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Computational Docking Study of the Phytochemical Constituent, Silybin (Silybum marianum) against SARS-CoV-2 Omicron Variant Spike Glycoprotein: An *In-silico* Approach

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Abstract

SARS-CoV-2 is continually evolving with the emergence of new variants with increased viral pathogenicity. The emergence of heavily mutated Omicron (B.1.1.529) with spike protein mutations are known to mediate its higher transmissibility and immune escape that has brought newer challenges for global public health to contain SARS-CoV-2 infection. One has to come up with a therapeutic strategy against the virus so as to effectively contain the infection and spread. Natural phytochemicals are being considered a significant source of bioactive compounds possessing an antiviral therapeutic potential. Being a promising anticancer and chemo-preventive agent, Silybin holds a significant potential to be used as a therapeutic. In the present study, molecular docking of Silybin with Omicron spike protein (7QNW) was carried out. Molecular docking results showed greater stability of Silybin in the active site of the Omicron spike protein with suitable binding mode of interactions. The study reveals that Silybin has the potential to block the host ACE2 receptor-viral spike protein binding; thereby inhibiting the viral entry to human cells. Therefore, Silybin may be further developed as a medication with the ability to effectively combat SARS-CoV-2 Omicron.

Keywords: SARS CoV-2 Omicron, Spike Protein, Phytochemicals, Computational Approach, Antiviral Drugs

INTRODUCTION

The COVID-19 pandemic is a serious global menace that has been affecting the human population. Acute respiratory distress and infection caused by COVID-19, which is thought to have begun in Wuhan, China, in December 2019 and has since spread around the world, is the current leading cause of morbidity and mortality globally.[1,2] Infectious disease known as COVID-19 is caused by a newly described virus in the β-coronavirus genus of the coronaviridae family. This virus had phylogenic similarity with severe acute respiratory syndrome coronavirus (SARS-CoV), but later renamed as SARS-CoV-2.[3] Some mutations in SARS-CoV-2 have led to the emergence of new viral subtypes with unique properties. Vaccines and other pharmaceutical formulations often allow "variants of concern" (VOCs) or "variants of interest" (VOIs) to escape.[4] Moreover, they are more transmissible, and may lead to further severity of the disease.[5] In addition to being classified a new VOC by the World Health Organization on November 26, 2021, Omicron was also identified as a variant of SARS-CoV-2 called B.1.1.529.[6,7]

Omicron SARS CoV-2 known to be originated from South Africa, is a highly divergent variant of the virus with more than 60 mutations.[8] Amongst all the SARS-CoV-2 variants, Omicron has been seen to have many mutation prone sites with more than 50% mutations were detected in the spike protein regions.[9,10] There are 32 mutations in spike protein which have been confirmed to be involved in transmissibility, immune evasion and disease severity. Moreover, 15 out of 32 mutations have been identified at the spike protein receptor binding region, responsible for making the virus sensitive to neutralizing antibodies.[11,12] These mutations may allow the immune response to be bypassed, which could lead to multiple infections leading to several breakthrough infections or re-occurrence with mutant virus strains.[8,13] Because of this, the scientific community and pharmaceutical industry have been tirelessly searching for new medications that can effectively combat the infection.[14] One effective method of preventing viral infection is to block the process by which virus particles connect to host cells. As the virus spike glycoprotein is a major virulent factor for facilitating viral entry inside the human cells, it is considered as one of the primary drug targets to neutralise the variant of the virus.[3,15]

Natural products containing phytochemicals have been consistently being considered as primary source of drug development against the virus.[16,17] Many natural plant-based phytochemicals including alkaloids, flavonoids, and other compounds, have been reported to be active against SARS-CoV-2.[18,19] *In vitro* and even *in vivo* studies have revealed that flavonoids have the anti-viral potential as well.[20] Silybin, a component of flavonolignan extract isolated from *Silybum marianum*, has been shown to

have hepato-protective effects. Moreover, it has been reported to be a potent anticancer and chemo-preventive agent.[21] Silybin was also reported to inhibit Hepatitis C and Influenza A virus infection displaying its antiviral potential.[22] Molecular dynamics (MD) simulations are an effective approach in molecular docking for virtual screening of target-ligand binding ability prediction.[23,24] Silybin was molecularly docked with SARS-CoV-2 Omicron spike glycoprotein in the current investigation. The objective of the study was to discover Silybin as a potent natural compound that targets omicron spike protein to hinder its interaction with the cellular receptor of the host.

MATERIALS AND METHODS

Receptor preparation

The protein responsible for the virus pathogenicity especially the Omicron variant spike glycoprotein which facilitates the viral entry into the host cell, was selected as the target. The 3-D crystal structure of the target spike glycoprotein was retrieved from the Research Collaboratory Structural Bioinformatics-Protein Data Bank (RCSB-PDB) with PDB ID: 7QNW.[25,26] The water molecules and heteroatoms were excluded in order to clean the model.

Docking via Auto dock

We have selected Silybin as the lead molecule (Figure 1). The docking studies of the ligand design and its fitting in the binding pocket were performed using Autodock tools. The same is designed to check out the binding of drug candidates to a receptor of a known 3D structure.[27,28] Visualization studies at every level have been performed using Autodock, PYMOL and SPDBV tools.[29,30] It was found that the designed probable best ligand/compound has shown the best binding affinity of the ligand, i.e. ΔG-8.2Kcal/mole around the binding cleft, which proves that the more negative energy corresponds to the better ligand features (Table). Based on our experimental calculations on ligand binding free energy, favoured interactions with protein atoms, the results of the study are consistent with those found in the existing literature.[31]

Figure 1. Silybin-3D structure

MD simulation Studies

For MD simulations, GROMACS 2020.2 was used on the 7QNW-Silybin complex with the highest docking score, which demonstrates an exceptionally strong binding affinity. The purpose of this study was to learn more about the structural variations that occur in a fast-paced environment on a time scale of 10 ns.[32] Interaction energy, free energy of solvation (DGsolv), root mean square fluctuation (RMSF), solvent accessible surface area (SASA), radius of gyration (Rg), and root mean square deviation (RMSD) values as a function of time, and average angle and angle distribution of ligand in the active site of the receptor protein were used as the basis for the MD simulations. The RMSF, SASA values, radius of gyration, and RMSD obtained were in agreement with the previous studies.[33]

Table. Ligands run with Autodock, showing Binding Energies with RMSD values after docking

Ligand Mode	Binding Affinity	Rmsd/ub	Rmsd/lb
1	-7.6	12.785	9.204
2	-7.4	14.706	7.302
3	-7.3	10.423	5.437
4	-7.3	11.211	7.323
5	-7.2	8.156	6.144
6	-7.1	9.9	1.935
7	-7.1	11.562	5.918
8	-7.1	12.276	6.164
9	-8.2	16.988	14.213

RESULTS AND DISCUSSION

SARS-CoV-2 is continuously evolving into newer variants with its Omicron variant carrying an unusual number of mutations, mainly on the spike protein which could lead to altered transmissibility and immune evasion, imposing a newer challenge for the humanity.[34] Spike glycoprotein serves as the major virulence factor that facilitates virus entry into human cells (attached to the cellular receptor ACE2) and mediates its infectious cycle.[23] Therefore, the spike glycoprotein was looked at as

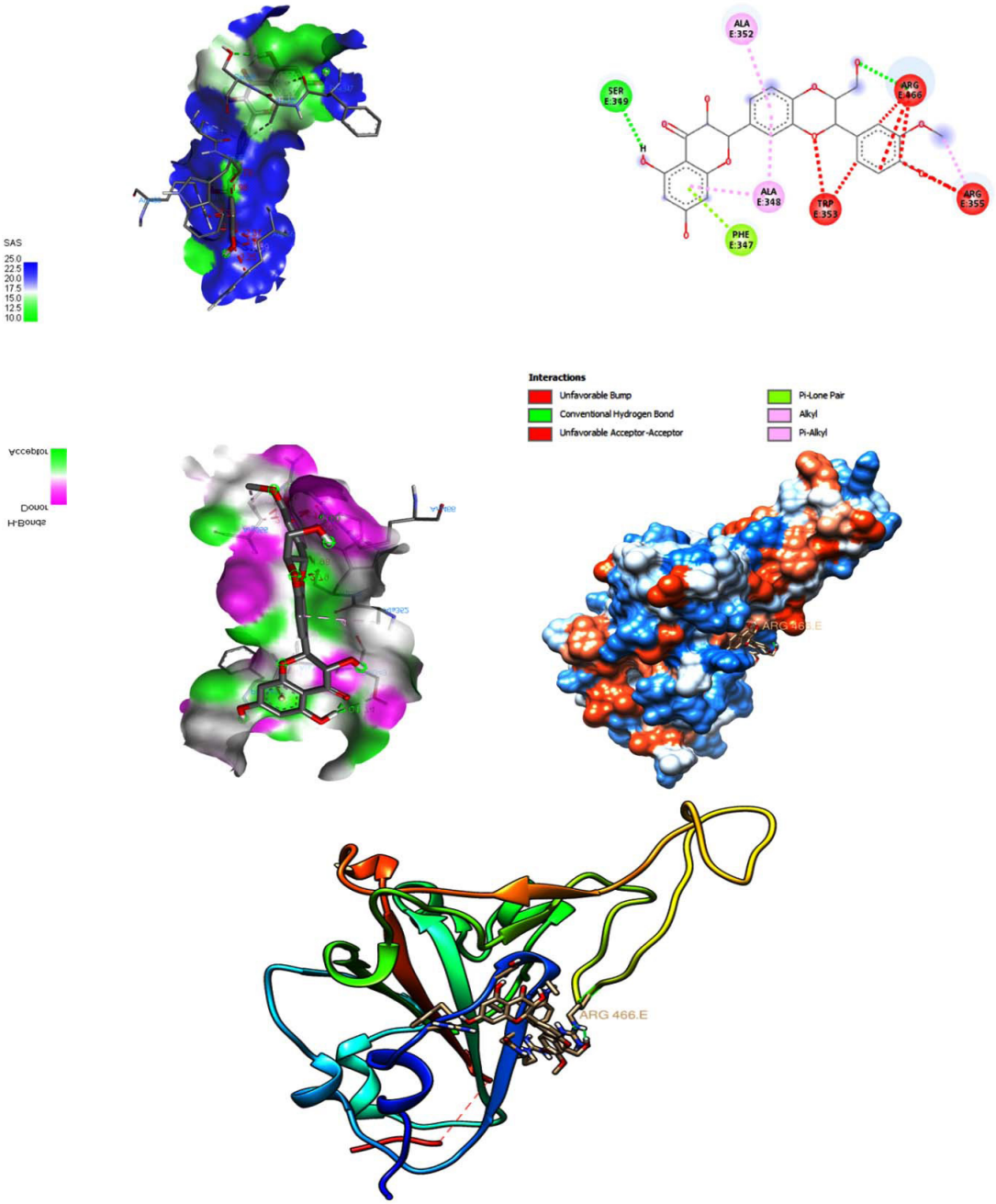

Figure 2. Docked complex structure with various conformations & interacting residues

a promising therapeutic target to prevent human viral entry and transmission. We adopted the *in-silico* approach as computational virtual screening has been very cost-effective and takes relatively lesser time to reach to the market.[18,35]

In the quest to produce effective antiviral drugs, natural substances are widely regarded as the most important resource due to their wide availability and low risk of side effects. Phytochemicals like flavonoids abundantly present in our daily diet, have been extensively studied as a possible therapeutic alternative against viruses.[20] Silybin (a flavonolignan extract component) has

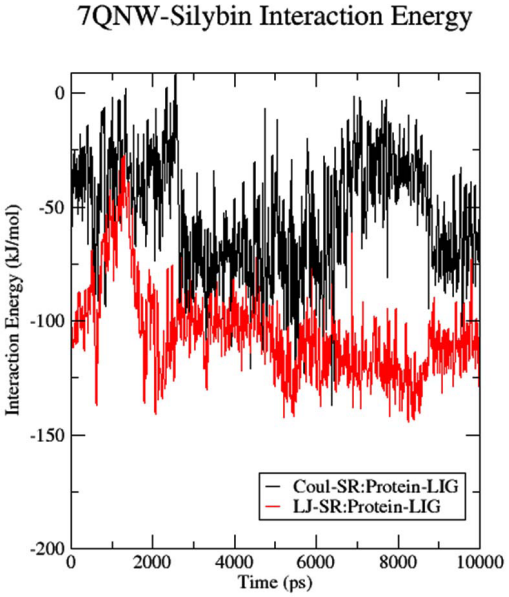

Figure 3. Interaction energy

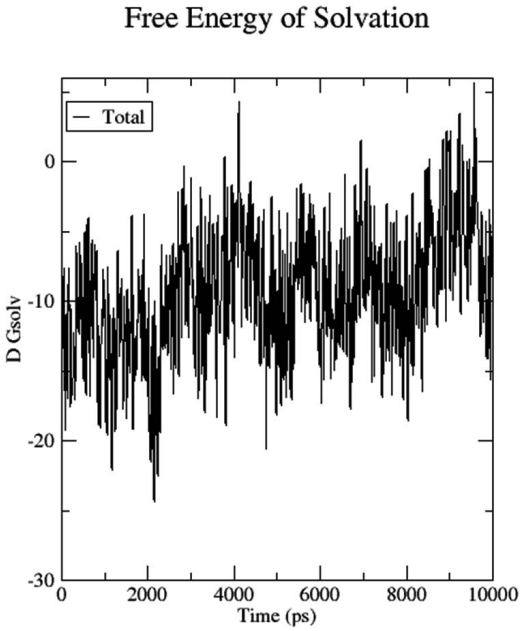

Figure 4. Free energy of solvation

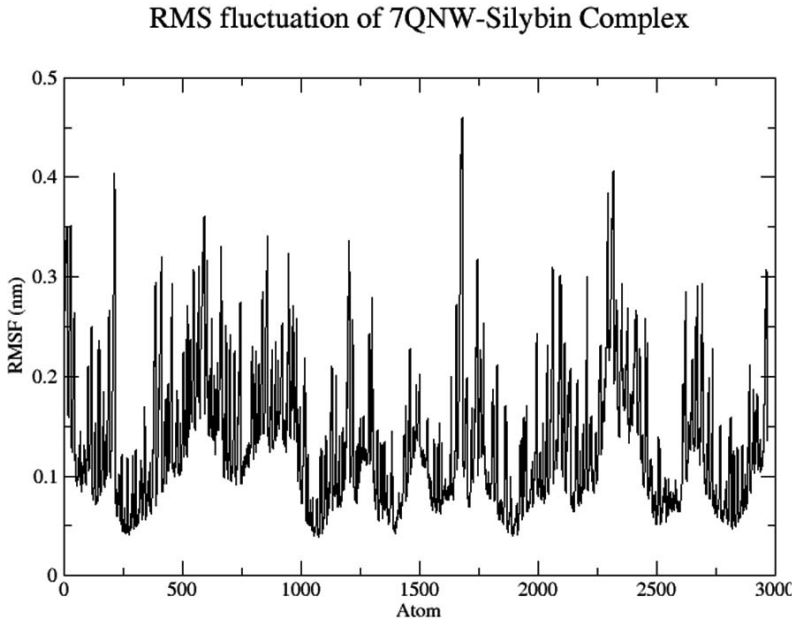

Figure 5. RMS Fluctuation of 7QNW-Silybin Complex

been reported as a potent anticancer and chemo-preventive agent.[21] The antiviral activities of Silybin showed its potential to be used as a broad-spectrum antiviral therapeutic.[22] Therefore, the molecular docking of Silybin with the target protein was carried out using the Lamarckian Genetic algorithm and Local Search default parameters.[24] Developed ligands displayed significant binding as evident from the Gibbs free energy values having lower RMSD values in comparison to the original conformation. Moreover, IC50 values (i.e. activity concentration) of analyzed ligands was also reported to be highly satisfactory consistent with earlier reports in literature (Table) (Figure 2).

Initial fluctuations in the simulations led to an average Coulomb's short-range (Coul-SR) value of -53.32 KJ/mol for the complex 7QNW-Silybin. As a whole, the complex has a Lennard-Jones short-range (LJ-SR) value of -105.69 KJ/mol. LJ-SR value was reportedly higher at the start of the simulation and got stable to -100 KJ/mol after two nanosecond simulations, indicating that the 7QNW-Silybin interaction got stable as the simulation proceeded (Figure 3). With an average solvation-free energy of -10 DGsolv, the complex has not changed (Figure 4). To insert a solute molecule into the solvent at constant temperature and pressure, the energy value obtained was reportedly significant and most prominent in keeping the complex static in an environment required.[36]

To gauge atomic malleability, we calculated RMSF values for ligand-protein complexes. Under 0.4 nm was maintained for RMSF values in the ligand-protein combination (Figure 5). At least in theory, the SASA can reveal how easily a protein can be dissolved in water. As the simulations progressed, SASA for the complex

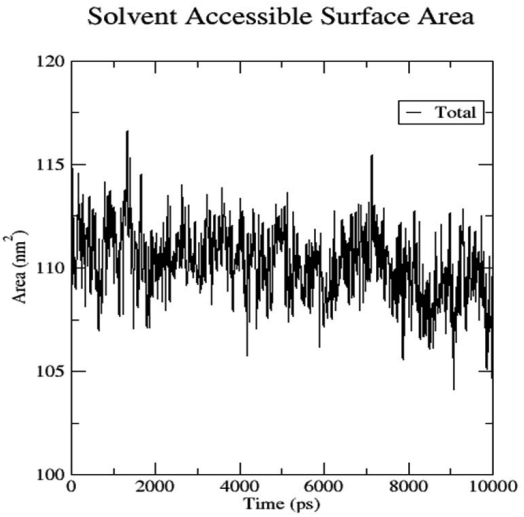

Figure 6. Solvent Accessible Surface Area

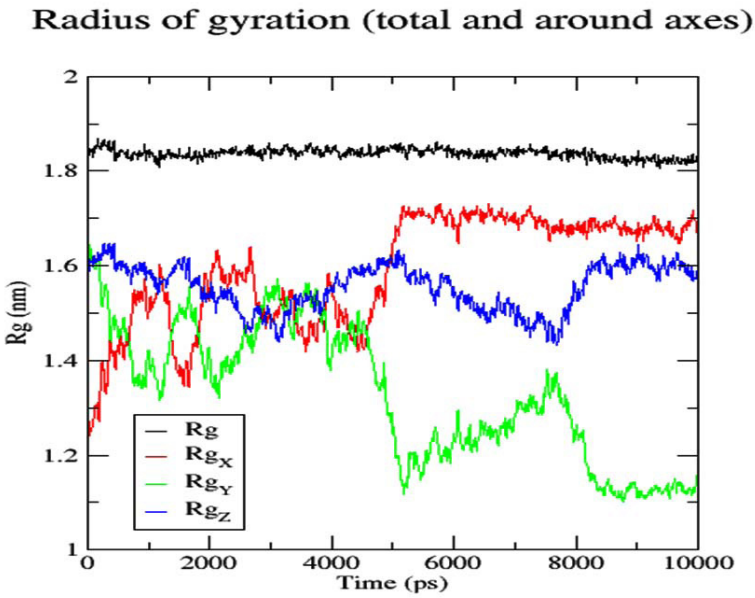

Figure 7. Radius of Gyration

stayed about 110 nm.[2] SASA values for the 7QNW-Silybin complex begin to lower down after 7 ns (Figure 6). Based on the decreasing SASA values, we can infer that the ligand Silybin interacts more with the system as the simulation progresses. The protein's compactness during MD simulations was visualized by analyzing a plot of Rg during a time interval of 10 ns.[37] The 7QNW-Silybin complex has a constant Rg of 1.8 nm, which is an indicator of its stability during simulations (Figure 7). The RMSD of ligands was tracked in order to monitor ligand stability in simulations. Average RMSD for Silybin was 0.5 nm, with a range of 0.3–0.7 nm suggesting the ligand's stability during the simulation (Figure 8). Literature also supports the range of RMSD values obtained as a generally acceptable

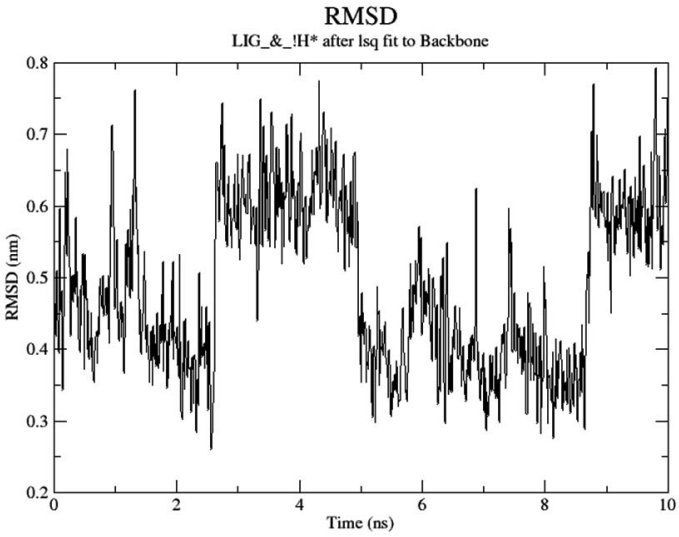

Figure 8. RMSD fluctuations

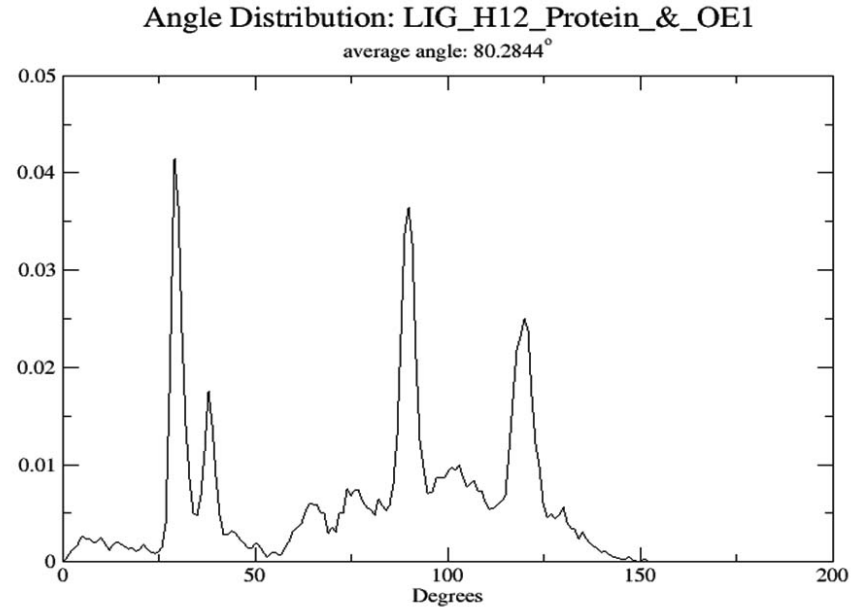

Figure 9. Angle Distribution among Ligand & Protein

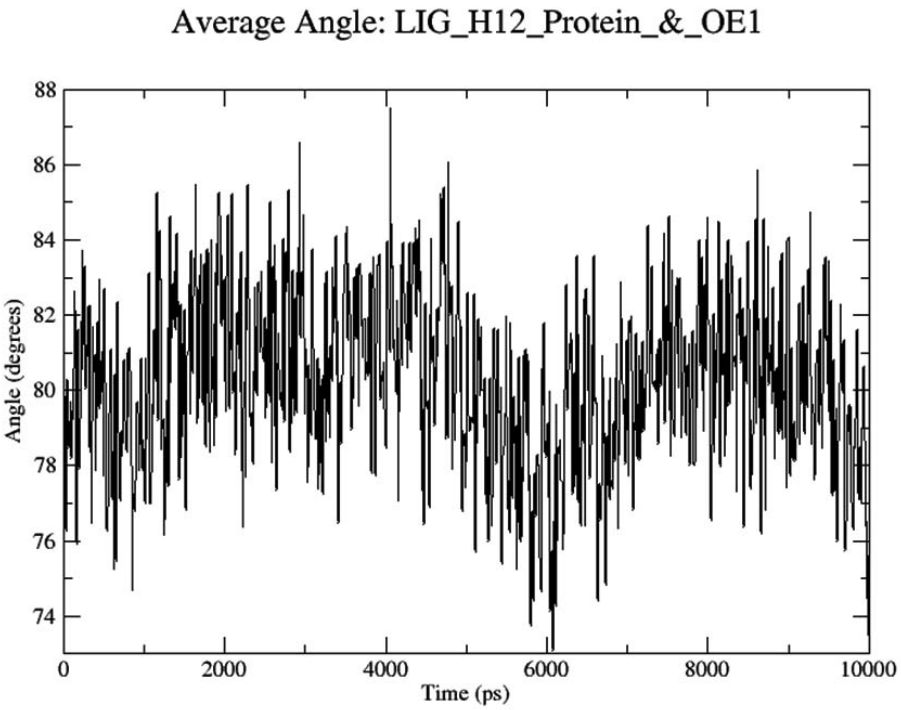

Figure 10. Average Angle of Silybin in the active site

range. The average angle of Silybin in the protein's active site was recorded as 80.28° (Figure 9, 10).[38]

Studies revealed that Silybin has many health-related beneficial properties. It has also been observed that silybin inhibits the NS5B RNA-dependent RNA polymerase of the Hepatitis C virus as well as the replication of the Influenza A virus.[21,22] Computational docking studies of Silybin resulted in binding with the receptor binding domain of Omicron glycoprotein which further supports its use as an adjuvant therapy to inhibit virus spike glycoprotein and angiotensin converting enzyme (ACE)-2 interactions after experimental confirmations.

CONCLUSION

Recent emergence of the Omicron variant has added new difficulties to the worldwide fight against SARS-CoV-2 infection because this variant is extremely divergent with a significantly higher number of mutations especially in the spike protein region, which are associated with its immune escape potential and higher transmission rate. Selecting or prioritizing possible drug-like compounds for experimental research has been facilitated by computer-aided drug development, which has proved both time- and cost-efficient. In the active site of the Omicron variant spike protein, MD analysis shows that Silybin has lower binding energy, more non-bonded interaction capability, and increased stability. The results suggested that Silybin could be utilized to inhibit the interaction between the spike glycoprotein of SARS-CoV-2 Omicron variant and ACE-2, hence reducing the prevalence and spread of the virus. Silybin shows promise as a potential treatment for treating COVID-19 viral infection, but further research is needed in the form of in-vitro and in-vivo trials.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

AS, SR and DC collected the literature and arranged references. PB and VS performed molecular docking part. AP, RK, DC and AD performed molecular simulation studies. AKS and KD critically analysed the data. PB, VS, AKS wrote the manuscript. KD critically edited the manuscript. All authors read and approved the final manuscript for publication.

FUNDING

None.

DATA AVAILABILITY

The datasets generated and/or analysed during the current study are available from the corresponding author on reasonable request.

ETHICS STATEMENT

This article does not contain any studies on human participants or animals performed by any of the authors.

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