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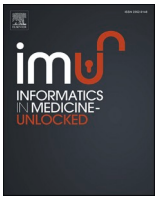

Virtual screening and molecular dynamics simulation study of plant protease inhibitors against SARS-CoV-2 envelope protein

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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.

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 going on 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

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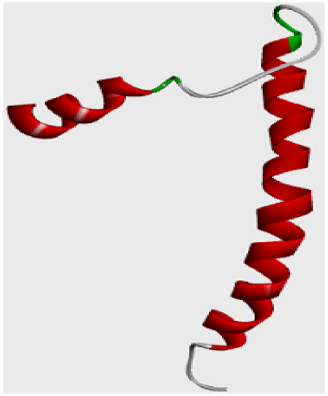

Fig. 1. 3-D structure of SARS- CoV- 2 envelope protein.

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 (anti-malarial 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 (Dlg1)/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, Bowanman-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/pdbby using PDB ID 2mm4 and the accession number is

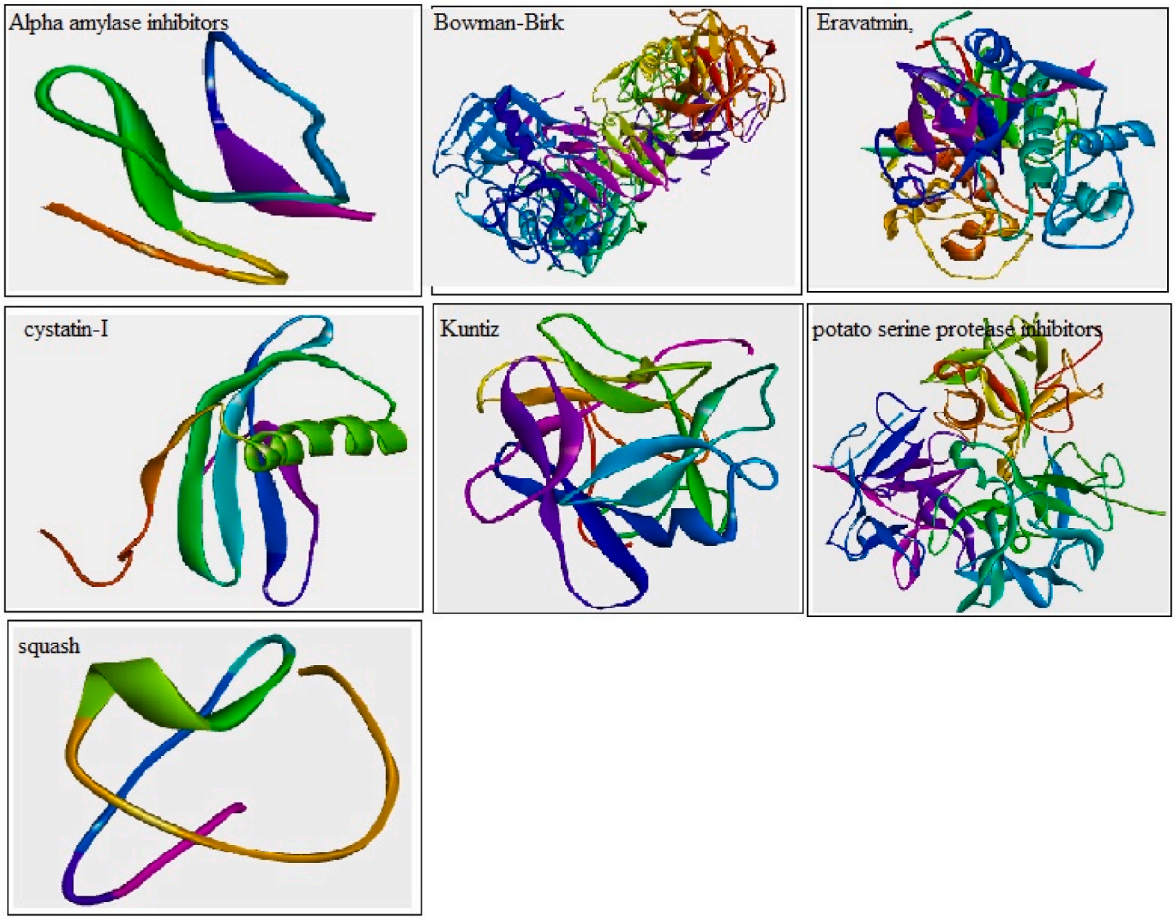

Fig. 2. 3-D structure of PPIs: Alpha amylase inhibitors (4BFH), Bowman- Brik (1TX6), Ervatamin (1o0E), Cystatin-I (1EQK), Kunitz (1R8N), Potato serine protease inhibitors (3TC2), Squash (3CTI).

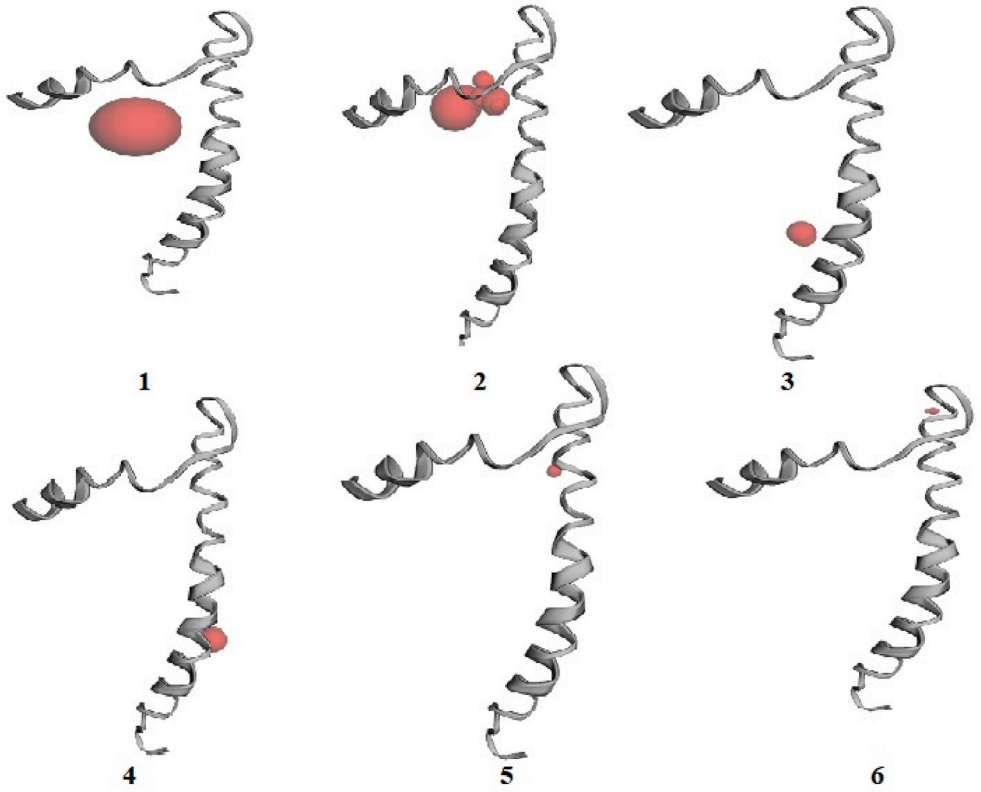

Fig. 3. Predicted active pocket site from CASTp software for SARS-Cov2 envelope protein.

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, (45) Val, (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)Try, (39 Ile, (40) Val, (42) Val**

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 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	−0.04	0.00	−0.10	0.00	1198.80
Trypsin:Bbi 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	−12.35	−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	0.00	157.40

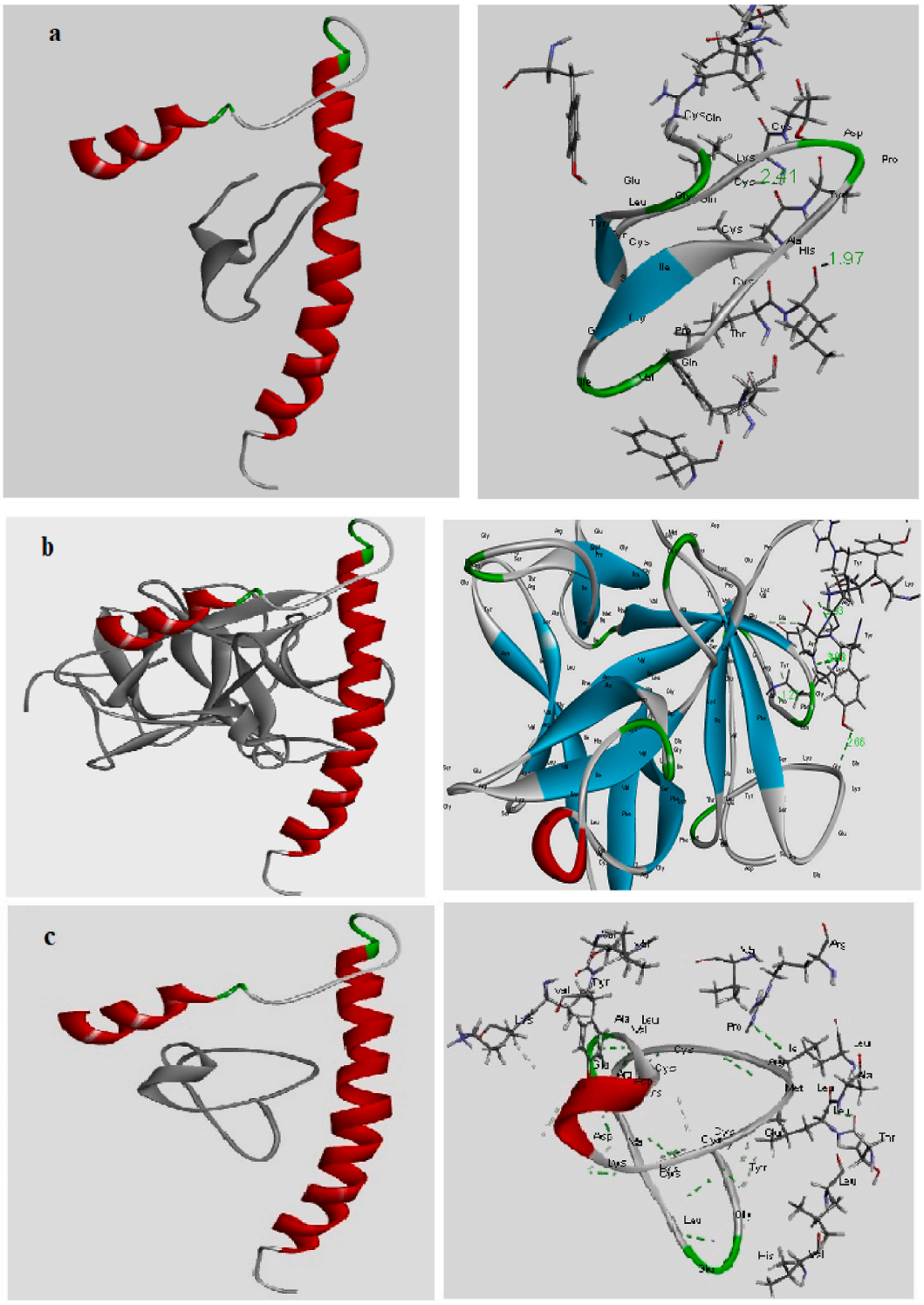

Fig. 4. Molecular docking and its binding pattern, in figure grey colour was used for ligand and red and green was used for envelope protein. In figures ligand alpha-amylase inhibitor wrightide r1 (Fig. 4a), kunitz (sti) type inhibitor (Fig. 4b) and Squash (Fig. 4c) are shown respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

evaluated for protein stability and motion of complex by molecular dynamic simulation. The molecular dynamics was performed by the mods server (http://imods.chaco nlab.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

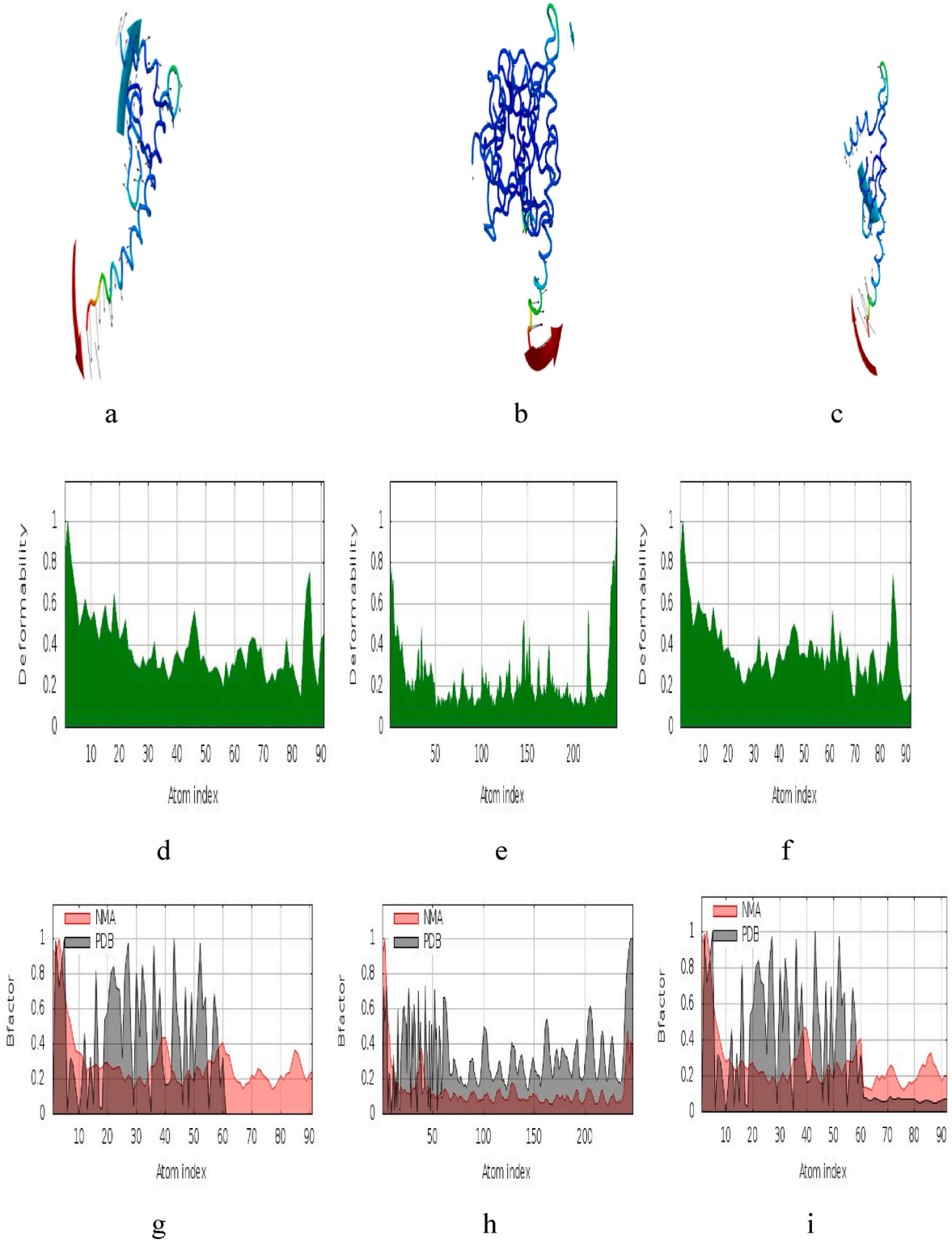

Fig. 5. Molecular dynamics simulation of squash, kunitz and alpha amylase proteases inhibitors docked with E protein of SARS CO-V. 3 D structure of interaction (a, b), deformability (c, d), B- factor (e, f), eigen value (g, h), variance map (I, j), correlation matrix (k, l), elastic network model (m, 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.)

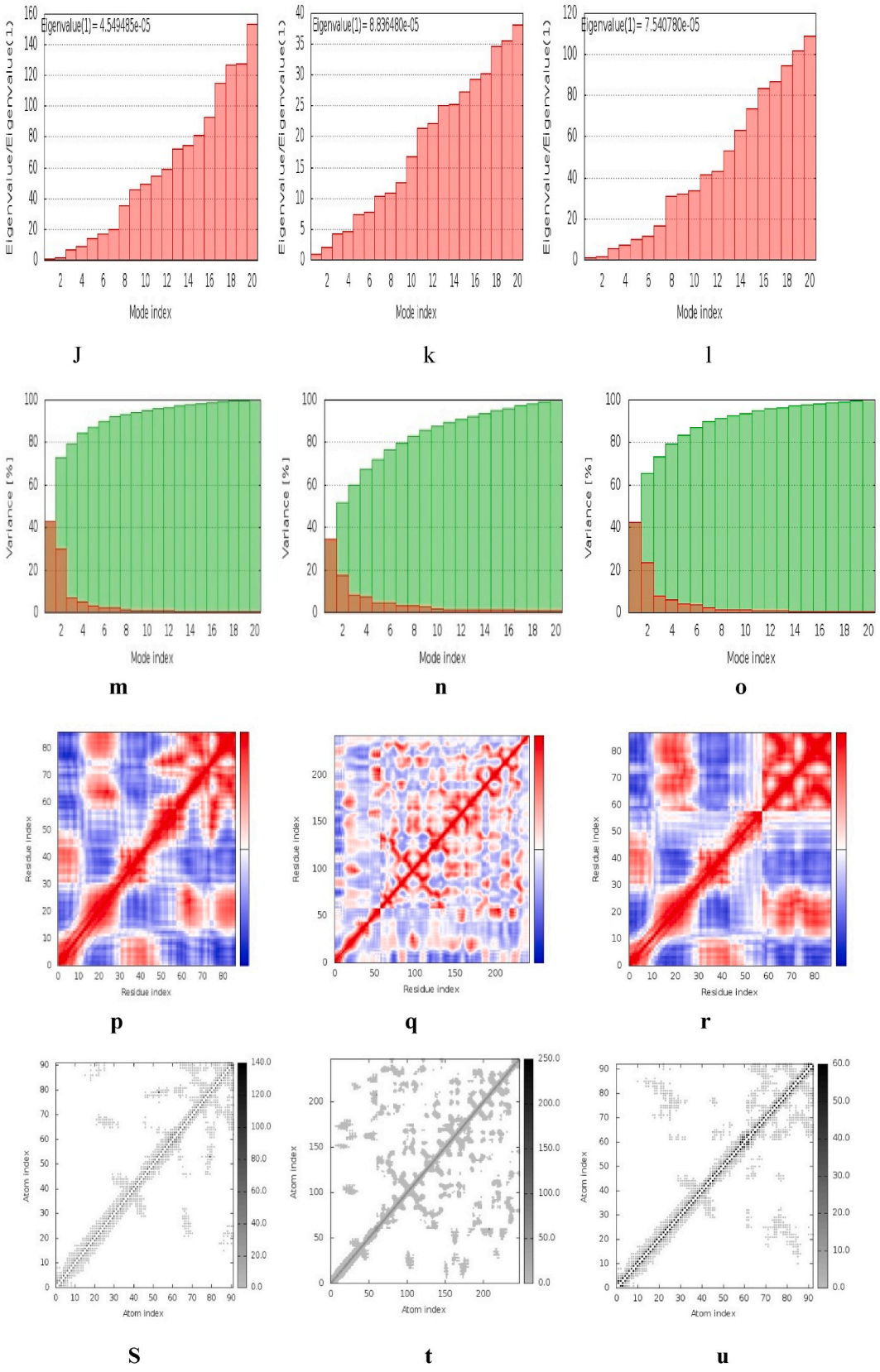

Fig. 5. (*continued*).

studio Visualizer.

3. Results

3.1. Active site and molecular docking for protein interaction analysis

Six active sites were predicted for interaction with other molecules by CASTp. The active site regions of envelope protein were shown in Fig. 3**.** These active sites were used for binding with ligands which are different from each in their size, area, and volume shown in (Table 1). The active pocket site 1 is the specific binding site of all the ligands **(24) Leu, (45) Val, (52) Tyr.**

The envelope protein was docked with all selected protease inhibitors. 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 (sti) 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 conformation. 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 inverserly 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 controversial 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 proteinase 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, PotatoI, Potato II, serpine, squash, rapeseed, mustard, cereal [45,46]. These inhibitors have been used as therapeutic agents [47–49]. Ervatamin 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 Plasma 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 Molitor 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. Variegate *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.

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