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Homology Modelling and Molecular Docking Studies of Spike Protein in SARS-CoV-2

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The COVID-19 pandemic, caused by SARS-CoV-2, has posed substantial global health challenges, highlighting the urgent need for effective antiviral treatments. This study utilizes homology and molecular docking to identify potential natural compound inhibitors targeting the SARS-CoV-2 spike protein. The spike protein sequence was sourced from the Swiss-Prot database and modeled using MODELLER 10.3, employing templates from the Protein Data Bank (PDB). The constructed model underwent validation via Ramachandran plot analysis and MolProbity scores, confirming its reliability for subsequent analyses. Virtual screening of database was performed using AutoDock Vina. Compounds exhibiting the highest binding affinities were subjected to MD simulations to evaluate their stability. Tetrandrine (L1) and Tubocurarine (L2) emerged as the top candidates, with Tetrandrine demonstrating the lowest binding energy and the best fit. The ADMET properties of these compounds were assessed using SwissADME, affirming their drug-like potential. Molecular dynamics simulations further substantiated the stability of the Tetrandrine-spike protein complex, revealing significant interactions. This study identifies Tetrandrine as a promising inhibitor of SARS-CoV-2, warranting further exploration for antiviral drug development.

Keywords: ADMET properties; COVID-19; SARS-CoV-2; Middle East Respiratory Syndrome Corona Virus MERS-COV.

Corona virus disease, commonly known as COVID-19, is a transmissible and infectious disease that is caused by the severe acute respiratory syndrome corona virus 2 (SARS-COV-2). It led to the devastating effect that led to the pandemic-like situation on the entire world, resulting in more than 6 million deaths all over the It is known to have caused the most impactful global health crisis since the era of the influenza pandemic that emerged in As a zoonotic virus, the two highly contagious diseases, severe acute respiratory syndrome (SARS-COV-2) and Middle East Respiratory Syndrome Corona Virus (MERS-COV) emerged in 2002 and 2012, respectively, in humans and caused fatal respiratory This makes the emerging corona viruses a new health concern for the human in the twenty-first The patients suffering from SARS and MERS showed symptoms of viral pneumonia, including fever, cough, and chest discomfort, and in severe cases, shortness of breath and bilateral lung

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(pulmonary) infiltration (Zhu, et al., Gralinski, L.E. et al. 2020).

After that, the first case of corona virus was reported on December 8, 2019, in Wuhan, Hubei Province, China (Wu, & Menachery 2020). On December 31, Wuhan Municipal Health Commission notified the people of a pneumonia outbreak due to unidentified organism cause and informed the World Health Organization (WHO) (Hu, B et 2020). The isolation of virus from the bronchoalveolar lavage fluid was taken from several patients and by metagenomic RNA sequencing the causative agent was identified as corona virus and the strain that was never seen before (Deng, Q. & Peng, H. J., National health commission, WHO 2020). The first genomic sequence of novel corona virus was published on the Virology on 10 January 2020, and nearly completed genome sequences carefully determined by many different research institutions were then released via the GISAID database on 12 January 2020 (Chan, J F et 2020). Later, many more patients without any pre-exposure to Huanan Seafood Wholesale Market were also Several familial clusters of infections as well as nosocomial infections also occurred in healthcare All the cases of infection provide very clear evidence of human-to-human transmission of this new virus (Wang, , et al.2020, Cui, , Li, F. & Shi, Z. L.2019, National Health Commission China 2020). The Lunar New Year coincides with the outbreak of new virus, people travelled one place to anther which makes the transmission of this virus easier and quick (World Health Organization 2020).

Further, the novel corona virus pneumonia was seen spreading to the entire city of Hubei and, since then to nearly 34 provinces of The confirmed cases increased suddenly and rapidly with thousands of daily new cases during January 2021(Coronaviridae Study Group of the International Committee on Taxonomy of Viruses 2020). Thereafter, on 30, January 30, WHO (World Health Organization) declared an outbreak, a public health emergency that became a real concern (Fisher, D. & Heymann, D 2020). The corona virus was named as SARS-COV-2 by the International Committee on Taxonomy of Viruses and the WHO named the disease 'COVID-19' (Lai, C., et al. 2020). By that time the outbreak of COVID-19 disease in China reached to an epidemic peak in The National Health Commission of China claimed that the total number of cases that were identified every day at an average of 3,000 To control the COVID-19 outbreak, Chinese government implemented strict public health The Wuhan city was shut down completely, and all travel and transportation connecting the city was stopped to check the spread of corona Common people witnessed high restrictions on all outdoor activities, public gatherings, and other facilities all over the country (World Health organization 2020). As people started to follow all the norms the daily number of cases began to But by the during late February that international spread of COVID 19 began from China in different countries and cases were now reported from there as well (Dong, E., Du, H. & Gardner, L. 2020). The rapid global spread of COVID-19 was made possible by the high transmission efficiency of SARS-CoV-2 and the prevalence of international The global COVID-19 epidemic was formally classified as a pandemic by the WHO on March 11, 2020 (Tian, et al. 2019). While there was a noticeable decrease in China, the number of cases skyrocketed in the USA, Europe, and other The Center for System Science and Engineering at Johns Hopkins University's COVID-19 dashboard indicates that as of August 11, 2020, 216 countries—which has since increased to over 229 countries— had been impacted by the Over 20 million COVID-19 instances have been documented from various places across all six continents, with over 733,000 individuals having died as a result of the virus (Kleiger, 2000). High mortality was particularly prevalent when there was a significant impact on health care The nation having the most cases to date is the United States of According to the most recent data, there were 768 million Covid-19 instances worldwide as of June 12, 2023 (Statista 2024).

Despite the unrivalled efforts of vaccine development against the prevention of COVID-19 and vigorous global mass vaccination drives, the existence of new SARS-CoV-2 variants threatens to rule out the progress made in restricting the spread of this disease. It has already been established that ACE2 protein is having a very important role in developing the disease in humans by providing the channels of bonds for spike protein of Corona virus (Rahul Dev Ambedkar 2021). Currently, there is an urgent need for the novel SARS-COV-2 inhibitor which is potent enough for the production of an

effective antiviral drug. The present study shows the use of a natural compounds database that were chosen and selected for virtual screening. The best compound with the highest binding affinity with one of the active sites of SARS-COV-2 spike protein was selected that needs to be further analysed (Dong, E. et al. 2020, Yao, X. et al 2020).

The Ministry of Health (MOH) is closely monitoring the recent increase in COVID-19 infections in Singapore. Although there is no evidence that the circulating variants are more transmissible or cause more severe illness compared to previous strains, population immunity may have decreased over time. The ministry of Health urged the public to stay up-to-date with COVID-19 vaccinations to protect against current and emerging virus strains and to practice personal and social responsibility to reduce transmission. https://www.moh.gov.sg/newsroom/update-on-covid-19-situation)

From 5 to 11 May 2024, the estimated number of COVID-19 cases rose to 25,900, up from 13,700 the previous The average daily COVID-19 hospitalizations increased to approximately 250 from 181 the prior week, while the average daily Intensive Care Unit (ICU) cases remained low at three, compared to two the previous week (Ministry of Health Singapore) (May 2024).

We suspect that SARS-COV-2 will remain in the environment and will continue to change its spike protein structure and the human will with newly appearing strains of SARS-COV-2, however, the virus will reduce its virulence and the human will acquire immunity against the virus, finally both will learn to live with each other.

MATERIALS AND METHODS

The query target sequences of spike protein was imported in F format followed by the specifying a template structure in build B concept to identify the most suitable template that locates the appropriate The templates were taken from Protein data bank (PDB) (PDBID: 8DT3) and aligned with the target protein and an initial model was created and after that refinement was done for the geometry, side chain conformation and uncertain areas were fine-tuned through molecular dynamics simulations or energy minimization.

Finally, the final model was put through a vigorous validation process such as Ramachandran plot analysis and MolProbity scores to confirm the model's dependability, ensuring that the model is reliable and appropriate for further studies, including drug design or other downstream

Data Collection

The single letter Fasta Sequence of SARS-CoV-2 is downloaded from Swiss-Prot A PSI-BLAST search was performed using the query sequence of SARS-CoV-2 (YP_009825051.1) against the PDB It was found that the search results many significant For our study we carefully choose the suitable similar PDB sequences from Bats (7CN4|A), Pangolin (7CN8|A) and Human (7AKJ|A). The ClustalX program was used for sequence alignment.

Homology Modelling

The three-dimensional (3D) structures of SARS-CoV-2 was modelled in a stepwise procedure using MODELLER 3. This software implements homology modelling of proteins by satisfaction of spatial Binding site identification

The CASTp tool (Computer Atlas of Surface Topography of Proteins) (Tian et al., 2018) was utilized to identify the binding site, a freely accessible web-based tool that offers functions for identifying, characterizing, and quantifying the geometric and topological characteristics of protein structures (Kleiger et al. (2000)). It provides the internal cavities, geometric, and topological properties of the protein's amino acids along with the number of active sites on the specific pocket ID, and it aids in the prediction of the best binding residues for the modelled target.

Protein and ligand preparation

First, the Protein structure was prepared using the OPLS (optimized potentials for liquid simulations) force field after being imported into the AutoDock Vina software (Morris et al.,2009; Trott and Olson, 2010). Using the AutoDock tool, the polar hydrogens and Kollman charges were added. Moreover, the pdbqt format was used to save the Protein. Ligands from the natural compound's library were also converted into pdbqt formate using the OpenBabel tool (O'Boyle et al.,2011) and then use with Auto dock vina for Virtual screening.

Generation of grid box

One of the most crucial processes in the binding of a ligand to its receptor is the creation of the grid. Grid is the ligand-binding site's three-

dimensional boundary. AutoDock tool was used in the study to generate the grid (Trott and Olson, 2010), molecular docking open-source program. The receptor grid box (spacing of 0.375 Å) was created around the amino acid residues at the active site.

Virtual screening studies of Protein with natural compounds

Virtual screening was used to identify the best-fit molecule after the ligands and receptors were prepared (van Santen et 2019). Using a Perl script and the AutoDockVina program, the ligands were docked to the target protein or During the docking analysis, the ligands were viewed as flexible molecules and the receptor as a rigid PDBsum and was further used to visualize the docking results.

ADMET properties

SwissADME was used to predict ADMET properties (Daina et al., 2017) that enables users to predict ADME parameters, pharmacokinetic features, druglike nature, and medicinal chemistry friendliness of one or more small molecules for drug discovery in addition to computing physicochemical descriptors.

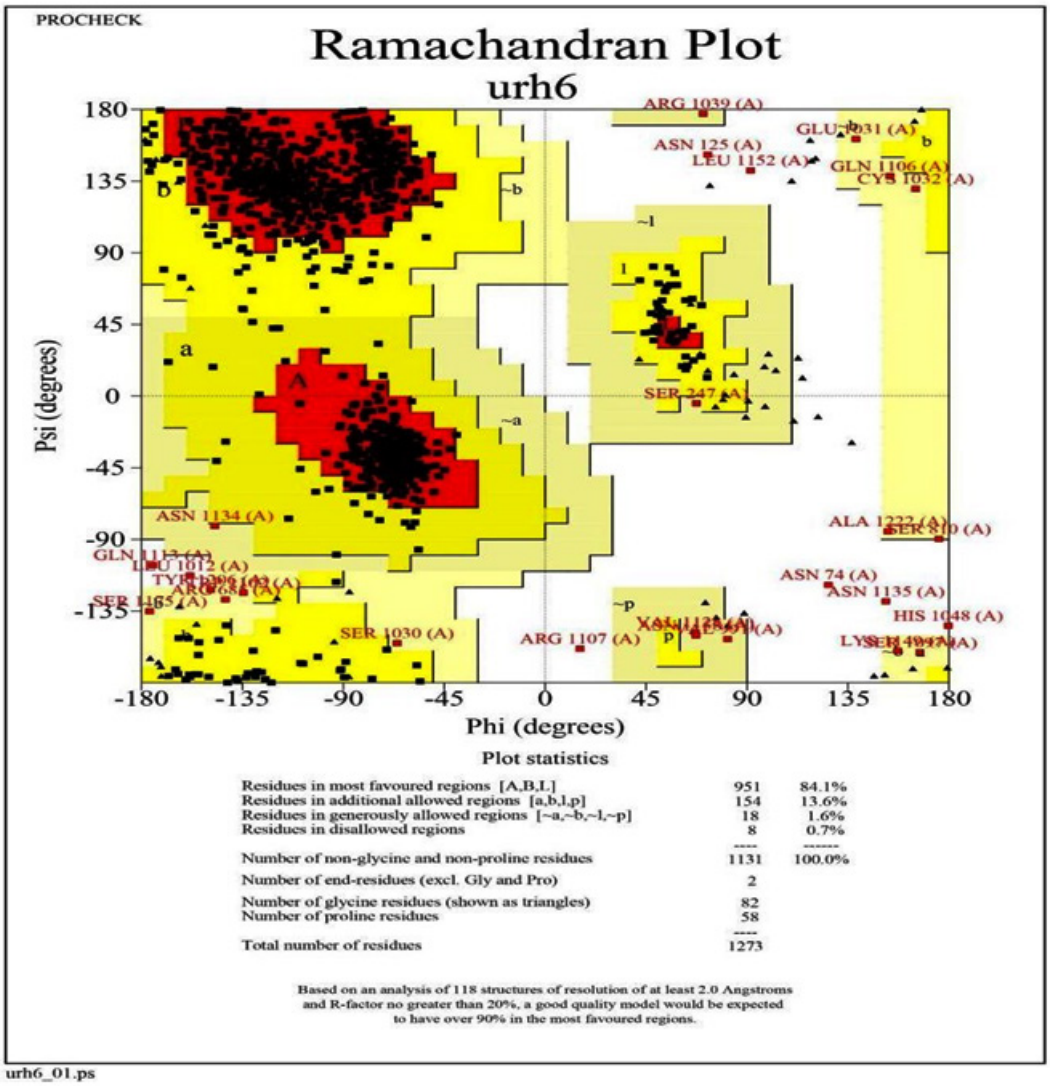

Fig. 1. Ramachandran plots for the protein theoretical 3D model of 1 protein The percentage of residues in the most favoured, allowed, and generously allowed regions are shown above in the Ramachandran

Molecular Dynamic Simulations

Molecular dynamics (MD) simulations explore the configuration space and produce a trajectory that captures molecular movements over Initially developed to investigate the properties of liquids, these simulations provide detailed insights into molecular An all-atomic MD simulation was carried out to demonstrate the binding stability of the Protein-ligand complex using the Schrodinger Desmond Before MD simulations, the complex structure was minimized using the protein preparation wizard of The hydrogen bond network was optimized at pH 4, and final restrained minimization was performed using the OPLS4 force Further minimized structures were incorporated into the system builder module to build an orthorhombic box solvated with TIP3P water model and further system neutralized by adding 15M NaCl as counter ions. The prepared system was relaxed before the MD simulation by

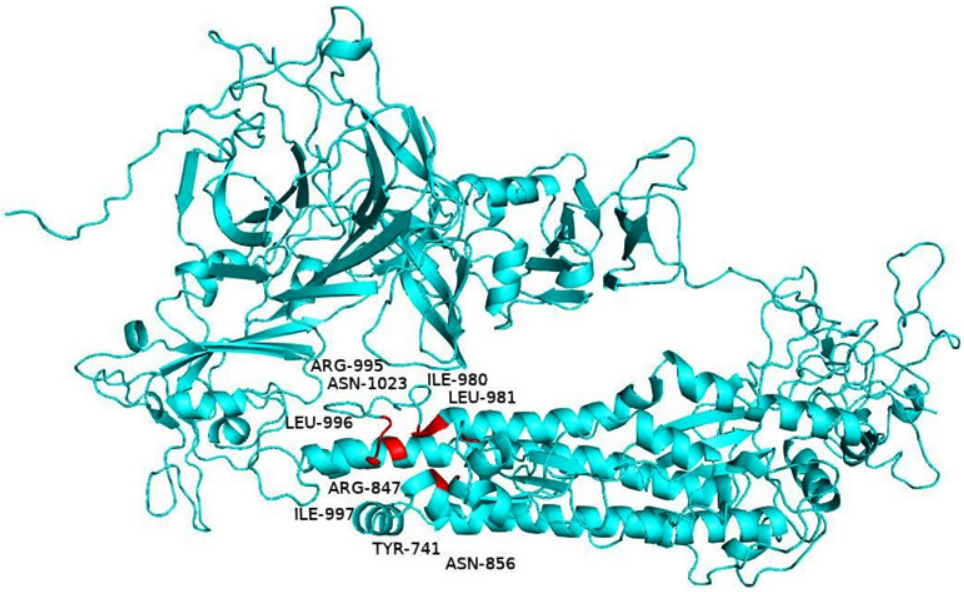

Fig. 2. Showing active site

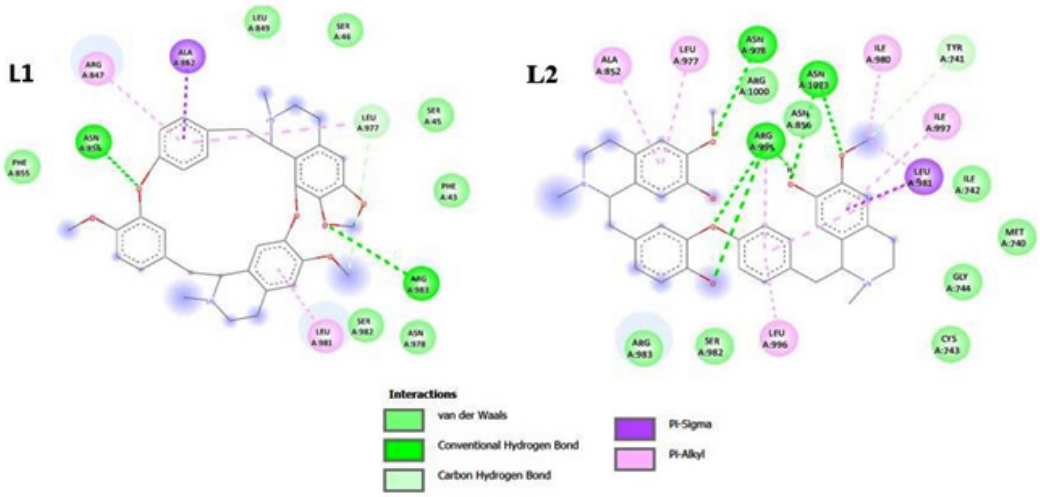

Fig. 3. Chemical structure of Tetrandrine (L1), Tubocurarine (L2)

a series of energy minimization and short MD Finally, the MD simulation was subjected to a 100ns time period and the coordinates were saved at an interval of 50 ps at 300 K temperature with 0325 bar pressure. The trajectory files were used to evaluate simulation results, e., Root Mean Square Deviation (RMSD), Root Mean Square Fluctuation (RMSF) and Ligand-protein interaction profile.

RESULTS

Homology modelling, Model quality assessment, and binding site prediction

The 3D structure was modelled by using Modeller 10.3. This software utilizes homology modelling of proteins by satisfying spatial restraints. We model the query sequence by searching suitable template using BLAST against pdb database. And carefully choose the model according to lowest DOPE score. After energy minimization 951 amino acids were in most favoured region, 154 in additional allowed region, 18 in generously allowed region and only 8 were in disallowed region.

The active site is predicted using CASTp tool, which shows that TYR 741, ARG 847, ASN 856, ILE 980, LEU 981, ARG 995, LEU 996, ILE 997, ASN 1030 are in the pocket of active site.

Molecular docking analysis

Natural compounds database was selected for virtual screening in the current These compounds were docked into the Protein active

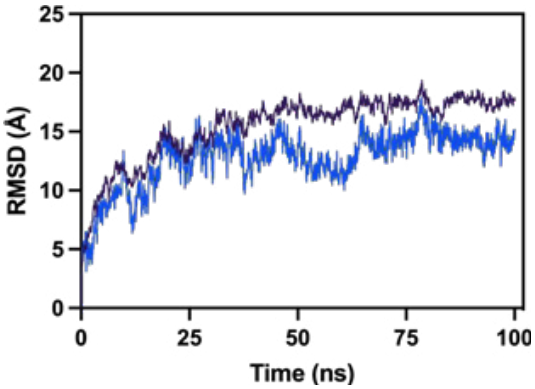

Fig. 4A. (RMSD of protein ligand complex)

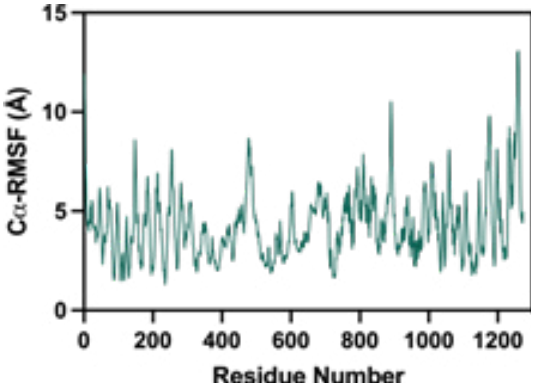

Fig. 4B. (Residue interaction with ligand)

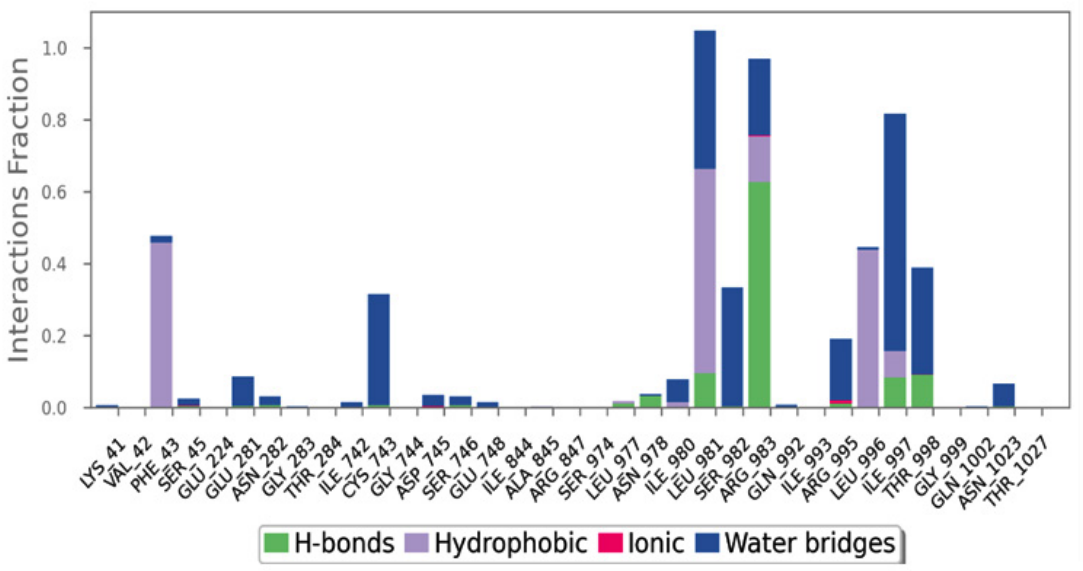

Fig. 4C. Stabilization through hydrogen bonding with Arg 983 and hydrophobic interaction with Leu 981

Table 1. Describing the comparison of the protein sequence from the three major models, SARS corona virus WH20, Bat corona virus RaTG13, and Pangolin corona virus, comparing the length of the protein sequence, positives, gas, and percentage identities

Protein sequence model	Sequence Id	Length	Score	Method	Identities	Positives	Gaps	Expect
Chain A, Spikeglycoprotein [SARS coronavirus WH20]	7AKJ_A	1193	1830 bits (4741)	Comparison matrix adjust.	872/1169 (75%)	1009/1169 (86%)	24/1169 (2%)	0.0
Chain A, Spike glycoprotein [Bat corona virus RaTG13]	7CN4_A	1267	2425 bits (6284)	Comparison matrix adjust.	1179/1213 (97%)	1190/1213 (98%)	4/1213 (0%)	0.0
Chain A, Glycoprotein [Pangolin corona virus]	7CN8_A	1295	2303 bits (5969)	Comparison matrix adjust.	1114/1216 (92%)	1157/1216 (95%)	6/1216 (0%)	0.0

site using the Autodock Vina program with a Perl The top Ten natural compounds were chosen from a natural compound library, based on their higher interactions with protein binding residues and lowest binding energy (high binding affinity). Out of the ten compounds, top five molecules (Figure 3) that followed the Lipinksi rule (drug-like properties), and out of five top two molecules (Tetrandrine (L1), Tubocurarine (L2) were selected for further Top one compound, Tetrandrine (L1) was the molecule with the lowest binding energy and the best fit, so it was chosen for the Molecular dynamic simulation Table 2 displays the binding energy, which represents their interactions with the binding residues.

Molecular Dynamic Simulations

To get insight into the protein-ligand binding stability of protein complex structure with ligand 100 ns of all atomic molecular dynamics To access the conformational stability of protein-ligand complex, the backbone and ligand root mean square deviation (RMSD) information was extracted from the MD simulation trajectory (Figure 4 A). System gets well equilibrated during the period of ~40 ns of MD simulation.

Protein-ligand complex attained stable RMSD throughout the MD simulation after equilibration (Fig A). The RMSD value for backbone and ligand movement was observed to be 4 ± 2.8 Å and 12.7 ± 2.4 Å. RMSF measurement shows that the residues interacting with ligands had lower fluctuation (Fig. 4 B). Further ligand stabilized through hydrogen bonding with Arg983 and hydrophobic interaction with Leu 981 (Fig. 4 C).

DISCUSSION

The studies show that the SARS-CoV-2 (YP_009825051.1) is one of the prominent targets for the antiviral drug treatment and some various natural products or resources can be exploited for their pharmacological importance. In order to do so, a combination of computational biology like pharmacophore-based virtual screening molecular docking, MD simulation, and Autovina program with pearl script were used to conduct the protein docking in the active site of SARS-COV-2. There have been various natural products with potential anti-COV activity analysis through

Table 2. ADMET properties of natural compounds using SwissADME

Properties	Tetrandrine	Tubocurarine
Molecular weight	570.42 g/mol	560.43 g/mol
Num. rotatable bonds	2	8
Num. H-bond donors	2	4
Num. H-bond acceptors	6	7
TPSA	79.44 Å²	103.65 Å²
Drug likeness		
Properties	Tetrandrine	Tubocurarine
Lipinski	Yes, 1 Violation	Yes, 1 Violation
Bioavailability Score	0.55	0.55
Water Solubility	Poorly soluble	Poorly soluble

Table 3. Top-screened hits showing interacting residues, binding energies and Lipinski rule of Five

Compounds	Amino Acids	Binding Energy (KCal/mole)	Lipinski Rule follow
Ligand 1	SER 45, SER 46,PHE 43, ARG 847,LEU 849, ALA 852,PHE 855, ASN 856,LEU 977, ASN 978,LEU 981, SER 982ARG 983	-10.1	Yes
Ligand 2	MET 740, TYR 741,ILE 742, CYS 743, GLY 744, ALA 852,ASN 856, LEU 977, ASN 978, ILE 980,LEU 981, SER 982,ARG 983, ARG 995,LEU 996, ILE 997,ARG 1000, ASN1023	-9.6	Yes

virtual screening against natural product databases. Therefore, generated pharmacophore models were used as filters for screening the selected natural product databases- Top Ten natural compounds were chosen from a natural compound library, based on their higher interactions with protein binding residues and lowest binding energy (high binding affinity). Out of the ten compounds, the top five molecules (Figure 2) that followed the Lipinksi rule (drug-like properties), and out of the five top two molecules (Tetrandrine (L1), Tubocurarine (L2) were selected for further Further stability was analysed using MD simulations. The results showed that there top compound, the Tetrandrine (L1) molecule with the lowest binding energy and the best fit, so it was chosen. Table 2 displays the binding energy, which represents their interactions with the binding residues. The results reveal that PDB sequences from Bats (7CN4|A) have the highest percentage identity and are perfectly aligned with the target sequence of SARS-COV-2 using the ClustalX program. Further, Ligands from the natural compound's library were also converted into pdbqt format using the OpenBabel tool (O'Boyle et ,2011) and then used with Auto dock vina for Virtual screening. Thereafter, the grid is created to analyze the bonding of the ligand to the Grid is the ligand-binding site's three-dimensional AutoDock tool was used in the study to generate the grid (Trott and Olson, 2010). Virtual screening was used to identify the best-fit molecule after the ligands and receptors were prepared (van Santen et 2019). SwissADME was used to predict ADMET properties (Daina et , 2017). Finally, after the docking analysis, the best compounds were screened and analyzed.

Ramachandran plot is a space-filling model of peptides used to visualize energetically possible (i.e. sterically permitted) values for dihedral angles ? against F for a polypeptide It is a space-filling model of peptides used to visualize energetically possible (i.e. sterically permitted) values for dihedral angles ? against F for a polypeptide The Ramachandran plot analysis of the protein query sequence 1 (done by PDBsum) depicts that the structure is stable with only a few

conserved cysteine residues in the disallowed Since the cysteine residues contain the disulfide bonds it provides more stability to the protein Therefore, these are the conserved regions that don't ought to any changes in the There are other amino acid residues present in the disallowed regions which are alanine, Aspargine, Histidine, Glutamate, alanine, and Homology modelling, Model quality assessment, and binding site prediction were done using the Ramachandran plot, and the one with the least binding energy was selected which can be the most potent inhibitor for the active site of the spike protein of SARS-COV-2.

CONCLUSION

After the molecular docking analysis using the Autodock Vina program the natural compound databases were selected for virtual screening and all ten compounds were selected further analysis resulted in the top two natural compounds namely Tetrandrine (L1), Tubocurarine (L2) which showed the lowest binding energy and highest The topmost compound with the highest affinity with the active sites of SARS-COV-2 is Tetrandrine (L1). Homology modelling, Model quality assessment, and binding site prediction were done using the Ramachandran The final model was put through a rigorous validation process such as Ramachandran plot analysis and MolProbity scores were done to confirm the model's dependability, ensuring that the model is reliable and appropriate for further studies, including drug design or other downstream This compound can be the most potent inhibitor against the SARS-COV-2 active sites and needs to be further

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Conflict of Interest

The authors do not have any conflict of interest.

Data Availability Statement

This statement does not apply to this article.

Ethics Statement

This research did not involve human participants, animal subjects, or any material that requires ethical approval.

Informed Consent Statement

This study did not involve human participants, and therefore, informed consent was not required.

Author Contributions

Rahul Dev Ambedkar: Conceptualization, Methodology, Writing – Original Draft, Data Collection, Analysis, Writing – Review & amp; Editing; Prof. Amar P Garg: Supervision; Prof. Payal Mago: Supervision.

REFERENCES

1. Ambedkar, R.D., Role of ACE2 in infection by Corona Virus. Annals of Romanian Society for Cell Biology ISSN:1583-6258, Vol. 25, Issue 5, 2021, Pages. 2941 – 2961
2. Chan, J. F. A familial cluster of pneumonia associated with the 2019 novel Corona virus indicating person-to-person transmission: a study of a family cluster. Lancet 395, 514–523 (2020)
3. Coronaviridae Study Group of the International Committee on Taxonomy of Viruses. Th species severe acute respiratory syndrome- related Corona virus: classifying 2019-nCoV and naming it. SARS- CoV-2. Nat. Microbiol. 5, 536–544 (2020)
4. Cui, J., Li, F. & Shi, Z. L. Origin and evolution of pathogenic Corona viruses. Nat. Rev. Microbiol. 17, 181–192 (2019).
5. Daina, A., Michielin, O., & Zoete, V. (2017). SwissADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. *Scientific Reports*, *7*(1), 42717. https://doi.org/10.1038/srep42717
6. Deng, S. Q. & Peng, H. J. Characteristics of and public health responses to the Corona virus

disease 2019 outbreak in China. J. Clin. Med. 9, 575 (2020)

7. Dong, E., Du, H. & Gardner, L. An interactive web- based dashboard tommm track COVID-19 in real time. Lancet Infect.Dis. 20, 533–534 (2020).
8. Dong, E., Du, H. & Gardner, L. An interactive web- based dashboard to track COVID-19 in real time. Lancet Infect.Dis. 20, 533–534 (2020). Wang, M. et al. Remdesivir and chloroquine effectively inhibit the recently emerged novel Corona virus (2019-nCoV) in vitro. Cell Res. 30, 269–271 (2020).
9. Fisher, D. & Heymann, D. Q&A: the novel corona virus outbreak causing COVID-19. BMC Med. 18, 57(2020). ·
10. Gralinski, L. E. & Menachery, V. D. Return of the Corona virus: 2019-nCoV. Viruses 12, (2020).
11. https://www.statista.com/statistics/1103040/cumulative-Coronavirus-covid19-cases-number-worldwide-by-day.
12. Hu B, Guo H, Zhou P, Shi ZL. Characteristics of SARS-CoV-2 and COVID-19. Nat Rev Microbiol. 2021 Mar;19(3):141-154. doi: 10.1038/s41579-020-00459-7. Epub 2020 Oct 6. Erratum in: Nat Rev Microbiol. 2022 May;20(5):315. PMID: 33024307; PMCID: PMC7537588.
13. Kleiger, G., Beamer, L. J., Grothe, R., Mallick, P., & Eisenberg, D. (2000). The 1.7 A crystal structure of BPI: a study of how two dissimilar amino acid sequences can adopt the same fold. *Journal of Molecular Biology*, *299*(4), 1019–1034.
14. Lai, C. C., Shih, T. P., Ko, W. C., Tang, H. J.& Hsueh, P. R. Severe acute respiratory syndrome Corona virus 2 (SARS- CoV-2) and corona virus disease-2019 (COVID-19): the epidemic and the challenges. Int. J. Antimicrob. Agents 55, 105924(2020).
15. Ministry of Health Singapore (May, 2024). https://www.moh.gov.sg/news-highlights/details/update-on-covid-19 situation#:~:text=The%20average%20daily% 20COVID% 2D19,the%20trajectory% 20of%20this%20wave.
16. Morris, G. M., Huey, R., Lindstrom, W., Sanner, M. F., Belew, R. K., Goodsell, D. S., & Olson, A. J. AutoDock4 and AutoDockTools4: Automated docking with selective receptor flexibility. *Journal of Computational Chemistry*,*30*(16), 2785–2791.(2009).
17. National Health Commission of the People's Republic of China. Briefing on the latest situation of the novel Corona virus pneumonia epidemic. http://www.nhc.gov.cn/xcs/yqtb/list_gzbd.shtml (2020).
18. O'Boyle, N. M., Banck, M., James, C. A., Morley, C., Vandermeersch, T., & Hutchison, G. (2011). Open Babel: An open chemical toolbox. *Journal of Cheminformatics*, *3*(1), 33.
19. Studer, G., Rempfer, C., Waterhouse, A. M., Gumienny, R., Haas, J., & Schwede, T. (2020). QMEANDisCo—distance constraints applied on model quality estimation. *Bioinformatics*, *36*(6), 1765–1771. https://doi.org/10.1093/bioinformatics/btz828
20. Tian, W., Chen, C., Lei, X., Zhao, J., & Liang, J. (2018). CASTp 3.0: computed atlas of surface topography of proteins. *Nucleic Acids Research*, *46*(W1), W363–W367. https://doi.org/ 10.1093/nar/gky473
21. Tian, Wei; Chen, Chang; Lei, Xue; Zhao, Jieling; Liang, Jie. CASTp 3.0: computed atlas of surface topography of proteins. Nucleic Acids Research, 46(W1), W363–W367. doi:10.1093/nar/gky473 (2018).
22. Trott, O., & Olson, A. J. AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. *Journal of Computational Chemistry*, *31*(2), 455–461. https://doi.org/10.1002/jcc.21334 (2010).
23. van Santen, J. A., Jacob, G., Singh, A. L., Aniebok, V., Balunas, M. J., Bunsko, D., Neto, F. C., Castaño-Espriu, L., Chang, C., Clark, T. N., Cleary Little, J. L., Delgadillo, D. A., Dorrestein, P. C., Duncan, K. R., Egan, J. M., Galey, M. M., Haeckl, F. P. J., Hua, A., Hughes, A. H., … Linington, R. G. (2019). The Natural Products Atlas: An Open Access Knowledge Base for Microbial Natural Products Discovery. *ACS Central Science*, *5*(11), 1824–1833. https://doi.org/10.1021/acscentsci.9b00806
24. Wang, R., Zhang, X., Irwin, D. M. & Shen, Y. The emergence of SARS- like Corona virus poses a new challenge in China. J. Infect. 80, 350–371 (2020).
25. World Health Organization declares novel Corona virus (2019-nCoV) sixth public health emergency of international concern. Euro. Surveill. 25, 200131e(2020).
26. World Health Organization. Corona virus disease 2019 (COVID-19). Situation report – 51. https://www.who.int/docs/default-source/Coronaviruse/situation- reports/20200311-sitrep-51-covid-19.pdf?sfvrsn=1ba62e57_10 (2020).
27. Wu, F. et al. A new Corona virus associated with human respiratory disease in China. Nature 579, 265–269 (2020). Zhou, P. et al. A pneumonia outbreak associated witha new Corona virus of probable bat origin. Nature 579,270–273 (2020).
28. Wu, Z. & McGoogan, J. M. Characteristics of and

important lessons from the Corona virus disease 2019 (COVID-19) outbreak in China: summary of a report of 72314 cases from the Chinese Center for Disease Control and Prevention. JAMA 323, 1239–1242(2020).

29. Yao, X. et al. In vitro antiviral activity and projection of optimized dosing design of hydroxychloroquine for the treatment of severe acute respiratory syndrome Corona virus 2 (SARS- CoV-2). Clin. Infect. Dis. 71, 732–739 (2020).

30. Zhu, N. et al. A Novel Corona virus from patients with pneumonia in China, 2019. N. Engl. J. Med. 382,727–733 (2020).