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Original article

In silico evaluation of flavonoids as effective antiviral agents on the spike glycoprotein of SARS-CoV-2

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ABSTRACT

The novel coronavirus pandemic has spread over in 213 countries as of July 2020. Approximately 12 million people have been infected so far according to the reports from World Health Organization (WHO). Preventive measures are being taken globally to avoid the rapid spread of virus. In the current study, an *in silico* approach is carried out as a means of inhibiting the spike protein of the novel coronavirus by flavonoids from natural sources that possess both antiviral and anti-inflammatory properties. The methodology is focused on molecular docking of 10 flavonoid compounds that are docked with the spike protein of SARS-CoV-2, to determine the highest binding affinity at the binding site. Molecular dynamics simulation was carried out with the flavonoid-protein complex showing the highest binding affinity and highest interactions. The flavonoid naringin showed the least binding energy of −9.8 Kcal/mol with the spike protein which was compared with the standard drug, dexamethasone which is being repurposed to treat critically ill patients. MD simulation was carried out on naringin-spike protein complex for their conformational stability in the active site of the novel coronavirus spike protein. The RMSD of the complex appeared to be more stable when compared to that of the protein from 0.2 nm to 0.4 nm. With the aid of this *in silico* approach further *in vitro* studies can be carried out on these flavonoids against the novel coronavirus as a means of viral protein inhibitors.

1. Introduction

Coronavirus disease 2019 (COVID-19) or 2019 novel coronavirus (2019-nCoV) is a severe lower respiratory tract infection caused in humans. The deadly scenario of COVID-19, started in the month of December 2019, was unraveling in Wuhan city of China and spread across the globe like a wildfire. In the month of January, WHO declared the outbreak as global emergency (Cascella et al., 2020). According to the International Committee on Taxonomy of Viruses (ICTV), Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the present reference name for the virus (Y. Wu et al., 2020b). Compared to the 2002 SARS epidemic, COVID-19 is apparently less lethal but far more contagious as it spreads with ease among individuals. This novel coronavirus is believed to have instigated as a zoonotic virus that has mutated or adapted to human pathogenicity (Ye et al., 2020). The coronaviruses are enveloped single-stranded RNA viruses that can cause several symptoms including fever, troubled respiration, pneumonia and inflammation of the lungs. There are six known coronaviruses, other than SARS-CoV-2, in humans: HCoV-229E, HCoV-OC43, SARS-CoV, HCoV-NL63, HCoV-HKU1, and MERS-CoV (Adhikari et al., 2020). It has been officially declared by the WHO as an international public health emergency of concern. Higher transmissibility and pandemic risk levels were found to be higher than SARS-CoV, as the effective reproductive number (R) of COVID-19 (2.9) was estimated at the early stage to be higher than that of SARS-CoV (1.77). Early patterns of the Covid-19 pathogen have shown similarity to the viral agents of Severe Acute Respiratory Syndrome (SARS) and Middle East respiratory syndrome (MERS),

Abbreviations: **SARS-CoV-2**, Severe Acute Respiratory Syndrome Coronavirus 2; **CoVs**, Coronaviruses; **COVID-19**, Coronavirus Disease 2019; **PDB**, Protein Data Bank; Rg, Radius of Gyration; RMSD, Root Mean Square Deviation; RMSF, Root Mean Square Fluctuation.

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the two major coronaviruses that had caused pandemics over the last two decades. Since emerging in China in December 2019, Covid-19 has spread across the globe like a wildfire, claiming approximately 10,25,700 deaths and infecting more than 34 million people (Fig. 1) as of 2nd October 2020 (WHO Weekly operational update and Epidemiological update October 2020 reports).

The spike (S) protein, nucleocapsid (N) protein, and envelope (E) protein are main structural proteins encoded by the viral genome of coronavirus. However, for some CoVs, full ensemble of structural proteins is not necessarily a requirement to form a complete, infectious virion (Schoeman and Fielding, 2019). The clinical response spectrum of SARS-CoV-2 is quite broad, ranging from completely asymptomatic to death suggesting variability in either the configuration or the number of ACE-2 targets from person to person. The S1 subunit of the spike protein of coronavirus contains the receptor-binding domain that attaches the virus to the ACE-2 receptor molecule on the host cell membrane. ACE-2, angiotensin converting enzyme-2 is a transmembrane zinc metalloprotein found in the lungs, GI tract, kidneys and blood vessels (Li, 2016).

Each of the spike protein is composed of 3 domains. The large outermost ectodomain is referred to the receptor-binding domain which is responsible for binding to the ACE-2 receptor on host cells as well as getting cleaved off in order to activate underlying domains within the spike protein. This domain also consists of a membrane-fusion subunit S2 which is activated by the S1-ACE2 binding and helps to fuse the virus to the host cell, resulting in the entry of viral genome. The other two segments are- a short intracellular tail and a single-pass transmembrane anchor. The spike protein has thus been the focus of vaccine and antiviral drug development (Ou et al., 2020).

The goal of this virus is to reproduce and infect as any hosts as possible to ensure its existence. The new guidelines from the Chinese health authorities outlined three main COVID-19 transmission routes: 1) droplet transmission, 2) contact transmission and 3) aerosol transmission. If respiratory droplets are swallowed or inhaled in close proximity by people nearby, droplets transmission occurs; contact transmission arises if a person touches a virus-contaminated surface and then touches the 'T-zone' on their face, primarily the eyes, nose or mouth; and aerosol transmission can happen when respiratory droplets blend in the air, form aerosols and cause infection in a considerably confined area when a large portion of aerosols is inhaled into the lungs. The critically ill patients apart from respiratory failure have other common features which include- elevated inflammatory parameters (CRP, IL-6, IL-8, etc.), diminished lymphocytes in lymphoid organs, unrestrained release of pro-inflammatory cytokines (cytokine storm) and multiple organ failure (R. Wu et al., 2020a).

Hence, in order to halt this human-to-human transmission chain, many preventive, non-pharmaceutical measures have been implemented. Due to the urgent nature of the pandemic, several companies and researchers are working on the development of COVID-19 related therapies. There are drugs that have been repurposed and have gained emergency use authorization (EUA) from the FDA. Recently WHO welcomed the initial clinical trial results from the United Kingdom that show dexamethasone, a corticosteroid has the potential to save lives of critically ill SARS-CoV-2 patients who have cytokine storm leading to ARDS (Acute Respiratory Distress Syndrome) (Horby et al., 2020; Robinson, 2020). It exhibits both immunosuppressive and anti-inflammatory effects and halts two phases of inflammation- migration of immune cells and vasodilation. Compounds having such properties also inhibit over activation of macrophages which is one of the main culprits of SARS-Cov-2 (Zhang et al., 2020). Such chemical-based drugs can initiate many side effects in human body. Usage of dexamethasone for a longer period of time can weaken the capability of body's adrenal glands for producing corticosteroids (BC Cancer Agency, 2014). For this reason, natural sources need to be promoted that can act as a safe alternative to these drugs (Xian et al., 2020).

Flavonoids from natural sources have gained extreme importance in the field of research because of their versatile benefits. Several flavonoids have shown to have anticancer activities, anti-inflammatory activity, antimicrobial activity, antioxidative activity and few flavonoids also exhibit antiviral property (Cushnie and Lamb, 2005; Kumar et al., 2020; Zandi et al., 2011). For this study, few flavonoids have been listed out having both anti-inflammatory as well as antiviral properties which exhibited high binding affinity with the spike protein of the SARS-CoV-2 (Cheng et al., 2020; Rathee et al., 2009; Stebbing et al., 2020; Zakaryan et al., 2017). Function of enzyme systems involved in the generation of inflammatory processes can be affected by the flavonoids (Middleton and Kandaswami, 1992). They can be classified into various classes such as flavones, anthocyanidins, flavonols, isoflavonoids, flavanones etc. that vary in the oxidation levels and pattern of replacement of the pyrene ring, while within each class individual compounds vary in the substitution of benzene rings. Specific flavonoids have been reported to have exhibited antiviral and

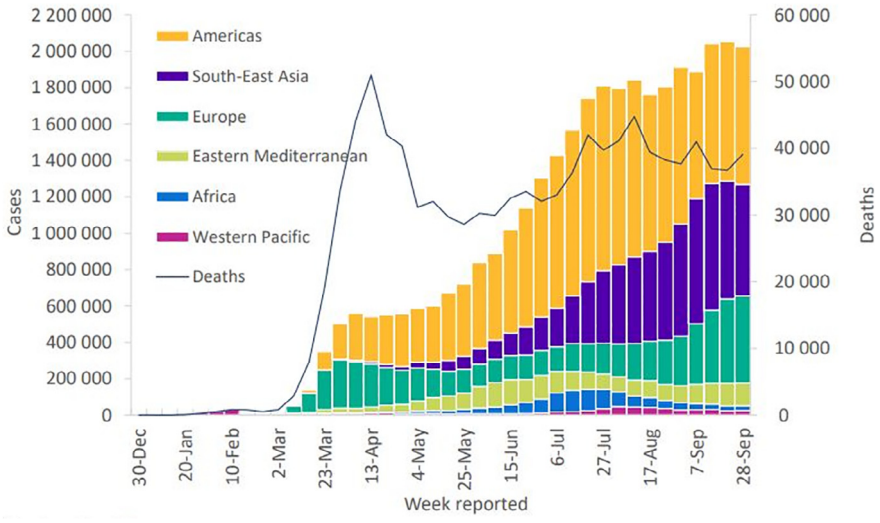

Fig. 1. COVID-19 confirmed cases reported weekly by WHO region and worldwide deaths, 30 December 2019 through 04 October 2020.

anti-inflammatory property very effectively (Dai et al., 2019; Middleton and Kandaswami, 1992). Table 1 gives the list of compounds to carry out virtual screening to look for the best flavonoid compound having the highest binding affinity with the spike protein of SARS-CoV-2.

A bioinformatics approach can help predict the interactions of the listed flavonoid compounds with the spike protein of SARS-CoV-2. These Interactions are carried out by molecular docking process of the small ligand molecules with the neighboring amino acid residues of the receptor protein (Hosseini and Amanlou, 2020). These docked compounds can be analyzed based on the estimated binding energy, bonded and non-bonded interactions. Lesser the binding affinity, the more strongly the target molecule and the ligand are attached to and bind to one another (Radwan and Alanazi, 2018). Molecular dynamics simulation for the best docked protein–ligand complex was carried out to simulate the atomic motions based on Newton's second law for determining the stability of the complex. There are several force fields that govern the molecular dynamics such as AMBER and CHARMM (Abraham et al., 2015).

Following is the list of software/tools/webservers used in this study to carry out the *in silico* work:

1.1. Protein data bank (PDB)

It is a repository of structural information of protein macromolecules obtained by X-ray crystallography and nuclear magnetic resonance (NMR) spectrometry. To the global community, all the 3D structures of the proteins are freely and publicly available (Flower, 2014)

1.2. PubChem

It is an open chemistry repository that contains information on small molecules and even large molecules such as peptides nucleotides etc. This repository is systematized in three discrete databases: PubChem substance

PubChem compound and PubChem bioassay (Flower 2014)

1.3. Castp

It stands for Computed Atlas of Surface Topography of Protein**.** To measure and detect the binding pockets of the proteins, the alpha shape theory from computational geometry is being applied. It returns the exact binding residues forming the pocket mouth and the pockets are ranked based on their volume and area (Binkowski et al., 2003).

1.4. Rampage

Ramachandran plot of the proteins is analyzed using the Rampage software. The 2-dimensional plots of the protein backbone ϕ-ψ dihedral angles represent the Ramachandran plot. It shows the conformation of proteins as very residues fall in favored allowed and disallowed regions (Lovell et al., 2003).

1.5. Open babel GUI –2.4.1

To interconvert the chemical file formats, this computer software is used.

1.6. Argus lab

This program has the ability to perform semi-empirical geometry optimization using UFF force field and molecular mechanics. The geometry of a molecule plays a major role in energy level and its properties including both physical and chemical.

1.7. PyRx 0.8

It is virtual molecular screening open source software that docks ligands with receptor macromolecules to find lead compounds. The input files are converted to PDBQT files and then docking is carried out by AutoDock Vina. The ligand is rotated and moved to find the best binding pose within the grid which surrounds the binding sites of the receptor. The structure data file (SDF) format stores arbitrary data along with data types and their coordinates. The excel format file of the results saves the best predicted binding poses along with the corresponding binding energy values. The more negative is the values, the better and stronger is the binding between ligand and receptor (Dallakyan and Olson, 2015).

1.8. Gromacs

Free software to perform molecular dynamics on protein–ligand complex. Simulations with velocity verlet, leap-frog verlet, stochastic and Brownian dynamics are supported along with calcu-

Table 1
Classification of dietary flavonoids along with their class, source and biological activities.

Sl. No.	Flavonoid	Class	Source	Biological activity
1.	Apigenin (Salehi et al., 2019; Wu et al., 2017)	Flavone	Chamomile tea, leaves of spinach, parsley, oregano, oranges, celery, onions, red wine	Anti-oxidant, anti-carcinogenic, anti-proliferative, anti-inflammatory, antiviral
2.	Chrysin (Schnitzler et al., 2010)	Flavone	Honey, passion flowers, propolis, carrots	Anticancer, anti-inflammatory, anti-proliferative, neuroprotective
3.	Fisetin (Jash and Mondal, 2014)	Flavonol	Grapes, acacia leaves, strawberry fruits	Anticancer, antiviral, immunosuppressive, anti-inflammatory
4.	Galangin (Meyer et al., 1997)	Flavonol	Propolis, leaves of lesser galangal	Anti-inflammatory, antiviral, antibacterial
5.	Hesperetin (Ahmadi et al., 2016; Xiao et al., 2018)	Flavanone	Lemon, sweet oranges, peppermint	Anti-oxidant, anti-inflammatory, anti-allergic, anti-carcinogenic, antiviral
6.	Luteolin (Cushnie and Lamb, 2005; Kumar et al., 2020; Zandi et al., 2011)	Flavone	Green pepper, chamomile tea, thyme, celery, spinach	Anti-inflammatory, anticancer, anti-allergic, anti-oxidant, anti-viral
7.	Morin (Choudhury et al., 2017)	Flavonol	Almond, fig, Indian guava, white mulberry, fruits and leaves of osage orange	anti-angiogenic, neuroprotectant, anti-oxidant, anti-inflammatory, antibacterial, antiviral
8.	Naringin (Alam et al., 2014; Chen et al., 2016; Inês Amaro et al., 2009)	Flavanone glycoside	Grapefruit, pummelo, grapefruit juice, cherries, tomatoes, red and white wine	Antineoplastic, anti-inflammatory, antioxidant, anti-asthmatic, antiviral
9.	Quercetin (Anand David et al., 2016; Wu et al., 2015)	Flavonol	Fennel, seeds of pepper apples, onions, berries, broccoli	Anti-inflammatory, antihypertensive, anti-obesity, anti-atherosclerotic, antiviral
10.	Rutin (Ganeshpurkar and Saluja, 2017; Ku, 2014)	Flavonol	Buckwheat, apricots, green tea, fig, unpeeled apple	Antiarthritic, antiulcer, antimicrobial, anti-inflammatory, neuroprotective, anti-fatigue

lations that do normal-mode analysis, simulated annealing and energy minimization. All available and largely used molecular mechanics force fields are validated and included in the package (Abraham et al., 2015).

1.9. Visualizing software

Following software were used in this study for visualization and analysis of molecular data of the best docked compounds obtained from PyRx software and to analyze the simulation results.

1.9.1. PyMol

This Python-based software, along with several Python plugin tools, helps in simplifying the drug design process. It has the ability to give high-quality images and movies of the compounds in several different representations (ribbon, cartoon, mesh. etc.). For *in situ* design, the stereo view obtained is of much importance. The bond distances and angles can be measured and H-bonds can also be created by highlighting the amino acid residues.

1.9.2. LigPlot+

A program that generates 2D representation of the interactions of a protein–ligand complex. It determines the hydrogen bonds between the ligand and the binding residues as well as the hydrophobic residues of the active sites are highlighted.

1.9.3. Visual molecular dynamics (VMD)

This is a molecular modeling and visualization program, mainly used to analyze molecular dynamics simulation results. Large biomolecular are systems being analyzed using built-in scripting and 3-D graphics.

2. Materials and methods

2.1. Ligand preparation

All the 3D structures of the flavonoids listed in table 1 were retrieved from PubChem in .sdf file format. As X-ray diffraction/NMR structure data for these ligands were already available in the small molecules database, energy minimization step was slashed out. These files were further converted to pdb format using OpenBabel GUI −2.4.1. Before carrying out the molecular docking studies, the geometry of all the structures was cleaned using ArgusLab program (Fig. 2).

2.2. Protein preparation

The above retrieved compounds were subjected to dock in the active site of the spike protein SARS-Coronavirus-2. The previously reported 3-dimensional Crystal structure of SARS-CoV-2 spike receptor-binding domain bound with ACE2 (Yu et al., 2020) was retrieved from RCSB Protein data bank (Fig. 3). The retrieved protein structure was validated using Rampage tool to determine the allowed regions for torsion angles ψ against φ of amino acid - residues (Fig. 4).

2.3. Binding site prediction

The active sites of the receptor were predicted using the *in silico* tool, CASTp that offers several pockets that are likely to bind the receptor molecule and their expected binding poses were provided. The possible ligand-binding pockets were ranked based on area and volume which are solvent-accessible surface area/volume (Kumar et al., 2020).

2.4. Molecular docking

Autodock Vina 1.1.2 in PyRx 0.8 software was used to predict the protein–ligand interactions of the flavonoid compounds against the SARS-Coronavirus-2 spike protein. The protein structure was prepared for docking by eliminating molecules of water and attached ligands and by the addition of polar atoms of hydrogen. The protein and ligand files were loaded to PyRx as macromolecule and ligand, respectively which were then converted to PDBQT files used for docking. These files were similar to pdb with an inclusion of partial atomic charges (Q), atom types (T) for each ligand. The binding pocket ranked 1, predicted from CASTp was chosen as the other predicted pockets were relatively small and had lesser binding residues (Fig. 5). The active sites of the receptor molecules were selected and were enclosed within a three-dimensional affinity grid box. The grid box was centered to cover the active site residues, with dimensions × = -30.05 Å, y = 15.81 Å, z = -9.20 Å. The size of the grid wherein all the binding residues fit had the dimensions of × = 44.34 Å, y = 70.98 Å, z = 44.58 Å. This was followed by molecular interaction process initiated via the AutoDock Vina from PyRx (Radwan and Alanazi, 2018). The exhaustiveness of all the three proteins was set at 8. Nine poses were predicted for each ligand with the spike protein. The binding energies of nine docked conformations of each ligand against the protein were recorded using the Excel (Office Version) by Microsoft, USA.

2.5. Molecular interaction analysis

All the obtained poses from molecular docking were carried out using the PyMOL software. Out of the nine poses generated from PyRx for each flavonoid, the best pose having the least binding energy and high interactions was analyzed using LigPlot+ tool (Elmezayen et al., 2020). One out of the 10 flavonoid-receptor complex having considerably high hydrophobic interactions and hydrogen bonds with high binding affinity was saved as a pdb file for further analysis.

2.6. Molecular dynamics simulation

The protein–ligand complex having the least binding energy with the best docked pose and more binding interactions was considered to carry out molecular simulation. The simulation was carried out using the GROMACS 2020 package. The charmm27 all-atom force field, which uses empirical, semi-empirical and quantum mechanical energy functions for molecular system, was applied. The topology and parameter files for the input ligand file were generated SwissParam server. TIP3P water model was used to incorporate the solvent and counter ions were added to neutralize the system. The total number of atoms of the system was 182816. The protein–ligand complex was equilibrated and steepest descent minimization algorithm was used to carry out energy minimization at 50,000 steps. The system was then heated gradually at 300 K using 50 ps in the canonical ensemble (NVT) MD with 2 fs time step. For the isothermal-isobaric ensemble (NPT) MD, the atoms were relaxed at 300 K and 1 atm using 50 ps with 2 fs time step. After equilibrating the system at desired temperature and pressure, the MD run for the system was carried out at 1 ns with time step of 2 fs at 5,00,000 steps (Elmezayen et al., 2020). The coordinates and energies were saved at every 10 ps for analysis.

3. Results

Validation of the crystal structure of SARS-CoV-2 spike glycoprotein using Rampage tool showed that 97.6% that is 768 residues

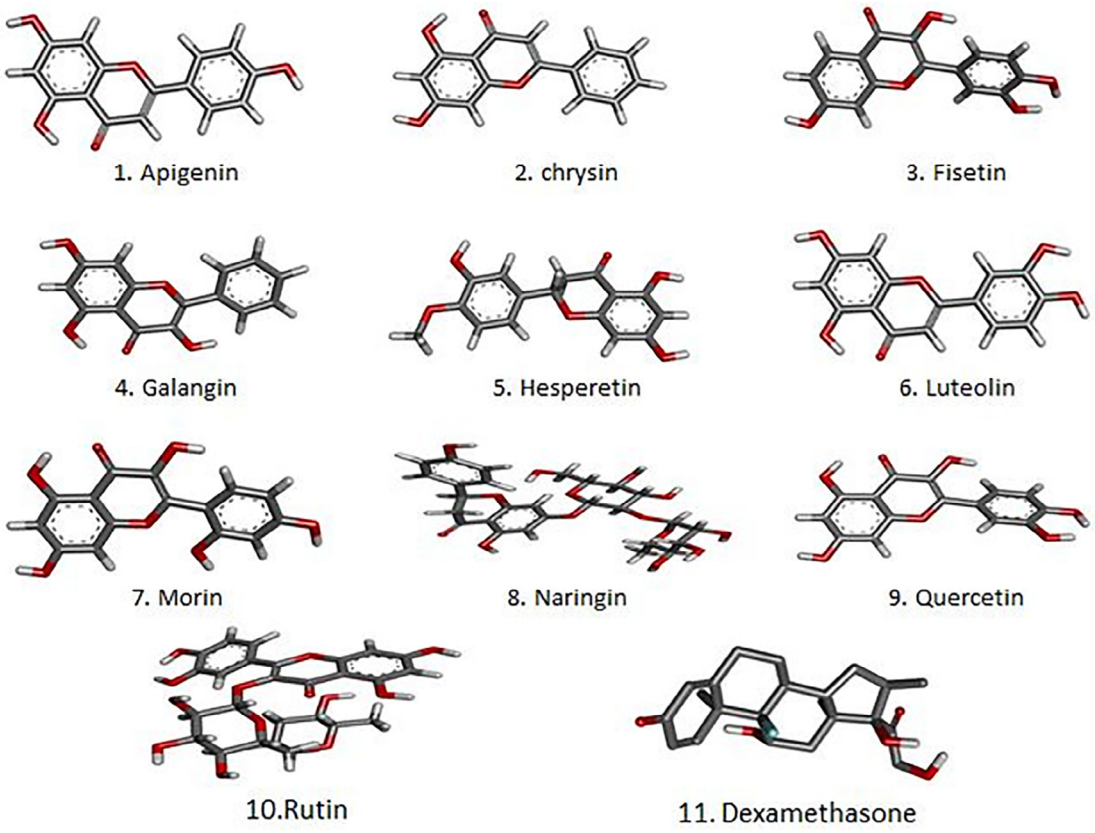

Fig. 2. 3-dimensional structures of Flavonoids and the structure of dexamethasone drug retrieved from PubChem.

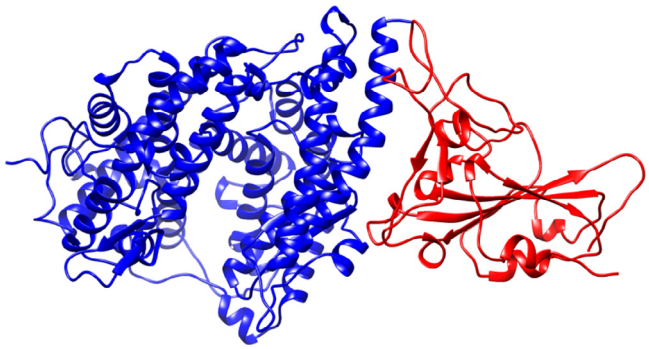

Fig. 3. 3-dimensional Crystal structure of SARS-CoV-2 spike receptor-binding domain bound with ACE2. PDB ID: 6m0j.

fall in favored region. Table 2 and Fig. 6 shows the estimated binding energies of the "best" pose out of nine poses having the least binding energy of the flavonoids with the active sites of the receptor molecule. A low binding energy value indicates a good affinity between protein and ligand.

3.1. Molecular docking analysis

Molecular docking was also carried out for the drug, dexamethasone which was taken as a reference and to compare the binding affinity of this compound with the flavonoids. From the above graph, it can be clearly predicted that the flavonoid, naringin has the highest binding affinity for the active site of the spike protein of novel coronavirus. Visualizing the interactions of the docked complex using LigPlot+ (Fig. 7) and PyMOL (Fig. 8) showed that naringin shared hydrophobic interactions with the residues-Asn290, Ile291, His374, Leu370, Leu410, Ala413, Pro415, Phe438 and Gln442. Along with that naringin interacted with the binding residues Asp367, Thr371, Lys441, Glu406, Ser409 via hydrogen bonds.

3.2. Molecular simulation analysis

Post molecular docking studies, a molecular simulation was carried out on the complex molecule of naringin flavonoid and spike protein of SARS-CoV-2. The simulation was performed for 1 ns to analyze the conformational stability of the complex. The data extracted from the trajectory was used to determine the stability of the secondary structure of the complexes by plotting Root Mean Square Fluctuation (RMSF) and Root Mean Square Deviation (RMSD). It showed the movement of the ligand within the binding pocket in 1 ns. The plots were made using QtGrace software. Fig. 9 shows the RMSD graph comparing RMSD plots of spike protein bound to naringin ligand and the spike protein without any bound ligands. For the complex, RMSD ranges from 0 to 0.5 nm and for the protein the variation was in range from 0 to 0.42 nm which was comparatively lower. The RMSD of the complex appeared to be more stable when compared to that of the protein from 0.2 nm to 0.4 nm. Root Mean Square Fluctuations (RMSFs) of the docked complex and protein were analyzed to characterize the fluctuations of residues during the 1 ns MD simulation (Fig. 10). It was observed that C terminal residues of protein and docked complex had high fluctuations at RMSD 0.4 nm and around 0.15 nm respectively. The radius of gyration of protein–ligand complex fluctuated up to 700 ps and then gradually reduced at the end of the simulation (Fig. 11). Solvent Accessible Surface Area (SASA) is the surface area of a molecule accessible by a solvent molecule(water)

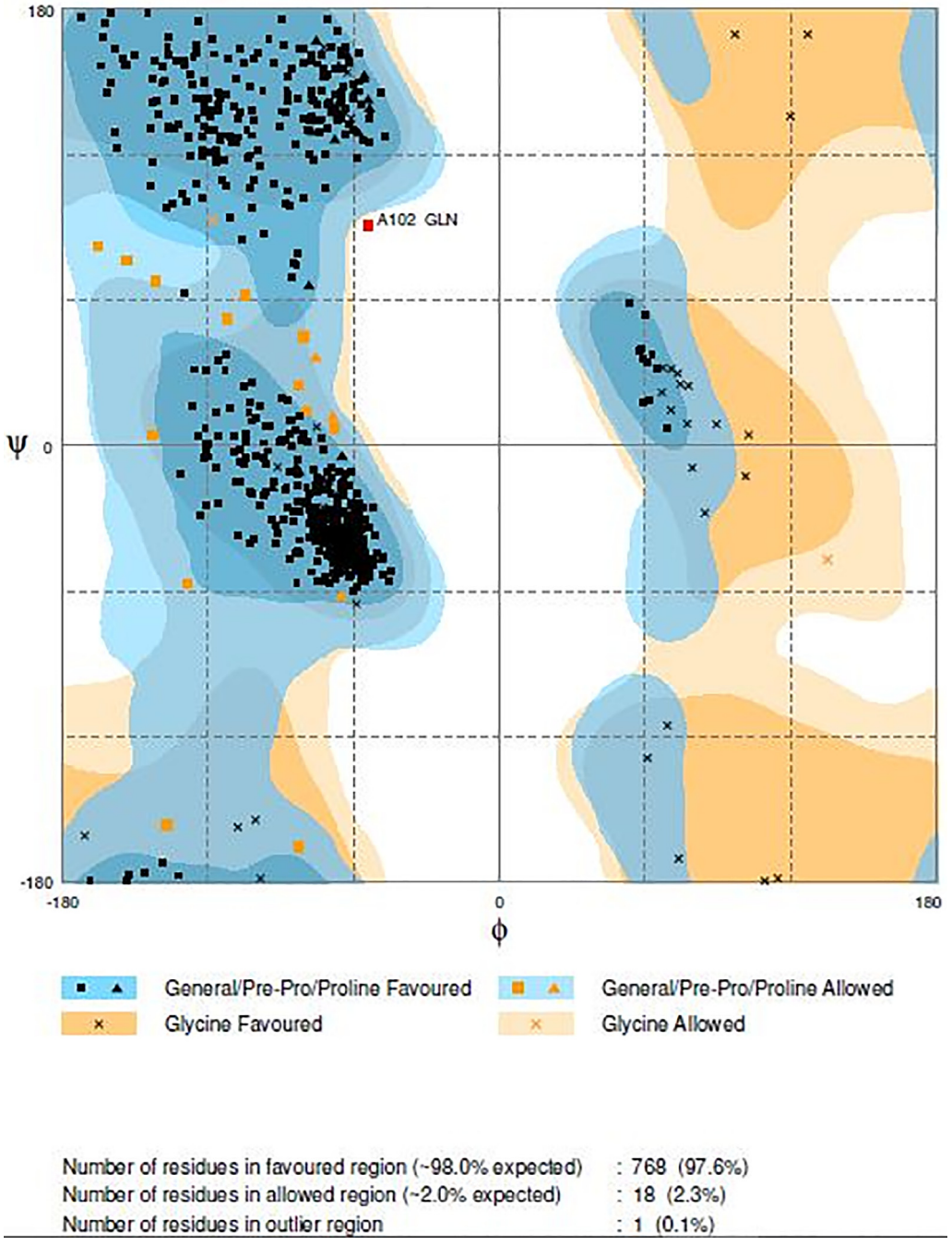

Fig. 4. Ramachandran plot of the spike protein showing 97.6% residues is favored region.

(Kumar et al., 2020). Naringin flavonoid showed a range of 7.6 to 9 nm^2 area and high fluctuations (Fig. 11). The area was least at around 850 ps and the equilibrium was achieved at a range of 8.2–8.6 nm^2.The H-bond interaction between the spike protein and flavonoid during the simulation time is shown in Fig. 12. It is clearly observed that a maximum of 5 hydrogen bonds and an average of 2H bonds were observed during I ns for the complex.

4. Discussion

The occurrence of a novel coronavirus discovered at the end of December 2019 has created a created a major impact globally taking the lives of millions (Kumar et al., 2020). The purpose of this study is to provide an insight on how flavonoids that possess antiviral properties can effectively inhibit this novel coronavirus named as COVID-19. It has created a major havoc and still continues to because of lack of vaccine and effective antiviral potent drug. There is absolutely an unmet need for an essential and effective treatment against the SARS-CoV-2.

In silico approaches using bioinformatics tools and software have accelerated the discovery of novel compounds to inhibit major proteins of viruses to treat viral diseases (Yu et al., 2020). In this study, spike protein of SARS-CoV-2 is considered as the target protein to which 10 flavonoids having antiviral and anti-inflammatory properties are docked to analyze the binding mode having the least binding energy. Interestingly, all of them showed extremely great binding affinity when compared to that of the standard current drug, dexamethasone. Molecular

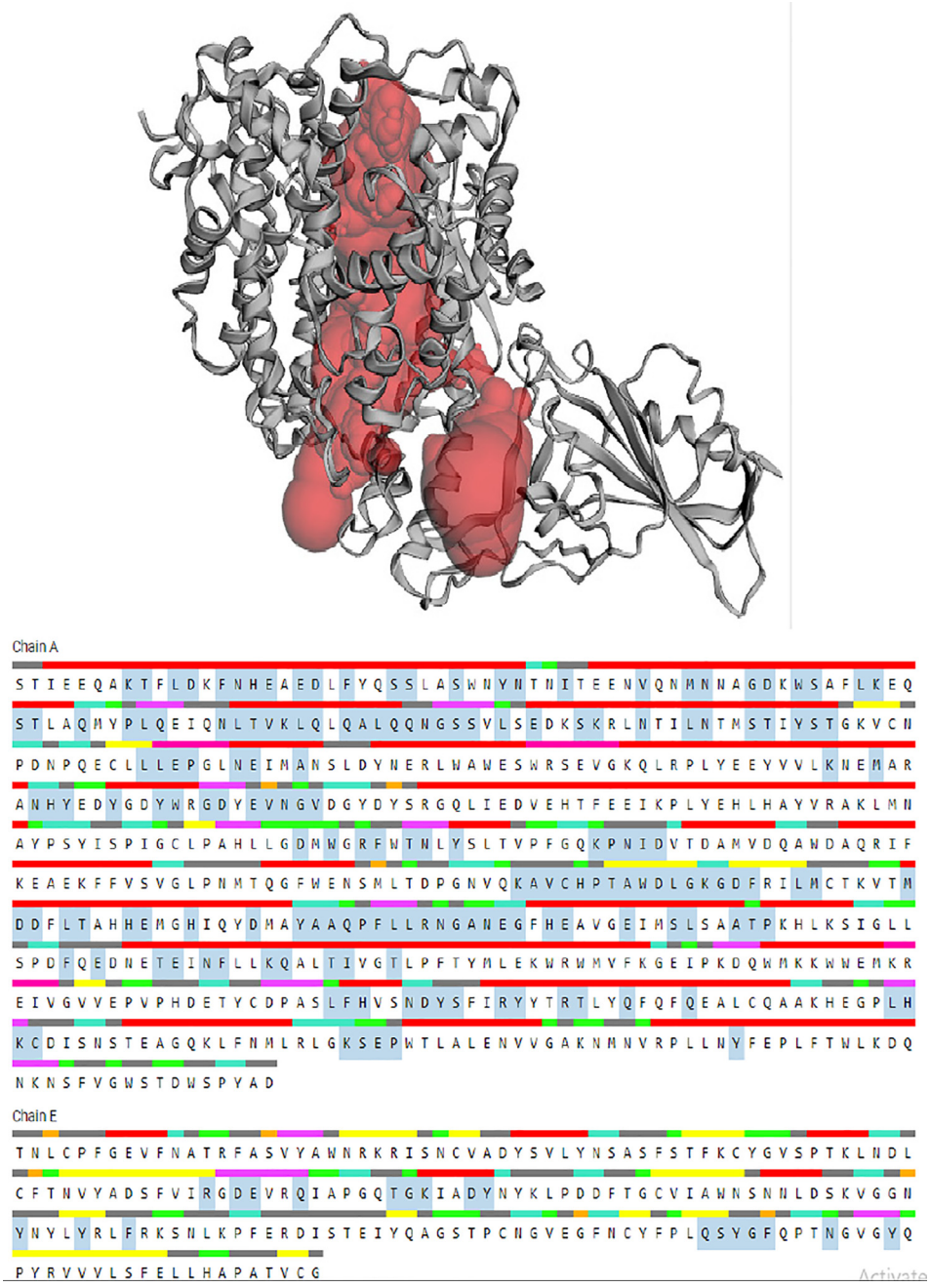

Fig. 5. Binding pocket (red color) obtained from CASTp tool along with the sequence which shows the highlighted residues forming the binding pocket.

Table 2
Estimated binding affinity (Kcal/mol) of the flavonoids with SARS-CoV-2 protein.

Flavonoid	Apigenin	Chrysin	Fisetin	Galangin	Hesperetin	Luteolin	Morin	Naringin	Quercetin	Rutin	Dexamethasone
Binding energy value (Kcal/mol)	−7.8	−8.1	−8.3	−8.2	−7.7	−8.0	−8.1	−9.8	−8.2	−9.2	−7.9

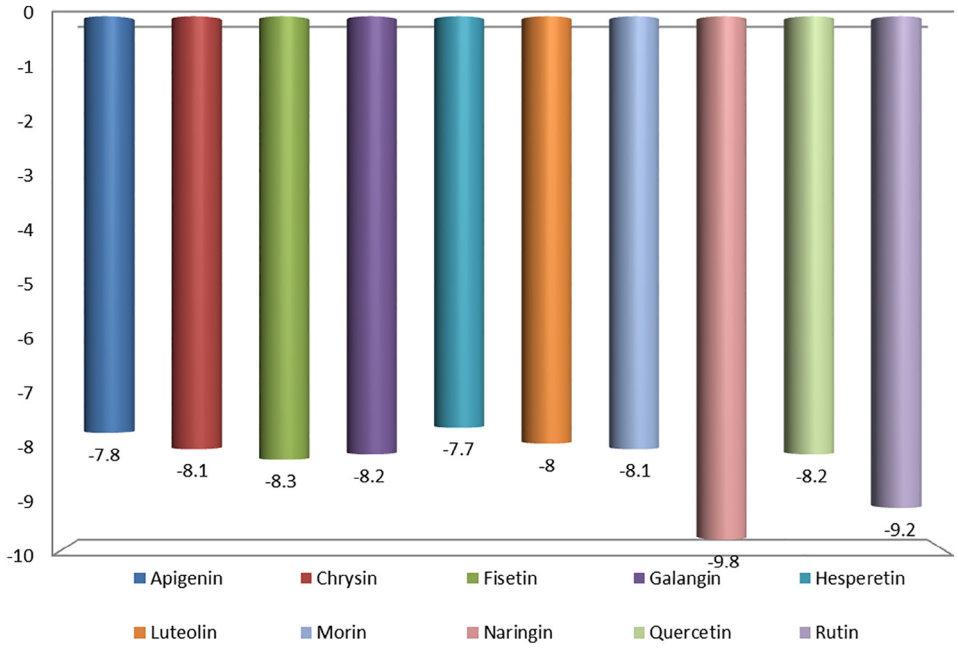

Fig. 6. Column bar graph showing the negative binding energies (Kcal/mol) of each flavonoid with the spike protein active site.

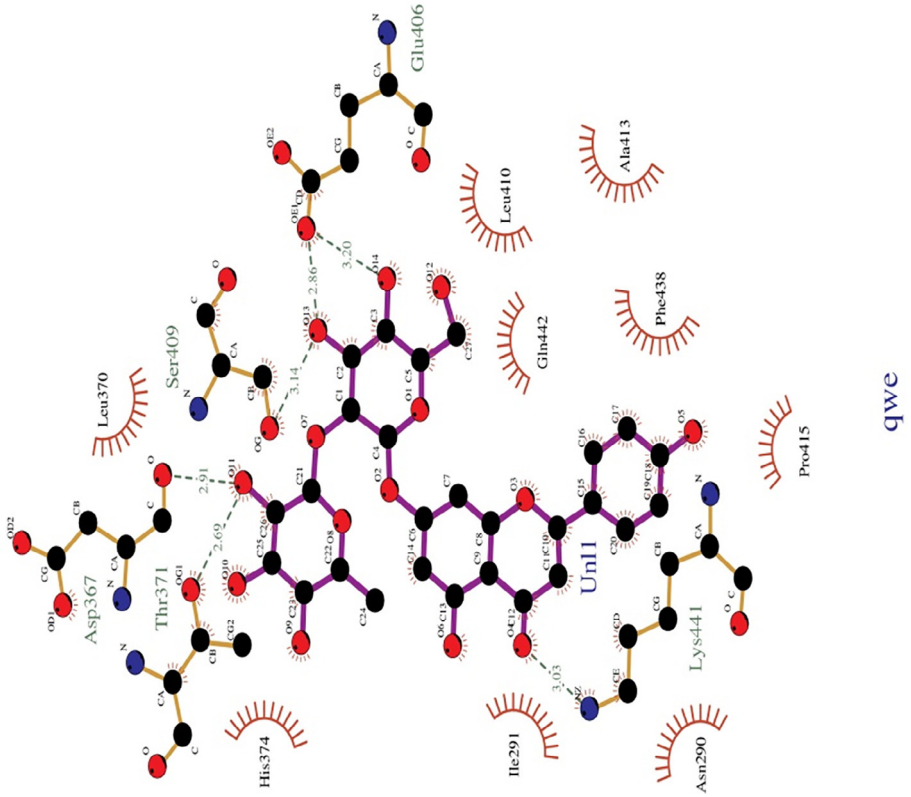

Fig. 7. Two-dimensional representation of the best docking pose of naringin flavonoid with the binding amino acid residues of the spike protein of novel coronavirus.

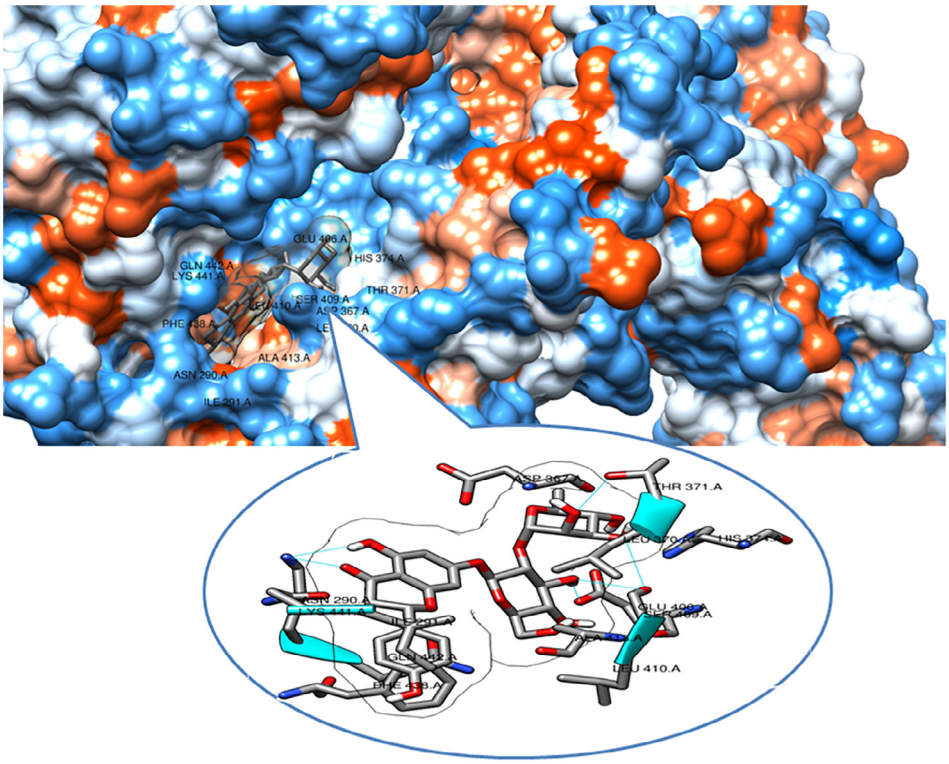

Fig. 8. Pictorial 3D representaion of naringin flavonoid buried within the active pocket of the spike protein bound to the interacting binding residues.

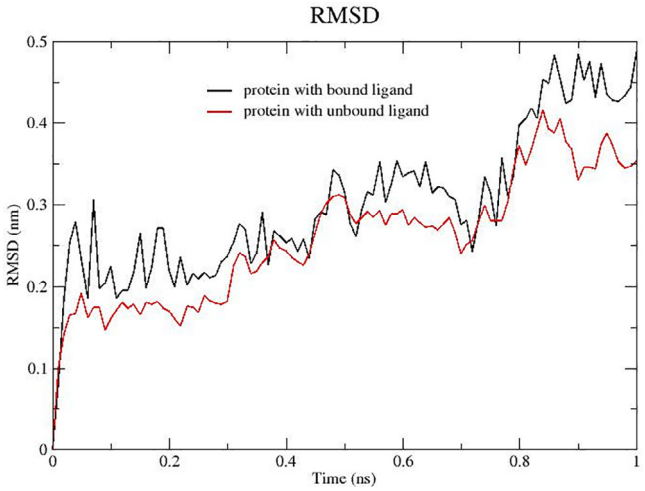

Fig. 9. RMSD of 6m0j protein with bound ligand naringin in comparison with protein without any bound ligands.

docking analysis proved naringin to be a promising compound of all the other flavonoids having the least binding energy with the binding residues of the SARS-CoV-2 spike protein. Simulating this docked compound determined how stable the docked compound would be in a solvent. The protein backbone RMSD analysis of the naringin-spike protein complex was performed, which revealed that the complex was stable for the given time. RMSF analysis was performed to determine the effect of the flavonoid compound on the spike glycoprotein of SARS-CoV-2. Overall, the molecular docking and MD simulation analysis exhibited a promising binding stability of the flavonoid compound, naringin with the active site of SARS-CoV-2.

5. Conclusion

This study sought to analyze and compare various flavonoids that are known to possess anti-inflammatory as well as antiviral properties in an effort to inhibit the predominant spike glycoprotein of SARS-CoV-2 using an *in silico* approach. All the flavonoids showed considerably high binding affinity when compared to dexamethasone, which is an anti-inflammatory drug. Naringin compound showed the highest binding affinity of all with the active site of the spike protein and hence further molecular dynamics simulation was conducted on this complex using GROMACS to study the dynamic characteristics. Analyzing the graphs obtained from 1 ns simulation, we concluded that the complex was stable for a time period but not throughout the simulations. By exploring several ligand properties, protein–ligand interactions and the protein stability, the docking study was validated by molecular simulations. Further *in vitro* analysis can be carried out to prove the effectiveness of naringin flavonoid extracted from natural sources as an inhibitor of the spike protein of SARS-CoV-2. The complete study concludes that flavonoids with anti-inflammatory properties can be a good alternative to the standard drugs.

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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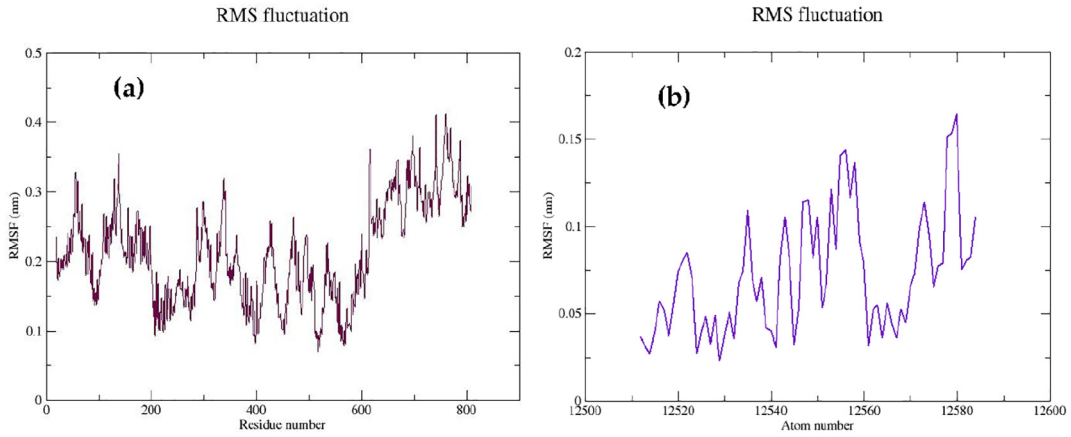

Fig. 10. RMSF of the Cα atoms of (a) protein and (b) protein–ligand complex characterizes the atomic fluctuation during the 1 ns MD simulation.

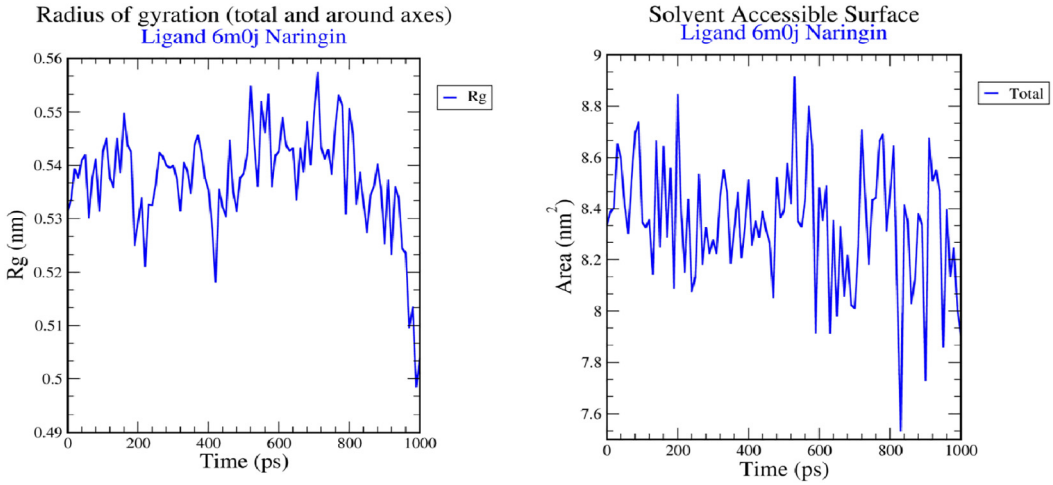

Fig. 11. Radius of gyration (right) and Solvent accessible surface area (left) of protein–ligand complex during the 1000 ps time frame.

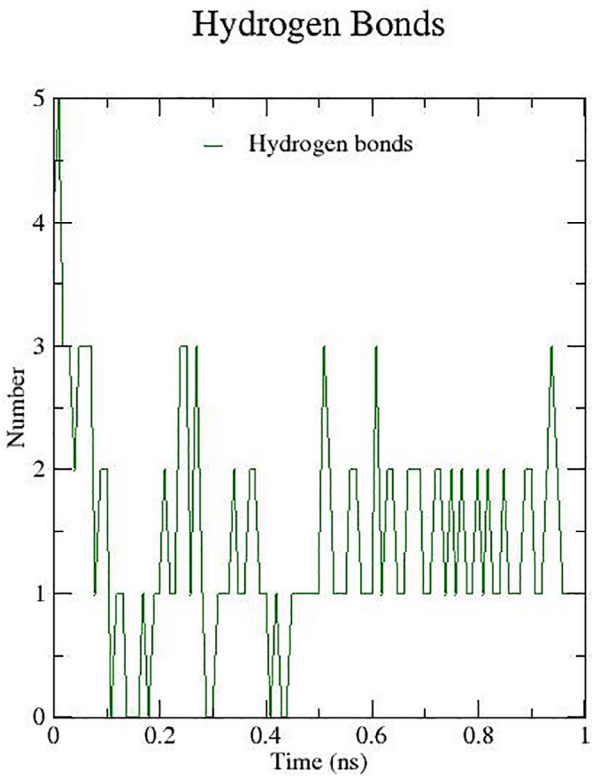

Fig. 12. Variation in hydrogen bond number with respect to simulation time.

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