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MOLECULAR DOCKING AND MOLECULAR DYNAMIC SIMULATIONS FOR NATURAL ANTIVIRAL COMPOUNDS AGAINST SARS-COV-2: COMPUTATIONAL INVESTIGATION

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ABSTRACT

A new coronavirus was identified as the COVID-19 virus; it is the etiological agent responsible for the 2019-2020 viral pneumonia outbreak. The infection began in Wuhan, China At the end of December 2020. Currently, there is no antiviral drug available to control the infection, and options in medical treatments are limited. We have used a computer-aided drug design approach to find active compounds against the virus. We have identified a total of seventy-eight natural compounds with antiviral properties. The PubChem database is used to retrieve the structures in 2D format. We have used the main protease named 5REH as a target. We have used PyRx to dock using a standard protocol. We have further investigated compounds with good binding affinity for their ADMET profile using computational approaches. This study leads us to have three compounds named Lycorine, Corylifol, and Broussochalcone. Molecular dynamic simulation studies were done to check the stability of the protein and ligand complex during a simulation. Parameters like RMSD, RMSF, and radius of gyration were observed to understand the fluctuations. We also have performed the simulation for standard ligand AWP to compare the stability with test ligands. All the results obtained were showing deviations from the standard values but all the deviations were not statistically significant. Protein-ligand interaction studies also reveal that enough hydrogen and hydrophobic bonds are present to justify our results. The three compounds namely lycorine, Corylifol, and Broussochalcone A identified can come out as a potential candidate for the treatment of Covid -19 infections.

KEYWORDS: Molecular Docking, MD simulation, Covid-19, Protease, VMD and NAMD, RMSD.

INTRODUCTION

The world witnessed the novel coronavirus diseases (Covid-19), which began from Wuhan city, Hubei, China, and declared as public health emergency of international concern by the world health organization on (WHO) on 30th January 2020. It is caused by severe acute respiratory syndrome coronavirus (SARS-CoV-2). The various symptoms include in the diseases are sore throat, pneumonia, difficulty in breathing, fever, lung infection, and several of the times gastric related symptoms are also present.[1,2] WHO on 12th January named the coronavirus as the 2019 novel coronavirus (2019-nCoV), but later on 11th February 2020, WHO, officially declared its name as novel coronavirus diseases 2019. On the same day, the coronavirus study group of the international committee on taxonomy of virus proposed the name SARS-CoV-2.[3]

Coronaviruses are a group of highly diverse enveloped, positive sense, and single-stranded large RNA viruses. They are known to cause a variety of diseases including hepatic, respiratory, neurological, and enteric in animals and humans with various severity. Four different types of coronaviruses exist, they are alpha, beta, gamma, and delta. Alpha and beta are known to originate from bats, whereas gamma and delta have pigs as an origin.[4] Over the past two decades two novel coronaviruses named severe acute respiratory syndrome Cov (SARS-CoV) and Middle East respiratory syndrome CoV (MERS-CoV), have emerged and caused severe human diseases[5] Covid-19(SARS-CoV-2) belongs to the family of Coronaviridae, genus betacoronavirus, subgenus Sarbacovirus. SARS-CoV and MERS-CoV both belong to the betacoronavirus genus. Genome comparison with both showed that Covid-19 belongs to the SARS-CoV with an identity of 82%. The main protease (M^{pro}) is also known as chymotrypsin-like serine protease (3CL Pro) and it plays an important role in the replication and gene expression of the virus. The essentiality of the main protease for the virus makes it a very suitable target to design an effective drug against it. The crystal structure PDB ID 5REH from the protein data bank is used here to find a suitable ligand against it.[6]

Centre for diseases control and prevention already issued that the COVID-19 is spreading rapidly through the respiratory droplets from an infected individual while coughing and sneezing. Guidelines are also issued to avoid public gatherings and to maintain social

distance with wearing a nose mask compulsory. These all precautions have to be taken seriously as we have not had any antiviral drug or any therapy which proves its effectiveness confidently. More specifically the people above the age of 50 and 55 are more prone to get the infection to compare to young individuals.

As, it is very much clear that if we follow the traditional drug discovery approach to find a suitable drug for COVID-19, it may take several years and we cannot afford to lose many of our lives during such a long process. Today we have high computation power with all the necessary information available online and most of them are free. We can fasten the discovery process by using molecular docking and molecular dynamic simulation studies to narrow down our selection for the antiviral compounds at the very initial stage of our process. Even we don't need to have those compounds physically to test their effectiveness and also, we don't need to handle such an infectious virus.[7] It is being always observed that natural compounds so far have been found to have many of the therapeutic antiviral effects.[8] We have searched the literature and found 78 natural compounds, which can come out as a potential therapeutic candidate for COVID-19. We have used binding affinity and simulation parameters to scrutinizes our selected 78 natural ligands.

MATERIAL AND METHODS

Protein structure preparation

The X-ray diffraction-based crystal structure of COVID-19 main protease (PDB ID:5REH) in a complex with ligand AWP (1-cyclohexyl-3-(2-pyridine-4-ylethyl) urea) with a resolution of 1.80 Å was taken from the protein data bank. The structure was cleaned to ensure maximum quality and reliability.[9] The bound ligands, water molecules were removed and missing atoms and residues were added. Steric clashes were minimized and hydrogen atoms were added. Formal bond orders were determined, side chains were optimized and fixed, charges added using program implemented in chimera, SWISS PDB viewer, and Chiron minimization and refinement tool.[10,12]

Computational screening

A literature survey was conducted to find out the natural compounds having said antiviral properties. A total of 78 compounds were identified and the structures of the identified compounds were retrieved from the PubChem database. The compounds were imported in to the PyRx (V 8.0) and energy minimization was done using Open Babel (Version 2.3.1).[13] module of the same software. Energy minimization was done via the Universal force field

(UFF) using the conjugate gradient algorithm. A total number of 200 steps were set and the number of steps to update was set to 1. The minimization was set to stop at an energy difference of less than 0.1 Kcal/mol.[14]

Docking studies

Molecular docking was performed with PyRx (V 8.0), which is an extension of the python molecular viewer. A Lamarckian genetic algorithm was used to perform the automated molecular docking of the protein with each ligand. The AWP ligand which is a bound ligand to the original structure was docked to compare the binding affinity with test ligands. The torsion bonds and side chains were kept to rotate freely, while the protein structure was kept rigid. Gasteiger charges were computed, and all the charges of non-polar hydrogens were assigned.[15] The grid map was set at 60×60×60× and the grid was spaced at 0.375Å. Both selected ligand and protein were converted in to pdbqt structure format. Protein and ligands were loaded in to PyRx as macromolecule and ligand, respectively. All the compounds were docked and affinity was calculated in kilocalories per mole.[16]

Table 1: Molecular Properties & Drug likeness of selected ligands.

Molecule Name	PubChem ID	Number of HBA	Number of HBD	Molecular weight	cLog P	Drug likeness
10207	10207	5	3	270.24	1.7447	-1.1908
5281600	5281600	10	6	538.5	4.671	0.28194
5280443	5280443	5	3	270.24	2.3357	0.28194
6438825	6438825	5	4	340.4	3.9435	-0.47336
6419835	6419835	10	7	442.4	2.4	-0.32874
5276890	5276890	11	8	458.4	2.0543	-0.32874
475277	475277	18	14	864.8	3.4428	0.50761
25056407	25056407	4	2	390.5	6.1672	-1.9187
10472405	10472405	5	4	340.4	3.9435	-0.47336
3008868	3008868	18	11	742.5	6.1241	-2.2054
145937	145937	9	6	372.3	3.0212	-2.2054
442879	442879	6	0	354.4	3.625	0.19401
135704008	135704008	16	11	716.6	2.8806	1.4858
5318717	5318717	10	6	418.3	0.5089	-1.6541
5280445	5280445	6	4	286.24	1.99	0.28194
72378	72378	5	2	287.31	1.2078	3.1877
5281672	5281672	8	6	318.23	1.1445	-0.082832
5321765	5321765	4	2	338.4	4.5649	-0.26787
130976	130976	14	9	602.5	5.1482	-2.0638
122738	122738	12	10	578.5	2.3016	0.31525
11250133	11250133	12	10	578.5	2.3016	0.31525
10480940	10480940	12	8	496.4	3.7254	-2.2054

5280343	5280343	7	5	302.23	1.4902	-0.082832
5280805	5280805	16	10	610.5	-1.2573	1.9337
21637642	21637642	13	9	781	1.7831	-10.972
5281697	5281697	6	4	286.24	1.99	0.28194
11787114	11787114	13	4	654.7	2.2501	-4.1179
21146795	21146795	20	13	868.7	3.6197	1.3554

ADME and Toxicity predictions

The selected compounds with good binding energies were further studied for their Adsorption, distribution, excretion, metabolism, and toxicity profile using SWISS ADME[17] and data warrior tools.[18,19] The predicted properties considered were blood-brain barrier penetration properties, Human intestinal absorption, inhibition to cytochrome P450 enzyme, bioavailability. Compounds showing satisfactory properties were further studied for their toxicity profile using data warrior tools. Toxicity profiles included were mutagenicity, tumorigenicity, irritability, reproducibility, Ames toxicity, and carcinogens.

Table 2: ADMET analysis with the lowest binding affinity.

PubChem ID	BBB Penetration	HIA	CYP 2D6 Inhibitor	BA	Mutagenic	Tumorigenic	Reproductive effect	Irritant	docking score
11250133	No	Low	No	0.17	None	None	High	None	-8.054
130976	No	Low	No	0.17	Low	None	None	None	-7.415
5318717	No	Low	No	0.55	None	None	None	None	-7.348
5281600	No	Low	No	0.17	None	None	None	None	-7.304
122738	No	Low	No	0.17	None	None	High	None	-7.079
21146795	No	Low	No	0.11	None	None	None	None	-6.786
135704008	No	Low	No	0.17	None	None	None	None	-6.758
5280805	No	Low	No	0.17	None	None	None	None	-6.585
5280443	No	High	Yes	0.55	High	None	None	None	-6.557
5321765	Yes	High	No	0.55	None	None	None	High	-6.55
6438825	No	High	No	0.55	None	None	None	None	-6.532
3008868	No	Low	No	0.17	Low	None	None	None	-6.452
21146795	No	Low	No	0.17					-6.447
442879	Yes	High	Yes	0.55	None	None	None	None	-6.441
5276890	No	Low	No	0.17	None	None	None	None	-6.425
145937	No	Low	No	0.55	Low	None	None	None	-6.417
6419835	No	Low	No	0.55	None	None	None	None	-6.408
475277	No	Low	No	0.17	None	None	High	None	-6.347
10480940	No	Low	No	0.17	Low	None	None	None	-6.323
11787114	No	Low	No	0.17	None	None	High	None	-6.202
5281672	No	Low	No	0.55	High	None	None	None	-6.199
10472405	No	High	No	0.55	None	High	None	High	-6.182
10207	No	High	No	0.55	High	None	None	High	-6.171
5280445	No	High	Yes	0.55	None	None	None	None	-6.149

475277	No	Low	No	0.11					-6.127
5281697	No	High	Yes	0.55	None	None	None	None	-6.088
72378	No	High	No	0.55	None	None	None	None	-6.067
5280343	No	High	Yes	0.55	High	High	None	None	-6.064
25056407	No	High	No	0.55	None	None	None	None	-6.03
21637642	No	Low	No	0.17	None	None	None	None	-6.018

Molecular dynamic simulation

Docked protein and ligand complexes were subjected to molecular dynamics simulation using NAMD software.[20] The success of MD simulation depends on the selection of the initial protein and ligand structures. Initially, the structure was checked for inconsistencies. Out of 30 selected compounds from the docking results, we have selected the final three compounds having a PubChem number 6438825, 72378, and 25056407. The docked complexes were studied for their stability during the simulation. The root means square deviation, root mean square fluctuation, and radius of gyration was studied for protein backbone residue and ligand within the binding site of the simulated system.[21,23] The stabilities of the complexes were examined by monitoring their root mean square deviation (RMSD) during 50,00,000 steps for a 10 ns simulation. MD simulations were performed using the CHARMM36 force field.[24] Visual molecular dynamics (VMD) was used to generate PSF files for all complexes.[18] all complexes were solvated in cubic water boxes containing transferable intermolecular potential with 3 points (TIP3P) water molecules. The box size was chosen to match the molecular dimensions so that there was a distance of 5˚A between the protein surface and the edges of the periodic box. A 5˚A cut off distance was used to calculate short-range nonbonded interactions. The particle mesh Ewald (PME) method was used to calculate long-range electrostatic interactions. The SHAKE method was used to constrain all bonds involving hydrogen atoms. A conjugated gradient system was used for energy minimization, with all parameters set to default. The system first performed 10000 steps of Conjugated gradient with energy minimization. We used Langevin Dynamics with pressure control so our system was not an NVT ensemble. The Nose–Hoover method was used to maintain a constant temperature. The time step of each simulation was set to 2 fs[20,25,26] Visualizations and data analysis were performed with VMD software.[27]

RESULTS AND DISCUSSION

Virtual screening and docking results

Virtual screening helps us to screen the biological molecules with good binding affinity. In this study, we have used PyRx 8.0 tool to screen out the molecules. A total of 78 natural

ligands were selected and were docked to the target protein.The docked compounds were examined in the Auto dock tool and binding free energy was calculated.[28,29] We have selected a total of 30 compounds on their binding affinity ranging from -8. 054 to -6.084 kcal/mol (Table 2).

ADMET analysis

We have selected thirty compounds and the same compounds were studied for their ADMET properties. The properties like Human intestinal absorption, irritability, reproductive effect, inhibition to cytochrome p450 enzyme, and several others were predicted. It was clear from the results that compound number 5321765, 6438825, 10472405, 10207, 72378, and 25056407 have a high intestinal absorption value. From the Table 2, it was also observed that we had several compounds like 528044, 442879, 5280445, 5281697, and 5280343 have high intestinal absorption values but at the same time they are also showing their inhibitory properties towards the Cytochrome P450 enzymes, so such compounds were removed from the further selections. Similarly, from the selected compounds with high intestinal absorption values and negative inhibitory actions to cytochrome P450 enzymes, compounds were also studied for their mutagenic, tumorigenic, irritability, and reproductive effect. Table no 2 indicates that compounds number 5321765, 10472405, and 10207 have either of the said effects, so these compounds were also removed from the study. Finally, we selected three ligands namely Lycorine_CID_72378, Broussochalcone A_CID_6438825, and Corylifol A_CID_25056407 for further analysis. The ligand selected for further study were having either hydrogen bonds or presents a hydrophobic interaction with the protein Table & Table 4.

Protein-ligand interaction

The hydrogen bond and hydrophobic interactions of protein-ligand complexes were analyzed by LigPlot+ (v 1.4.5)38 and Protein-ligand interaction profiler. “LigPlot+” is a graphical system that generates multiple two-dimensional (2D) diagrams of ligand-protein interactions from docked complexes. PLIP is complementary to another state of the art tools like a swiss dock, galaxy site, or ProBis and thus it can be used to study the protein-ligand complex. The server allows comprehensive detection and visualization of protein and ligand complexes along with interaction patterns.

Table 3: Hydronic interaction between Protean and Ligand complex.

Ligand	Residue	AA	Distance	Ligand Atom	Protein Atom
Lycorine	25A	THR	3.76	9391	707
	41A	HIS	3.94	9390	1221
	142A	ASN	3.85	9392	4444
	166A	GLU	3.87	9396	5123
Corylifol A	25A	THR	3.63	4756	355
	142A	ASN	3.89	4735	2222
	166A	GLU	3.57	4748	2562
	166A	GLU	3.99	4759	2563
Broussochalcone A	25A	THR	3.94	4742	355

Table 4: Hydrogen bond interaction between Ligand and Protein complex.

Ligand	Residue	AA	Distance H-A	Distance D-A	Donor Angle	Protein donor	Side chain	Donor Atom	Acceptor Atom
Lycorine	142A	ASN	2.39	3.03	119.38	✓	✓	4450 [N3]	9376 [O2]
	142A	ASN	2.94	3.53	119.92	✓	✓	4445 [O3]	9376 [O2]
	142A	ASN	2.08	3.03	155.51	✓	✓	4446 [N3]	9376 [O2]
	166A	GLU	2.75	3.67	149.68	✓	×	5115 [N3]	9379 [O2]
	166A	GLU	3.04	3.67	121.73	✓	×	5116 [N3]	9379 [O2]
Corylifol A	44A	CYS	2.22	3.00	139.01	×	×	4734 [O3]	660 [O2]
	143A	GLY	2.84	3.83	165.61	✓	×	2232 Nam	4733 [O2]
	166A	GLU	2.92	3.61	130.91	×	✓	4732 [O3]	2565 [O3]
Broussochalcone	41A	HIS	3.21	3.88	128.89	×	✓	4733 [O3]	611 [N2]
	41A	HIS	1.89	2.80	159.88	×	×	4732 [O3]	608 [O2]
	140A	PHE	2.60	3.30	130.36	×	×	2182 [O2]	4736 [O3]
	142A	ASN	2.46	3.16	126.46	✓	✓	4734 [O2]	2225 [Nam]
	144A	SER	3.64	4.07	111.10	✓	✓	4736 [O3]	2244 [O3]
	163A	HIS	2.76	3.73	158.77	✓	✓	4736 [O3]	2512 [Npl]
	166A	GLU	1.83	2.77	165.63	×	✓	2565 [O3]	4735 [O3]
	172A	HIS	3.44	3.92	111.00	✓	✓	4736 [O3]	2652 [Npl]

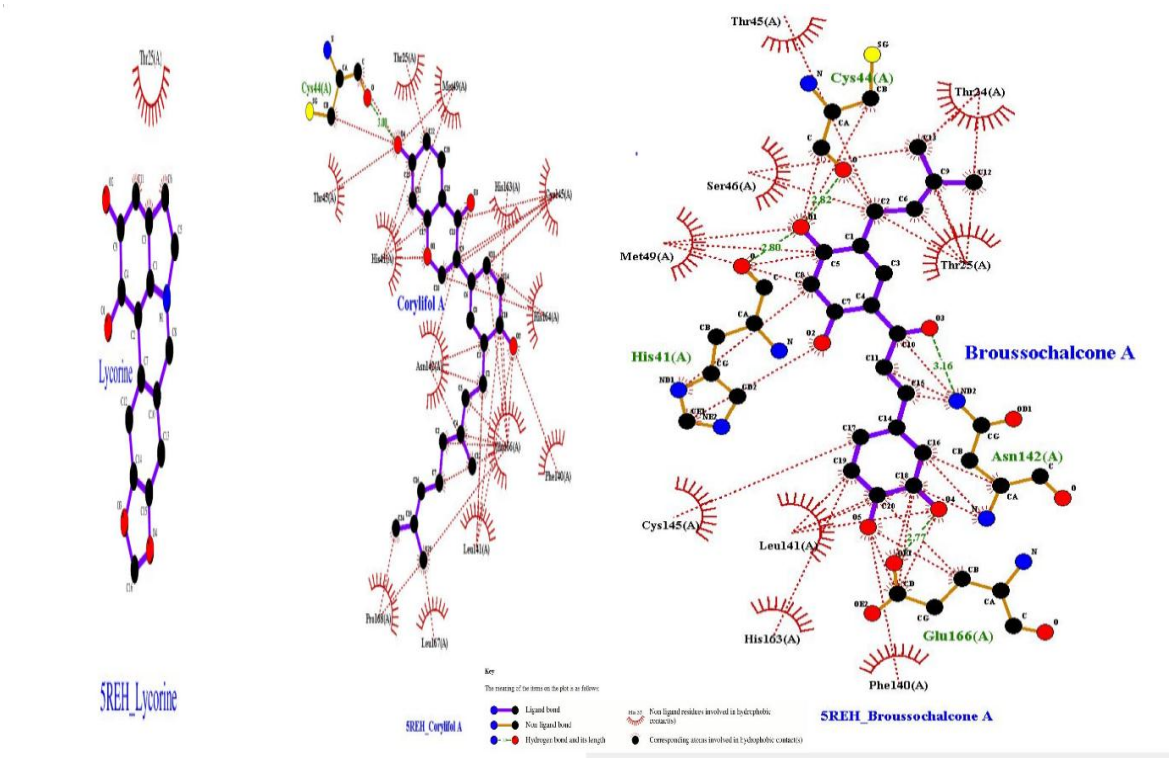

Figure 1: Protein and Ligand interaction diagram.

Molecular dynamic simulation studies

We assessed the residue RMSD to study the residue behavior of the protein during the simulations. In general, a residue's RMSD value was considered to represent the local flexibility of a protein and ligand complex. It reflected the mobility of an atom during the MD simulation trajectory. Therefore, a higher residue RMSD value indicated higher mobility; conversely, a lower residue RMSD value indicates lower mobility. To investigate the fluctuations in the ligand-binding energy as well as the motions of the amino acid residues within the complex during the simulation, the root means square fluctuation (RMSF) of the complex was also monitored. Besides, the compactness of each complex was determined by carefully examining how folded or unfolded the protein-ligand complex was by calculating the radius of gyration.[23] Based on the docking analysis 30 compounds were selected for further ADMET investigation and it leads us to select the final three compounds (Corylifol A_CID_25056407, Lycorine_CID_72378, Broussochalcone A_CID_6438825) to consider the structural stability of each protein-ligand complex by molecular dynamic simulation. MD simulations of standard (PDB ID-AWP) ligand bound to the protein also performed to compare the stabilities with three selected protein-ligand complexes. The stabilities of those four complexes (5REH-AWP, 5REH- Corylifol A_CID_25056407,

5REH- Lycorine_CID_72378, and 5REH- Broussochalcone A_CID_6438825 were monitored using root mean square deviation (RMSD) during 10 ns simulation studies.

Table 5: RMSD values for the simulated complexes.

Protein-Ligand complex	Mean RMSD (Å)	Min RMSD (Å)	Max RMSD (Å)
5REH_AWP	2.43	0.85	3.26
5REH_Lycorine	2.26	0.86	2.75
5REH_ Broussochalcone A	2.26	0.86	2.91
5REH_Corylifol	2.41	0.78	3.31

The values presented in (Table 5) for all the four protein-ligand complexes studied for their stabilities during 10 ns simulation. From the values, it is clear that the range of RMSD obtained for all the complex follows the acceptance range between 1 to 3. 5(Å). It is also observed from the graphs that the complex was also equilibrated as the average RMSD values are stabilized at the end of the 10 ns simulation. Further all the RMSD values obtained for all test ligands were showed some increasing or decreasing values of RMSD compare to the standard ligand but differences obtained were not statistically significant. This fixed range of RMSD was indicating the interaction between bound ligand and flexible loop region, as it reduces the flexibility of the protein-ligand complex.

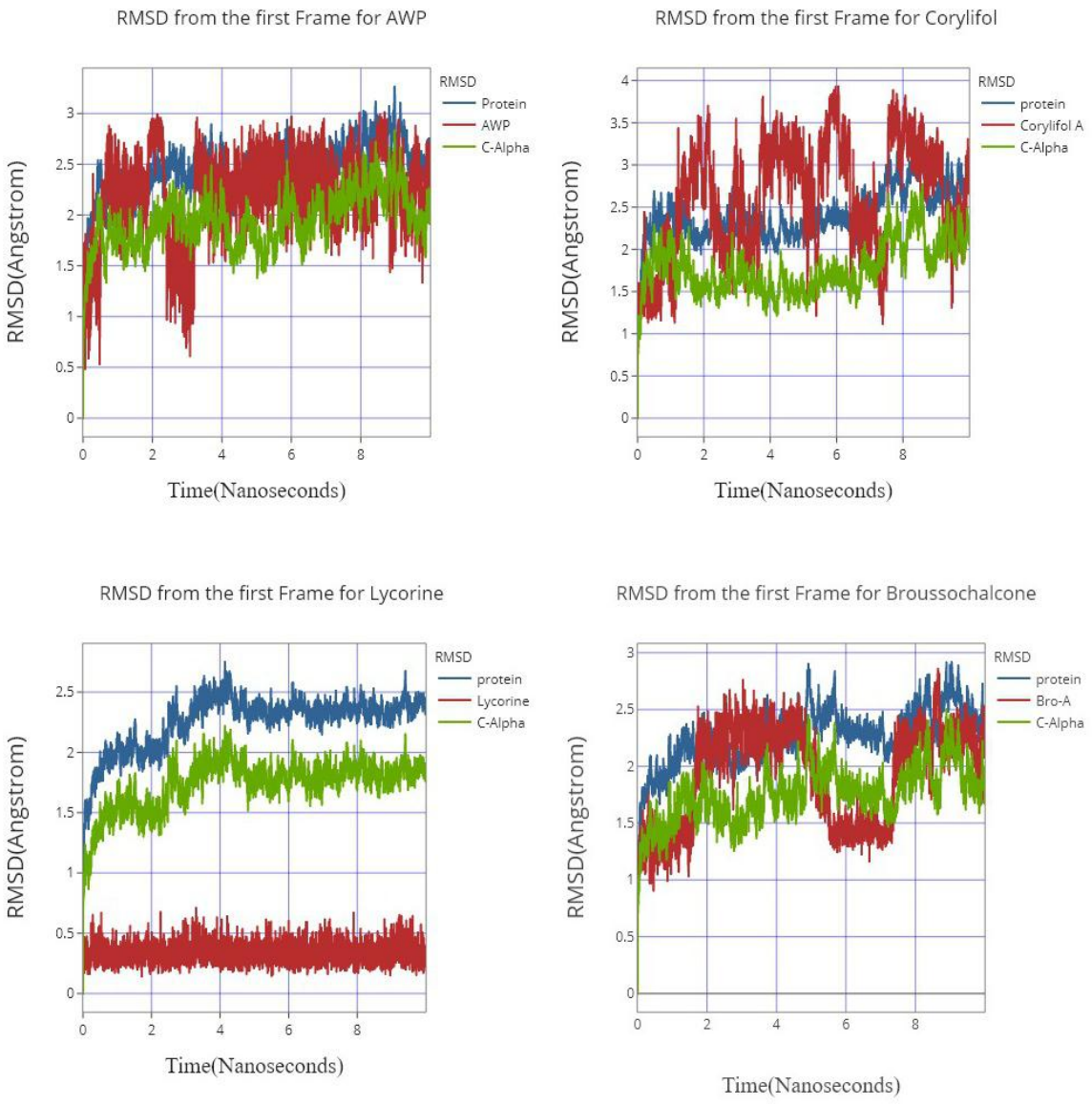

Figure 1: RMSD results for AWP, Corylifol, Lycorine, and Broussochalcone with 5REH protein, based on 10 ns simulation.

The root means square fluctuations (RMSF) were assessed and plotted to equate the flexibility of each residue in the–ligand-protein complexes. The RMSF of the protein-ligand complex denoted the minimized fluctuation for all the complexes. The RMSF did not deviate much during the simulation period of 10 ns and the average RMSF values were kept constant for all the complexes.

The radius of gyration was also monitored during the 10-ns MD simulation for each protein-ligand complex to ascertain whether the complex was stably folded or unfolded. If the radius

of gyration remained relatively constant, the complex was considered to be stably folded, otherwise, it was considered to be unfolded.

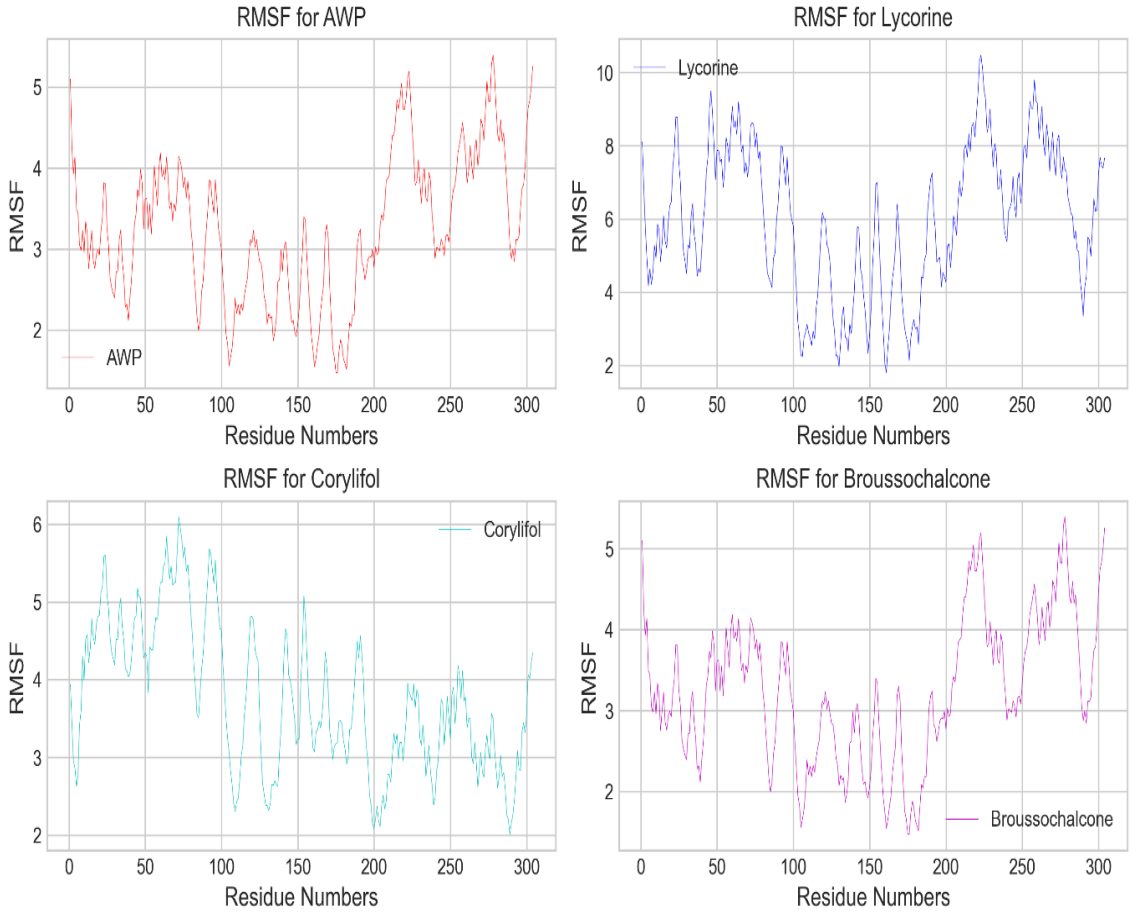

Figure 2: RMSF results for AWP, Corylifol, Broussochalcone, and lycorine with 5REH protein, based on the data from 10 ns simulation.

Table 6: The radius of Gyration for Protein-Ligand complexes.

Protein-Ligand Complex	Mean	Min	Max
5REH_AWP	22.1277	21.5693	22.5058
5REH_Lycorine	22.1774	21.6836	22.5126
5REH_ Broussochalcone A	22.1633	21.5523	22.5884
5REH_Corylifol	22.1888	21.7187	22.5877

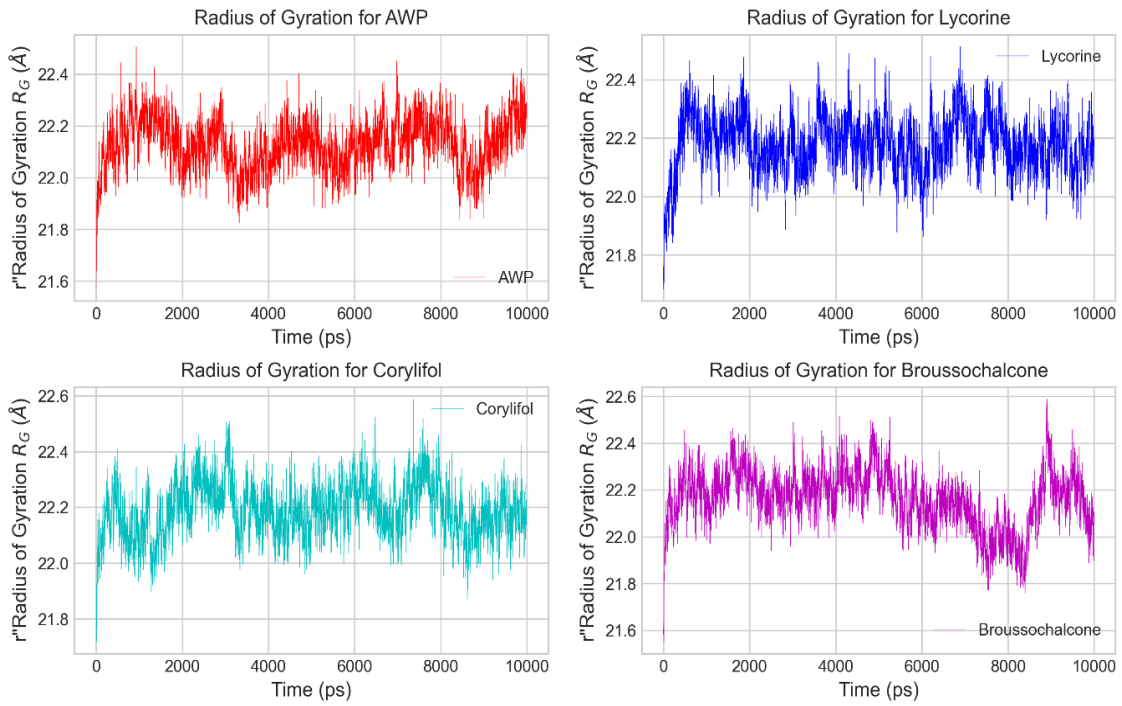

Figure 3: The radius of Gyration results for AWP, Corylifol, Broussochalcone, and Lycorine with 5REH protein, based on the data from the 10 ns simulation.

In this study, the radius of gyration values obtained is listed in Table 6. All the values obtained for the test ligand Lycorine, Broussochalcone A, and Corylifol showed a relatively constant radius gyration during the simulation are close to the mean radius of gyration values of protein and AWP complex. So, we can conclude that all of the complexes formed relatively stable folded polypeptide structures during the 10-ns MD simulation.

CONCLUSION

As our current understanding of the COVID-19, we know that today we are fighting without suitable and approved therapeutic guidelines. Until it is established, we have to survive with all the guidelines given and, on another hand, without delay, we have to be in search of the potential candidate that can help to control and prevent further spreading of infection. In this regard, we also have attempted an investigation by finding 78 natural compounds to selectively inhibit the main protease of the novel coronavirus. We also have adopted a computational method to screen ligands, as it requires less time, and produced results will help to reduce the failure of a drug candidate at their end-stage of development. The listed 78 compounds were docked, screened for their ADME profile. Further selected compounds were investigated for stability with protease by performing dynamic simulation studies. We found that three natural compounds namely lycorine, Broussochalcone A, and Corylifol A have

shown a good binding affinity towards the structure. They also have fulfilled the ADME criteria to come out as a novel therapeutic candidate. Finally, all three compounds were observed for their structural stability with protein by investigating their RMSD and RMSF values. Three compounds were also compared with standard ligand AWS with all the observed parameters. Three compounds showed deviations from the values compared to standard but were not statistically significant.

We submit the optimal binding affinity of lycorine, Corylifol, and Broussochalcone with the main protease target of nCoV-19 using molecular docking and simulation studies for further consideration by the scientific community.

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