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Original Research Article

Comparative *In-Silico* Molecular Docking of Silymarin for SARS-CoV-2 Receptor

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Abstract

The COVID-19 pandemic has spread worldwide in over 185 countries, with millions of infections and hundreds of thousands of deaths. The current pandemic has made the situation worse, forcing the development of better treatment. In this work, the binding ability of COVID-19 receptors with silymarin has been analyzed using AutoDock 1.4.6. Further, it is compared with the standard drug remdesivir. Silymarin, a potential phytochemical compound obtained from the seeds of the *Silybum marianum* (milk thistle) plant, has been documented as an antiviral agent against several viruses. So silymarin can also be an effective compound in the treatment of COVID-19. This study aims to determine the binding ability of COVID-19 receptors towards silymarin and further comparative analysis by remdesivir. Drug Discovery Studio version 2021 software was used to analyze ligands and targets. AutoDock 1.4.6 software was used to perform the docking study. Among the various receptors, 5N11 (Human beta1-coronavirus (β1CoV) OC43), 7MJP (SARS-CoV-2 receptor binding domain in complex with neutralizing antibody COVA2-39), 7JMO (SARS-CoV-2 receptor-binding domain in complex with neutralizing antibody COVA2-04) receptors showed the highest binding ability of -8.09, -7.23, -6.96 kcal/mol towards silymarin compared to the standard remdesivir having the docking score of -5.21, -3.76, -2.97 kcal/mol, respectively. By the comparative analysis, silymarin has a better and highest binding ability.

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INTRODUCTION

A novel coronavirus-causing pneumonia was identified in China in December 2019. On February 11, the Coronavirus Study Group (CGS) of the International Committee on Virus Taxonomy (ICTV) designated the virus as SARS-CoV-2 based on phylogeny and taxonomy. The same day, the Director General of the World Health Organization (WHO) established the disease caused by SARS-CoV-2, "coronavirus disease 2019" (COVID-19). On March 11, 2020, the WHO declared the COVID-19 outbreak a pandemic. As of May 2020, SARS-CoV2 has spread worldwide in over 185 countries, with millions of infections and hundreds of thousands of deaths[1]. SARS-CoV-2 belongs to the Coronaviridae family of enveloped single-stranded, positive-strand ribonucleic acid (RNA) structure. The structure of SARS-CoV-2 closely resembles that of SARS-COV. This SARS family contains 14 binding residues, of which eight amino acids are specifically conserved for SARS-CoV-2. Significantly, the binding residues of this family interact with the Angiotensin-converting enzyme-2 (ACE2) directly. Scientists in this field have suggested using known broad-spectrum antiviral drugs like Nucleoside analogs and HIV-protease inhibitors as a promising treatment methodology. As of now, the clinical management of COVID-19 patients is based on a trial-and-error basis with re-purposed antiviral drugs like

ritonavir-lopinavir (protease inhibitors), remdesivir (adenosine analog), the antiprotozoal hydroxy-chloroquine (endosomal inhibitor) and other[2]. RNA-dependent RNA polymerase (RdRp) and Angiotensin-converting enzyme 2 (ACE2) are viable drug targets for COVID-19 treatment. Some antiviral drugs like favipiravir, ritonavir, oseltamivir, lopinavir, ganciclovir, and remdesivir are clinically tested against COVID-19 infection. Chloroquine, an anti-malarial drug, has been proven to be effective in the treatment of COVID-19. Until any accurate treatment methodology is available for COVID-19, using derivatives of previously known antiviral drugs is a valuable strategy[3]. SARS-CoV-2 contains a spike (S) protein that helps viral entry into the host cell and releases virus particles by attaching to a cell surface receptor called human ACE2, which facilitates viral transcription and replication[4].

Studies by Tang *et al*.[5], Ou *et al*.[6], and Belouzard *et al*.[7] suggested that the S glycoprotein plays an essential role in virus attachment, fusion, and entry into the host cell. According to Hoffman *et al*.[8], The entrance of the virus into the cells is mediated by spike (S) glycoprotein; in particular, the spike1 (S1) surface unit allows the attachment of the virus to cellular receptors. To allow the entry of the viral particles, the S protein is cleaved by cellular proteases at the S1/S2 and the S20 site. Then, the viral capsid is fused with the cellular membrane, a process driven by the S2 subunit. It has been described that ACE2 mediates SARS-CoV-2 entrance and that the serine protease TMPRSS2 is responsible for the S protein cleavage. The analysis of the sequences of the receptor-binding motif (RBM) within the receptor binding domain revealed that it is responsible for the binding to ACE2 and that SARS-CoV and SARS-CoV-2 have conserved residues, suggesting that their binding with ACE2 could be similar. In contrast, identical residues are absent in other coronaviruses. Moreover, some antibodies developed against human ACE2 blocked SARS-CoV and SARS-CoV-2 infection[9].

The spike protein of SARS-CoV-2 bound with ACE2 has a higher binding affinity than that of SARS-CoV and ACE2[10]. ACE2 belongs to the renin-angiotensin-aldosterone system (RAAS), which plays essential roles in regulating blood pressure and body fluid, contributing to the pathophysiology of hypertension and cardiovascular/renal diseases by maintaining homeostasis of blood pressure, electrolyte balance, and inflammatory responses. The protease Renin, generated mainly in the kidney, cleaves angiotensinogen to generate Angiotensin I (Ang I); the angiotensin-converting enzyme 2 (ACE2) cleaves Ang I to produce Ang II, a key effector of the RAAS. Ang II induces two activations of Ang II type 1 and 2 receptors (AT1R and AT2R) to obtain vasoconstriction and inactivation of vasodilator bradykinin by cleavage. ACE2 is a terminal carboxypeptidase, a type I transmembrane glycoprotein, and a potent negative regulator of RAAS, localized on the apical surface of well-differentiated airway epithelia, especially ciliated cells[4].

Silymarin, a potential phytochemical compound obtained from the seeds of the *Silybum marianum* (milk thistle) plant, has been used as a hepatoprotective agent for over a decade. The primary bioactive components of the extract consist of several flavonolignans (silybin, silychristin, silydianin, isosilybin, and dehydrosilybin) and a few flavonoids, mainly taxifolin. The mixture of silybin A and silybin B (1 : 1) is also known as silibinin ($C_{25}H_{22}O_{10}$), which makes up the major active ingredient (roughly 50%) of silymarin. Recent studies documented the antiviral activities of silymarin against several viruses, including flaviviruses (hepatitis C[11,12] and dengue virus[13]), togaviruses (Chikungunya[14] and Mayaro virus), influenza virus, hepatitis B virus, and Human Immunodeficiency Virus (HIV); in addition to its anti-oxidative and anti-inflammatory role. And also, evidence suggests that the extract possesses potent antiviral activities against the hepatitis C virus (HCV)[11]. Consequently, silymarin is the most commonly consumed herbal product among HCV-infected patients in western countries. Silibinin exhibits highly efficient antiviral activity against HCV infection *in-vitro* and contributes to the anti-HCV effect observed from silymarin. The antiviral efficacy of silymarin has also been reported against epithelial malignancies[15].

Various antiviral activities of silymarin and derivatives have been shown against liver and non-liver pathogens, making them potential broad-spectrum antiviral for some of the enveloped viruses explored to date[16,17]. In addition, considering the polypharmacological activity of silymarin and derivatives towards multiple host cell targets, such as cell innate immunity and inflammation, oxidative stress production, and autophagy, all cell physiological processes are known to be elicited or subverted by many viral infections. These natural products will likely exert their antiviral activities by modulating the cellular environment and any potential direct antiviral function(s) against a specific viral protein[11]. Furthermore, a recent study demonstrated the role of silymarin in attenuating cigarette smoke extract-induced inflammation via simultaneous inhibition of autophagy and extracellular signal-regulated kinase/p38 mitogen-activated protein kinase (ERK/p38 MAPK) pathway in human bronchial epithelial cells, as well as

attenuating up-regulation of proinflammatory cytokines TNF-α, IL-6, and IL-8 and concluded that silymarin might be an ideal agent treating inflammatory pulmonary diseases[18]. So silymarin can also be an effective compound in the treatment of COVID-19.

In drug discovery, docking has become an inevitable tool[19]. The bioinformatic computer-aided modeling of the interaction between two is more molecules to form a stable adduct is known as molecular docking[20,21]. Molecular docking plays a vital and ever-increasing role in novel drug design. The binding properties of ligand and target determine the three-dimensional structure of a complex[22]. Its binding affinity with COVID-19 receptors is evaluated by docking studies, and further, silymarin is compared with the most potent currently used drug, remdesivir, for COVID-19 treatment.

Remdesivir is a direct-acting nucleotide prodrug inhibitor of the SARS-CoV-2 RNA-dependent RNA polymerase; it has potent nanomolar activity in primary human airway epithelial cells[23]. Remdesivir targets essential viral proteins involved in making three new copies of the virus and prevents them from working[24]. Remdesivir became one of the earliest direct-acting antiviral therapeutics to enter randomized clinical trials (RCTs) for COVID-19[25]. A phase III trial of remdesivir showed that both a 10-day and a 5-day course of remdesivir shortened the recovery time in patients hospitalized with COVID-19[26]. So remdesivir is considered as standard for silymarin. Petit *et al*. also used remdesivir as the control molecule in their docking study of *Arthrospira* compounds as potential antiviral agents against SARS-CoV-2[27]. This study is focused on finding the binding affinity of COVID-19 receptors with silymarin using AutoDock software and doing a comparative analysis of the same with the most potent drug, remdesivir, which is currently used for the treatment of COVID-19.

MATERIALS AND METHODS

Software

Drug Discovery Studio version 2021 software was used to analyze ligands and targets. AutoDock 4 and AutoDockTools 1.5.6 software was used to perform the docking study.

Accession of Target Protein

The three-dimensional structure of the COVID-19 receptors (PDB ID 6VYB, 7LMF, 6LU7, 4RNA, 7JMP, 6ML7, 6ZGE, 7JMO, 6ZGG, 5N11, 7LQW, 6VXX, and 6CRV) was retrieved from the RCSB protein Data Bank (https://www.rcsb.org/)[28,29].

Ligand Selection

The chemical structure of silymarin and remdesivir was obtained from the PubChem compound database. The collected Structure Data File (SDF) files of these compounds from the PubChem database were converted into PDB format using Online SMILES Translator (https://cactus.nci.nih.gov/translate/)[30].

Target and Ligand Optimization

For docking analysis, PDB coordinates of the target protein and silymarin, as well as remdesivir, were optimized by Drug Discovery Studio version 2021 software. If in case any ligand was attached to the target protein, they were deleted in the PDB coordinates. Energy minimization and stable conformation was the method of optimization[31].

Analysis of Target Active Binding Sites

The active sites are the coordinates of the ligand in the original target protein grids, and these active binding sites of the target protein were analyzed using the Drug Discovery Studio version 2021[32].

Protein-Ligand Docking

Molecular docking analysis was performed to evaluate the most preferred geometry of the protein-ligand complex. The docking phase is meaningless without its two components as the target protein and ligand. COVID-19 receptors were used for performing docking studies as target proteins. Docking results identify native or native-like

configurations of the protein-ligand complex—the selected proteins complex used after removing already bonded ligands and four water molecules[21].

The complete docking steps could be stated as follows: First, the water molecules were eliminated from the protein. After removing water molecules in the PDB file of those COVID-19 receptor proteins, which were provided as input to the software. Kollman charges were computed for the macromolecule by AutoDock 4. Then the macromolecule was checked for the missing atoms and repaired. After repairing missing atoms, the hydrogens were added by keeping all the parameters at default settings. After all these modifications, the macromolecule was saved as PDB in the same directory. Then the ligand preparation was carried out. Like macromolecule, Kollman and Gasteiger charges were computed for the ligand. Some of the torsions of the ligands were defined. The root was detected; the rotatable bonds were converted into non-rotatable bonds and vice versa. The number of active torsions was the most atoms rather than the fewest. A Protein Data Bank, partial charge (q), and atom type (t) (pdbqt) file was then created for the modified ligand with extension pdbqt[33].

After the preparation of a macromolecule and ligand, the rigid residue was prepared using the grid module provided in AutoDockTools 1.5.6. This program was run using a searching grid extended over ligand molecules with the dimensions of box spacing 90 × 70 × 60 Å; spacing was 0.808; x, y, and z coordinates were 8.485, -5.766, 15.737, respectively, for all the COVID-19 receptor proteins, while other parameters were default. The flexible macromolecule was saved with the pdbqt extension. For molecular docking, AutoDock 4 was used. It employed a configuration file referring to PDBQT files of macromolecules and compounds prepared using AutoDockTools 1.5.6 and grid properties. As an output, AutoDockTools 1.5.6 generated log and pdbqt files of energy models for the selected data set. The output file contained different energy models. Among these models, the lowest energy model against each ligand was selected and appended at the end of the original protein file. As a result of this step, docked files for the selected set were generated. For the interpretation of docking results, the target protein and protein docked with the data set of compounds, and the interactions between the active pocket of protein and compounds were found. The best docking poses were predicted to be the most stable conformation of each compound for binding to the protein active site. Consequently, the output of the docking process was analyzed utilizing AutoDockTools 1.5.6[34].

RESULTS AND DISCUSSION

In this study, silymarin was successfully docked with the COVID-19 receptor proteins. The binding affinities of silymarin to the target proteins are denoted in **Table I**. In this table, the different binding affinities of each receptor are denoted by each docking score. Among these receptors, those having a docking score of −5 or more are considered to have better binding affinity for COVID-19. The binding affinity data in **Table I** shows the code of 5N11, 7JMP, 7JMO, 6LU7, and 6VXX COVID-19 receptors shows the binding affinities of -8.09, -7.23, - 6.96, -6.55, and -6.31 respectively have higher the docking score than -5 and are further considered for the study. Despite the good binding ability of receptors 6LU7 and 6VXX with docking scores of more than -5, they are not selected for the study as only three receptors with high affinity are considered for further analysis. Hence, the receptors code of 5N11, 7JMP, and 7JMO with high binding affinity than other receptors are selected for further study, which is further compared with the standard remdesivir, and the results are shown in **Table II**. In this table, the selected COVID-19 receptors having high binding ability docking scores have been compared with remdesivir. From **Table II**, it is known that the binding affinity of these receptors 5N11, 7JMP, and 7JMO have higher docking scores for silymarin than with the standard remdesivir (-8.09 > -5.21 >, -7.23 > -3.76, -6.96 > -2.97), respectively. The comparative analysis study shows that silymarin has the highest docking score value and has a high binding affinity towards the COVID-19 receptors compared to remdesivir. The more binding affinity of silymarin is supported by the research work carried out by Ubani *et al*.[35], in which they reported that the binding affinity of silybin (silymarin) is -6 kcal/mol) and it can likely inhibit SARS-CoV-2 S glycoprotein and M^{Pro} targets, making it a drug to be considered with a possible multi-target activity against the SARS-CoV-2 virus. This high binding ability of silybin (silymarin) is also by the unpublished work of Pandit and Latha[36]; they have reported that silymarin has binding energy values of –11.928 kcal/mol with silybin-main protease complex, –10.572 kcal/mol with silybin-S spike glycoprotein complex, and –11.499 kcal/mol with silybin-RdRp. Wu *et al*.[37] also reported that remdesivir can bind to RdRp of SARS-CoV-2.

Table I. The binding affinity of silymarin with the COVID-19 receptors.

Receptors	PDB ID	Binding affinity (kcal/mol)
SARS-CoV-2 receptor binding domain in complex with neutralizing antibody COVA2-39	7JMP	-7.23
Human β1-coronavirus (β1CoV) OC43	5N11	-8.09
SARS-CoV-2 receptor binding domain in complex with neutralizing antibody COVA2-04	7JMO	-6.96
Middle East Respiratory Syndrome (MERS) coronavirus	4RNA	-5.25
SARS-CoV-2 spike ectodomain structure	6VYB	-5.32
SARS-CoV-2 main protease in complex with an inhibitor N3	6LU7	-6.55
SARS-CoV-2 $3C^{LPro}$ in complex with 2-(benzotriazol-1-yl)-N-[4-(1H-imidazol-4-yl)phenyl]-N-(3-thienylmethyl)acetamide	7LMF	-5.79
SARS-CoV-2 spike glycoprotein	6VXX	-6.31

Table II. The binding affinity of remdesivir with the COVID-19 receptors.

Receptors	PDB ID	Binding affinity towards remdesivir (kcal/mol)	Binding affinity towards silymarin (kcal/mol)
Human β1-coronavirus (β1CoV) OC43	5N11	-5.21	-8.09
SARS-CoV-2 receptor binding domain in complex with neutralizing antibody COVA2-39	7JMP	-3.76	-7.23
SARS-CoV-2 receptor binding domain in complex with neutralizing antibody COVA2-04	7JMO	-2.97	-6.96

The potential binding sites of remdesivir with 5N11, 7JMP, and 7JMO receptors are shown in **Figures 1** to **3**, respectively, and the potential binding sites of silymarin with 5N11, 7JMP, and 7JMO receptors are shown in **Figures 4** to **6**, respectively. The various amino acid binding sites of the COVID-19 receptor with silymarin and remdesivir have been represented in **Table III**. The possible binding modes of these receptors have active sites, and the protein residues with silymarin and remdesivir ligand molecule are shown in **Table III**. The possible binding modes of the 5N11 receptor having active sites to which the silymarin binds to this amino acid are TYR250, LN132, TYR232, VAL230, LEU251, TYR282, LE231, and MS249, while with remdesivir, the protein residues are LN132, LYS135, PRO309, PHE282, LYS135, ASN136, and GLU198. The binding modes of the 7JMP receptor having active sites ALA43, GLN105, PRO44, LYS45, TRP103, LEU46, ASP101, LEU4, and GLN3 with silymarin, and the remdesivir have the protein residues as LEU4, GLN3, VAL2, LE102, TRP103, LYS42, ALA43, PRO44, and LYS45. Lastly, for the receptor 7JMO the binding modes with silymarin, the protein residue is VAL62, MET4, THR97, GLY99, PHE98, GLN100, GLY44, and LYS43, and the protein residue GLY446, LEU452, PHE490, ASN450, ASN448, and LYS444 is the binding mode for remdesivir.

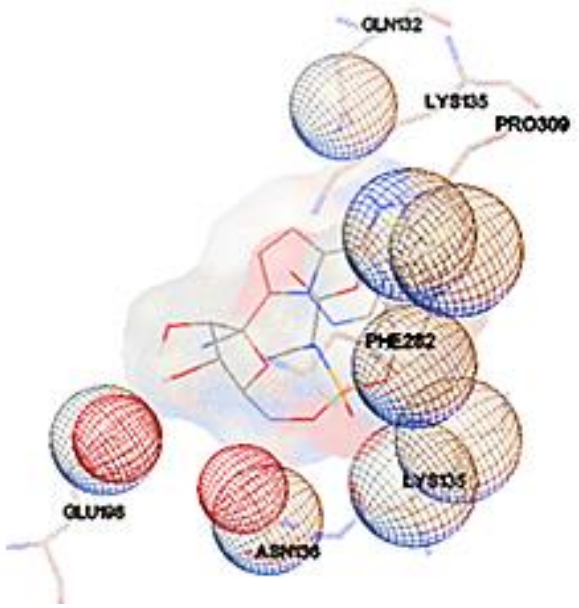

Figure 1. Potential binding sites of 5N11 receptor with remdesivir, with the various amino acid including glutamine, lysine, proline, phenylalanine, asparagine, and glutamic acid.

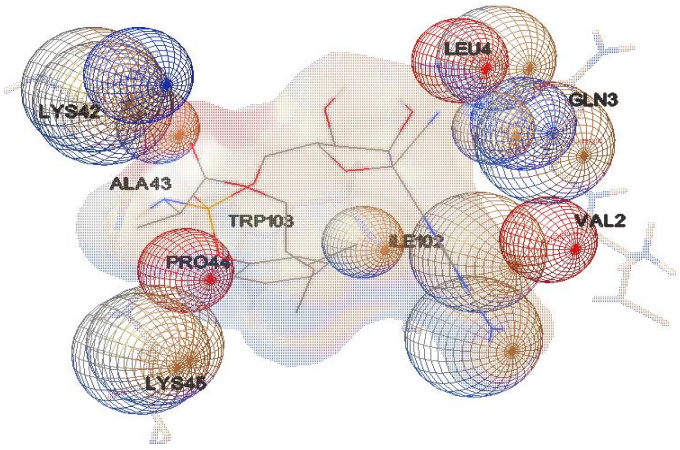

Figure 2. Potential binding sites of 7JMP receptor with remdesivir, with the various amino acid including leucine, glutamine, valine, isoleucine, tryptophan, lysine, alanine, and proline.

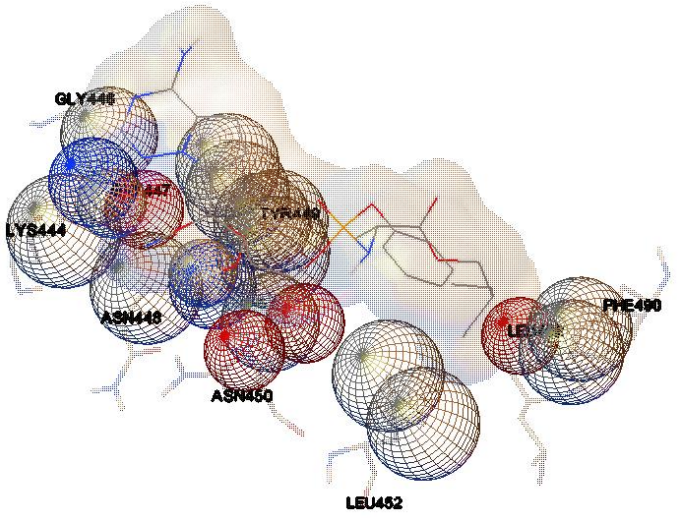

Figure 3. Potential binding sites of 7JMO receptor with remdesivir, with the various amino acid including valine, methionine, threonine, phenylalanine, glutamine, glycine, and lysine.

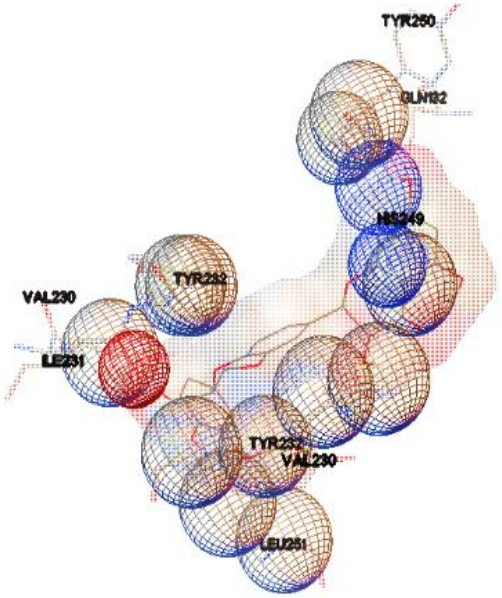

Figure 4. Potential binding sites of 5N11 receptor with silymarin, with the various amino acid including tyrosine, glutamine, valine, leucine, histidine, and isoleucine.

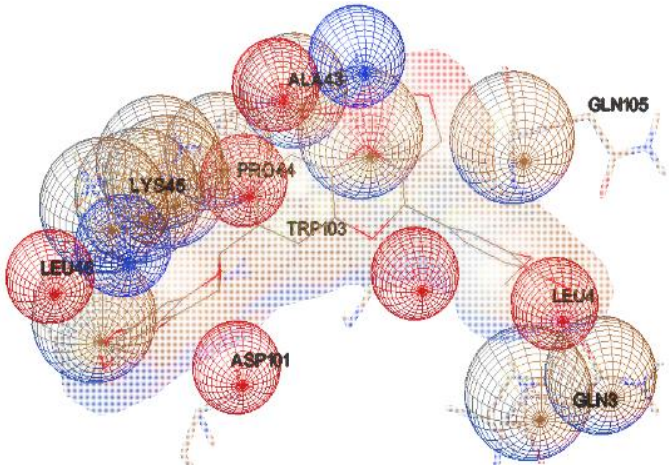

Figure 5. Potential binding sites of 7JMP receptor with silymarin, with the various amino acid including alanine, glutamine, proline, lysine, tryptophan, leucine, and aspartic acid.

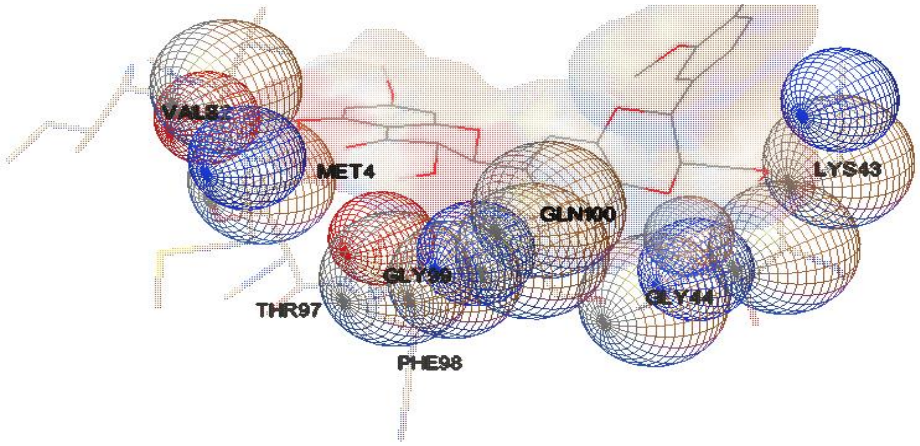

Figure 6. Potential binding sites of JMO receptor with silymarin, with the various amino acid including valine, methionine, threonine, phenylalanine, glutamine, glycine, and lysine.

Table III. The binding interaction with the amino acid residue of silymarin and remdesivir.

Receptors	PDB ID	Silymarin	Remdesivir
Human β1-coronavirus (β1CoV) OC43	5N11	TYR250, GLN132, TYR232, VAL230, LEU251, TYR282, LE231, HIS249, ILE231	GLN132, LYS135, PRO309, PHE282, LYS135, ASN136, GLU198
SARS-CoV-2 receptor binding domain in complex with neutralizing antibody COVA2-39	7JMP	ALA43, GLN105, PRO44, LYS45, TRP103, LEU46, ASP101, LEU4, GLN3	LEU4, GLN3, VAL2, LE102, TRP103, LYS42, ALA43, PRO44, LYS45
SARS-CoV-2 receptor binding domain in complex with neutralizing antibody COVA2-04	7JMO	VAL62, MET4, THR97, GLY99, PHE98, GLN100, GLY44, LYS43	GLY446, LEU452, PHE490, ASN450, ASN448, LYS444

CONCLUSION

This study was performed over the binding pocket of COVID-19 to find the potential small molecule docking to combat life-threatening coronavirus disease. This study finds that by comparative analysis, the docking score of the silymarin ligand is higher than the remdesivir and has better binding affinity than remdesivir. Therefore, silymarin will act as a better drug agent for the treatment of COVID-19. The antiviral activity of silymarin, which has been reported for various other viruses, can also act as a better antiviral agent for the better treatment of COVID-19 due to its high binding ability.

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CONFLICT OF INTEREST

The authors have no conflicts of interest to declare that are relevant to the content of this article.

FUNDING

None.

DATA AVAILABILITY

The docking data of this research work is available from the authors of the College of Pharmacy, Sri Ramakrishna Institute of Paramedical Sciences, Coimbatore, and the work is submitted to the Tamil Nadu Dr. M.G.R. Medical University as part of the dissertation work.

AUTHORS' CONTRIBUTIONS

All authors contributed equally in conceptualization, data curation, formal analysis, funding acquisition, investigation, methodology, project administration, resources, software, supervision, validation, visualization, writing - original draft, and writing - review & editing.

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