JL, Fernandez-Jordán JM, Núñez J, Ribas-Carbo M. Proteolytic enzymes and their inhibitors in plants. Annu Rev Plant Physiol Plant Mol Biol 1997;48:39-70.

[11] Sampaio CA. Purification and primary structure determination of a Bowman-Birk type inhibitor from Boronensis cereais seeds. Biol Chem 1997;378:273-81.

[12] Norioka S, Ikenaka T. Aminocidic sequence of tryptic coronavirus inhibitors (A-I, A-II and B-I) from peanut (Arachis hypogaea). A discussion on the molecular evolution of legume Bowman-Birk type inhibitor. Biochim Biophys Acta 1984;798:90-99.

[13] RyanGA. Proteinolytic enzymes, antiviral enzymes, and their inhibitors in plants. Annu Rev Plant Physiol Plant Mol Biol 1997;48:173-96. https://doi.org/10.1146/annurev.24.060173.01133v.

[14] Laskowski MJ. Plant kunitz inhibitors from Bauhinia bauhinioides and Bauhinia rufa on field bean protease inhibitor can function as an efficient tumor detecting agent. Biochim Biophys Acta 1993;1152:135-44.

[15] Thakurta PG, Sapa B, Chandana C, Monica S, Medicherla V, et al. Effect of 99mTc-labeled proteinase K on coronavirus 3-chymotrypsin-like protease (3CLPro): an in vitro study. J Radioanal Nucl Chem 2021;300(1):119-26.

[16] Jeong JH, Kim YS, Zhihong X, Yu ST, Keung CH, et al. Structural basis of the binding promiscuity of PDZ domains. PLoS Comput Biol 2012;8(11):e1002749.

[17] Ye Y, Hogue BG. Role of the coronavirus E viroporin protein transmembrane domain in virus assembly. J Virol 2007;81(7):3597-607.

[18] Meulenbroek EM, Ellen AJT, Laurice P, Jan PA, HGand Navraj SP. Structure of a 16 KDa coronavirus spike protein at 2.8 A resolution: insights into its unique conformation and glycosylation. PLoS Pathog 2021;17(6):e1009633.

[19] Jie C, Li F, Shi ZL. Origin and evolution of pathogenic coronaviruses. Nat Rev Microbiol 2019;17(1):1-22.

[20] Lim KP, Liu DX. The missing link in coronavirus assembly. Retention of the avian coronavirus infectious bronchitis virus envelope protein in the pre-Golgi compartment and physical interaction between the envelope and membrane proteins. J Biol Chem 2001;276:17515-23.

[21] Corse E, Machamer CE. Infectious bronchitis virus E protein is targeted to the Golgi complex and directly releases virus-like particles. J Virol 2000;74:4319–26.

[22] Ye Y, Hogue BG. Role of the coronavirus E viroporin protein transmembrane domain in virus assembly. J Virol 2007;81(7):3597-607.

[23] pangalosvm, pascalova V, Dooliana LM, lowe LA, dolzelka K, Hope BG. Coronavirus envelope (E) protein remains at the site of assembly. Virology 2015;478:75-85.

[24] Ruch TR, Machamer CE. The hydrophobic domain of infectious bronchitis virus E protein alters the host secretory pathway and is important for release of infectious virus. J Virol 2011;85:2677-84.

[25] Ran S, Ran B, Chen S, Gao Y, Sun J, et al. The PDZ-binding motif of severe acute respiratory syndrome coronavirus envelope protein is a determinant of viral pathogenesis. PLoS Pathog 2014;10(8):e1004320.

[26] Ye Y, Hogue BG. Role of the coronavirus E viroporin protein transmembrane domain in virus assembly. J Virol 2007;81(7):3597-607.

[27] Gerek ZN, Keskin O, Ozkan SB. Identification of specificity and promiscuity of PDZ domain interactions through their dynamic behavior. Proteins Struct Funct Bioinf 2012;80(10):2765-79.

[28] Ye Y, Hogue BG. Role of the coronavirus E viroporin protein transmembrane domain in virus assembly. J Virol 2007;81(7):3597-607.

[29] Le Deef, Volpillicia M, MicheliiI, Lani S, Gallerani R, CecilRI. PLANT-Pfic: a database for plant protease inhibitors and their genes. Nucleic Acids Res 2002;30:347-8.

[30] Laing WA, McManus MT. Protein interactions in plants, vol. 7. Sheffield Academic Press; 2002. p. 77–119.

[31] Tanaka AS, Sampaio MU, Marangoni S, de Oliveira B, Novello JC, Oliva ML, Fink E, Sampaio CA. Purification and primary structure determination of a Bowman-Birk tryptase inhibitor from Trtoraessa cereaisc cereais seeds. Biol Chem 1997;378:273-81.

[32] Kapoor LD. Handbook of ayurvedic medicinal plants. Boca Raton, Florida: CRC Press; 1990.

[33] Kapoor LD. Handbook of ayurvedic medicinal plants. Boca Raton, Florida: CRC Press; 1990.

[34] Jie C, Li F, Shi ZL. Origin and evolution of pathogenic coronaviruses. Nat Rev Microbiol 2019;17(1):1-22.

[35] Bellenchii D, Aliaga J, Quintero Dezes E, Chacón P. IMOSD: Internal coordinates normal mode analysis server. Nucleic Acids Res 2014;42:W271-6. https://doi.org/10.1093/nar/gkt339.

[36] Babare S, Nanjangud v, Anil K, Bilge S, Mehdi SR, Mehtap K, Gail BM, Sanja V, Morrelos L, Farrand K, Williams AA, Sneyd AY, Athar Aam Javed JR, Medicinal plants used in the treatment of human immunodeficiency virus. Int J Mol Sci 2018;19(5):1459.

[37] Jadina GM, Jacqueline FS, Paula R, Mark H. Plant-derived antivirals against hepatitis C virus infection. Virol J 2018;15:54. https://doi.org/10.1186/s12985-018-0945-5.

[38] Gideon AG, Alekebanek OA, Adegebono PA, Oluwadare MA, Osabode OA. Potential inhibitors of coronavirus 3-chymotrypsin-like protease (3CLpro): an in silico screening of alkaloids and terpenoids from African medicinal plants. J Biomol Struct Dyn 2021;39(9):3936-408.

[39] Moulin MM, Rodrigues R, Ribeiro SFF, Goncalves LSA, Bento CS, Sudre CP. Trypsin inhibitors from Capsicum baccatamcarvan. pendum leaves involved in pepper yellow mosaic virus resistance. J Virol Res 2014;21:925-9.

[40] De Leef, Volpillicia M, Liciafilii, Lani S, Gallerani R, CecilRI. PLANT-Pfic: a database for plant protease inhibitors and their genes. Nucleic Acids Res 2002;30:347-8.

[41] Murugesan S, Banerji AP, Samuel FD, Fernandes AO. Effect of the coronavirus E viroporin protein transmembrane domain on virus assembly. J Virol 2007;81(7):3597-607.

[42] Murugesan S, Banerji AP, Samuel FD, Fernandes AO. Effect of the coronavirus E viroporin protein transmembrane domain on virus assembly. J Virol 2007;81(7):3597-607.

[43] Murugesan S, Banerji AP, Samuel FD, Fernandes AO. Effect of the coronavirus E viroporin protein transmembrane domain on virus assembly. J Virol 2007;81(7):3597-607.

[44] Murugesan S, Banerji AP, Samuel FD, Fernandes AO. Effect of the coronavirus E viroporin protein transmembrane domain on virus assembly. J Virol 2007;81(7):3597-607.