RESEARCH ARTICLE

OPEN ACCESS

Structure and Ligand-based *In Silico* Studies towards the Natural Inhibitors against Receptor Recognition Spike Protein of SARS-CoV-2

Swati Srivastava[1], Mohammad Haneef[2,*], Vijay Laxmi Saxena[3], Mahvish Khan[5] and Saif Khan[4]

[1]*Department of Biosciences, Integral University, Hail, U.P. Lucknow, India*

[2]*Department of Bioengineering, Integral University, Hail, U.P. Lucknow, India*

[3]*Asutosh Mookerji Fellowship Indian Science Congress WT. Kolkata India*

[4]*Medical and Diagnostic Research Centre, University of Hail, Hail 55473, Saudi Arabia*

[5]*Department of Basic Dental and Medical Sciences, College of Dentistry, University of Hail, Hail 2440, Saudi Arabia*

Abstract:

Aim: *In silico* exploration, identification, and therapeutic potential of natural bioactive compounds as antiviral agents are compared with established FDA-approved antiviral drugs. The main aim of this study is to harness the best of plant-based bioactive compounds in the ongoing battle against viral infections.

Objectives: To investigate the specific amino acid residues within the spike (S) protein of SARS-CoV-2 that exhibit active sites. Furthermore, our objective is to evaluate the efficacy of molecular interactions between the active site residues of spike (S) protein of SARS-2 with FDA-approved drugs and screened bioactive compounds as a potential therapeutic antiviral agent employing *in silico* approach.

Background: The ongoing global pandemic, attributed to the SARS-CoV-2 virus, commonly known as severe acute respiratory syndrome, continues to spread, giving rise to various emerging variants. These variants, identified as variants of concern or interest (VOC/I), revealed a significant threat to global health, contributing to the severity of the catastrophe.

Methods: We explored how natural bioactive compounds derived from plants and certain FDA-approved drugs interact with the spike (S) protein of the virus. To do this, we employed techniques called molecular docking and molecular dynamics simulation (MD & MD Simulation) to evaluate the antiviral potential of screened bioactive compounds against spike (S) protein.

Results: In this study, Withanolide B and A bioactive compounds revealed the best molecular interaction (binding affinity) with spike (S) protein the SARS-CoV-2, with binding energies of -8.6 and -8.3 kcal/mol, respectively.

Conclusion: Unlike usually prescribed drugs, Withanolide B & A, derived from Withania *somnifera,* commonly known as ashwagandha, were found to be potential inhibitors of the spike (S) protein. This study indicates and emphasizes the promising role of natural compounds in the combat against COVID-19.

Keywords: SARS-CoV-2, Spike protein, Antiviral bioactive compounds, *In silico* approach, Antivirinformatics, Ligand based.

*Address correspondence to this author at the Department of Bioengineering, Integral University, U.P. Lucknow, India; Tel: +918960659834; E-mail: haneef@iul.ac.in

CrossMark

1. INTRODUCTION

The global pandemic problem with SARS-CoV-2 and its variants is still a major health issue, causing huge mortality [1]. The spread of the virus has led to many questions about how to treat affected patients and keep them healthy. It all started in Wuhan, China, in December 2019, and since then, this dangerous virus, called SARS-CoV-2, has quickly spread around the globe, infecting people worldwide [2-6]. Taxonomically, the SARS-CoV-2 belongs to the Coronaviridae family and Nidovirales order and is a kind of virus with an Enveloped (E) protein and a single-stranded RNA (ssRNA). It is a tiny virus, about 40-60 nm in diameter, and it looks like it has a crown on its surface, hence the name coronavirus [7, 8]. The coronavirus family has four main groups: alpha (α), beta (β), gamma (γ), and delta (Δ). The alpha (α) and beta (β) types are the ones that usually infect healthy persons, but we are not sure how the gamma (γ) and delta (Δ) modes of action yet. Interestingly, the newly emerged coronavirus shares 80% of its genetic material with the well-known SARS-CoV-2 virus, which first appeared in China in 2002–2003 [9, 10]. Recently, there have been different types of coronaviruses, like 229E (α-CoV), HKU1 (β-CoV), OC43 (β-CoV), and NL63 (α-CoV), causing mild infections in individual's upper respiratory tracts [11, 12]. However, there are two types of the virus, SARS-CoV and MERS-CoV, that have raised serious health concerns, and the latest one is SARS-CoV-2 (Beta-coronavirus) [13]. SARS-CoV-2 is highly contagious and can be harmful to the lungs and other important organs like the heart, liver, kidneys, gastrointestinal tract, and central nervous system. It often leads to failure of multiple organs [14, 15]. Additionally, SARS-CoV-2 has four main protein parts: spike (S) protein, envelope (E), membrane (M), and nucleocapsid (N) [16, 17]. Various types of polyproteins, both structural and non-structural, are required for SARS-CoV-2 to multiply into more copies of itself (Fig. **1**). Interestingly, the spike (S) protein attaches to a receptor protein called angiotensin-converting enzyme (ACE-2) on the cells of the host who got infected. The spike (S) protein cleaved into smaller parts, namely PPLA and IAB through viral proteases during pathogenicity established respectively. Additionally, the spike (S) protein is more important due to its mode of action, it acts as a virulence factor by interacting and adhering to ACE-2 proteins of host cells. Therefore, the spike protein of SARS-CoV-2 like a significant target could be hit by natural bioactive compounds to interfere with the pathogenesis mechanism of SARS-CoV-2 [18-20]. Currently, there are no FDA-approved drugs identified specifically for treating SARS-CoV-2 (COVID-19). In cases of SARS-CoV-2 infection, whether it is mild or severe, doctors usually focus on managing preliminary symptoms. They were commonly prescribed FDA-approved drugs like Remdesivir, EIDD-2801, Hydroxychloroquine, Dexamethasone, Rito navir, Favipiravir, and Chloroquine. However, these drugs have not shown effective results, and they might have side effects for patients [21]. Using an overdose of these aforementioned drugs in affected persons with corona virus has been associated with lower levels of anti-inflammatory substances, causing a problem known as cytokine storm syndrome (CSS) and acute respiratory distress syndrome (ARDS)., Dexamethasone, a type of corticosteroid, is one drug that has been used to reduce mortality in the early stages of treatment.
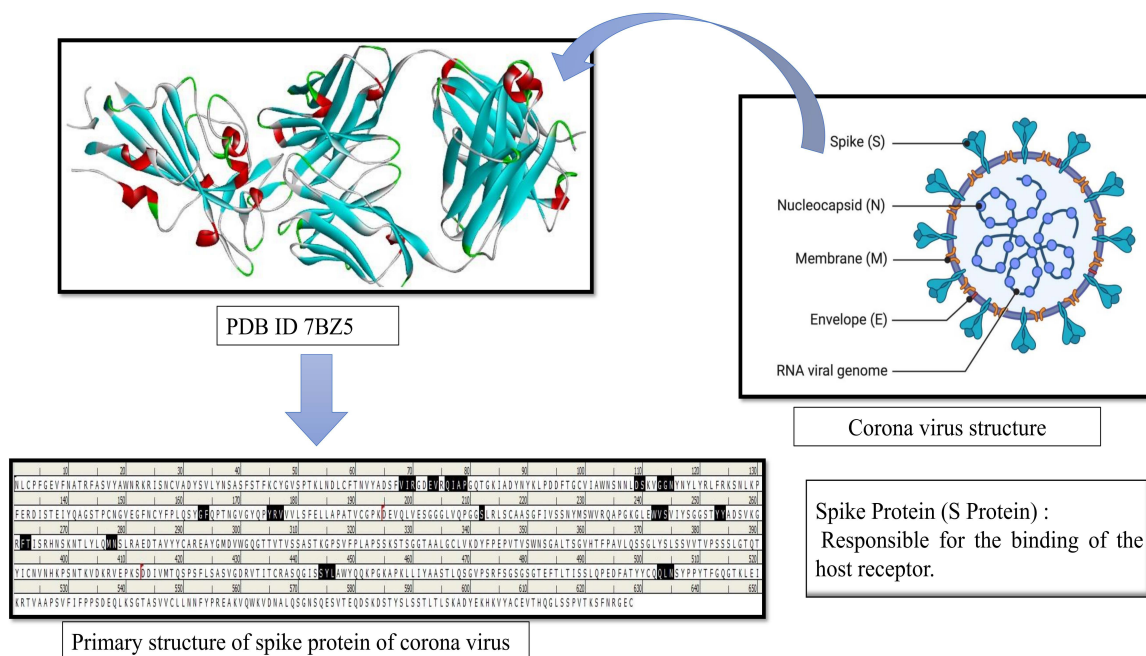

Fig. (1). Structural view of Spike (S) protein (PDB-ID: 7BZ5) and SARS-CoV-2.

While the adverse effects of dexamethasone are most frequently associated with anxiety, weight gain, fluid retention, hormonal imbalance, and insomnia [22]. The commonly used drugs such as hydroxychloroquine and chloroquine prevent virus and host cell interaction process and lead to control the activation of T-cells [23-25]. Drugs like Remdesivir, Favipiravir, and EIDD-2801 interfere with the activity of the RNA-dependent RNA polymerase of SARS-CoV-2, while the drug Ritonavir inhibits the activity of viral proteases, specifically cysteine protease PLpro and Mpro. Interestingly, natural herbs and their derivatives, known for minimal side effects and biocompatible, have also shown promising potential bioagents against viral infections [26-28]. Certain plants, like Ashwagandha (Withania *somnifera*), Neem (Azadirachta *indica*), and Giloy (Tinospora *cordifolia*), are reported in the literature and known to contain bioactive compounds with a wide range of therapeutic benefits like reducing inflammation, lowering fever, antioxidative properties, pain relief, immune system stimulation, and potentially antiviral effects [29-33]. These natural bioactive compounds might help lower mortality rates and block the chain of coronavirus infection. The aim of this study is to deliver a quick overview of the SARS-CoV-2 virus's therapeutic target sites. In addition, the research is aimed at exploring the potential efficacy of these natural chemicals as an alternative therapeutic agent through employing advanced computer-based techniques such as molecular docking and *in silico* high-throughput screening.

2. MATERIALS AND METHODS

2.1. Retrieval, Preparation and Optimization of Receptor Molecule Spike (S) Protein

The three-dimensional structure (3D) of the spike (S) protein (PDB-ID 7BZ5) [34], a key virulence factor of SARS-CoV-2, was retrieved from the RCSB Protein Data Bank (PDB). To ensure its relevance as a receptor molecule, the spike (S) protein underwent preparation, including the addition of charges and polar hydrogen atoms. Moreover, we marked a grid box to find potential binding sites on the protein. The receptor-binding site was specifically marked and secured at designated coordinates: x (79.684), y (18.727), and z (22.534), employing the Autodock Vina tool [35]. The prepared receptor molecule (spike (S) protein), along with its specified binding site, was then saved in the pdbqt file format for further molecular docking experiments.

2.2. Retrieval, Preparation, and Optimization of Selected Ligand Molecules Derived from Plants

We retrieved the ligand molecules, which are basically bioactive compounds and FDA-approved drugs, from reliable sources like the PubChem Database. Specifically, we selected commonly used drugs in treating SARS-CoV-2, like Remdesivir (PubChem-ID: 121304016), EIDD-2801 (PubChem-ID: 145996610), Dexamethasone (PubChem-ID: 5743), Ritonavir (PubChem-ID: 392622), Hydroxychloroquine (PubChem-ID: 3652), Favipiravir (PubChem-ID: 492405), and Chloroquine (PubChem-ID: 2711). We got these from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/). However, there was a slight setback because the SDF file format they were in could not be directly used with the Autodock Vina tool [36]. Therefore, we needed to change these SDF files into the PDB file format using the Open Babel GUI [37]. Subsequently, we further converted the PDB files into PDBQT file format and saved them for further *in silico* experimental analysis using the Autodock Vina bioinformatics software.

2.3. *In Silico* Parameters Applied for Molecular Docking

We used Autodock Vina for the molecular docking analysis of the screened bioactive compounds and selected FDA-approved drugs [38-40]. The outcomes of the docking indicate how these drugs mentioned above and compounds interact with the structural spike (S) protein of SARS-CoV-2 and exhibited their orientation patterns based on the calculated free binding energy scores in kcal/mol. From these results, we selected the best conformations that had the most favourable binding pocket sites for both drugs and bioactive compounds. Furthermore, these selected conformations were then examined more methodically and used Discovery Studio Visualizer version 2.5.5 and PyMol to find the best patterns in the docked structures. Additionally, we ran the screened bioactive compounds through the Lipinski rule of five (RO5), an assessment of their drug-like properties, using an online server [41]. Further, assessing how effectively these compounds qualified the RO5 prerequisites, our study has provided additional insights into their potential for anti-viral drug development.

2.4. Evaluating the Physiochemical Properties and Potential Toxicity of Screened Bioactive Compounds

In order to predict the ADMET characteristics of the top five bioactive compounds, the study examined their binding affinities [42].

2.5. Molecular Dynamics Simulation (*MD Simulation*) and *In Silico* Methodology of Screened Bioactive Compounds

To investigate the stability and flexibility of potent inhibitor bioactive compounds, particularly Withanolide B and Withanolide A, in how they interact with the spike (S) protein, generating a protein-ligand complex. For these simulations, MD simulation and GROMACS v2020 were employedand performed for 100 nanoseconds (100 ns) [43]. Employing MD simulations, researchers may acquire knowledge about the behaviour of biomolecules and the fundamental molecular interactions that impact their dynamics. The ligand compounds were developed by using a simple point charge (SPC) model in a triclinic box topology with the help of the ACPYPE web server [44]. Furthermore, to model how the spike (S) protein receptor acts, we used the CHARMM 27 force field [45], employing a dodecahedron as the simulation box and the TIP3P model for water. Throughout the simulations, we observed the root-mean-square deviation (RMSD) and root-mean-

square fluctuation (RMSF) values of the backbone atoms in both the spike (S) protein and the protein-ligand complex. In order to better understand the dynamic behaviour of tested biomolecules, furthermore, we evaluated solvent-accessible surface area (SASA), the radius of gyration (RG), and hydrogen bond (HB) formation.

3. RESULTS

3.1. *In Silico* Analysis of Certain FDA-approved Drugs and Screened Bioactive Compounds

In silico investigation intensive on the SARS-CoV-2 host receptor recognition spike (S) protein, which is essential for the virus's ability to become pathogenic by binding to and fusing with the host cell receptor ACE2. The results of the molecular docking analysis revealed insights into the binding affinities and molecular interaction patterns of screened bioactive compounds with the spike (S) protein. This study especially examined drugs that have been approved by the FDA, such as EIDD-2801,

Favipiravir, Ritonavir, Dexamethasone, Chloroquine, and Hydroxychloroquine (Table **1**). Apart from that, the plant-derived bioactive compounds, including Withanolide B, Withanolide A, Withaferin A, Gedunin, and Berberine, performed docking with selected receptor molecule spike (S) protein (Table **2**). To evaluate the ligands' affinity for the spike (S) protein, we assessed docking energies and scores. Interestingly, the bioactive compounds exhibited higher docked binding energies than the drugs approved by the FDA. Withanolide B, at -8.6 kcal/mol, had the highest docked binding energy, followed by Withanolide A at -8.3 kcal/mol and Withaferin A at -8.1 kcal/mol, respectively. These findings indicate that the bioactive compounds from Withania *somnifera* that have been evaluated showed excellent possibility of binding to the spike (S) protein's active site. Furthermore, the results validated and compared the molecular docking energies scores of docked bioactive compounds with the spike (S) protein to Dexamethasone, one of the most effective binding drugs (with a free binding energy of -8.0 kcal/mol), preferred as a positive control.

Table 1. Binding energy of FDA-approved drugs and their corresponding amino acid interaction with spike (S) protein of SARS-CoV-2 includes common side effects.

Sr. No.	Drugs Name	Pub Chem ID	Binding Energy (Kcal/Mol)	No. of HB with Amino Acids	No. Hydrophobic Bond	Common Side Effects
1	Dexamethasone ($C_{22}H_{29}FO_5$)	5743	**-8.0**	Ser 373, Ser 371	Asp 364, Val:362 Val:367	Adverse effects involving the hair, nails or skin
2	Ritonavir ($C_{27}H_{35}N_6O_8P$)	392622	**-8.0**	Val:367, Phe:338 gly:339	Leu 335	Gastrointestinal adverse effects like diarrhoea, nausea and vomiting
3	Remdesiver ($C_{27}H_{35}N_6O_8P$)	121304016	**-7.4**	ASN:343, ASN:440, SER:371, SER:373	TRP:436, PHE:342, LEU:368, LEU 441	Respiratory, Cardiovascular, Renal toxicity and Hepatotoxicity,
4	EIDD-2801 ($C_{13}H_{19}N_3O_7$)	145996610	**-6.3**	GLY:339, PHE: 342, ASN:343, SER373, SER371 ,TRP:436,LEU 368	VAL 367, PHE 338, PHE:374	Adverse effects on vital organs
5	Hydroxychloroquine ($C_{18}H_{26}ClN_3O$)	3652	**-5.6**	ASN:343, TRP:436, ASN:440SER:371	LEU :368, PHE:342, PHE:342LEU:441	Hypertension, cataract, neuropsychological disorder and osteoporosis
6	Favipiravir ($C_5H_4FN_3O_2$)	492405	**-5.3**	GLU:340, ASN:354, SER:399, ALA 348 PHE347	VAL:340, ALA:344	Hyperuricemia and Teratogenicity
7	Chloroquine ($C_{18}H_{26}ClN_3$)	2719	**-4.9**	SER 373, SER:371,TRP:436	PHE :342, PHE:374, LEU:368	Blurred vision, paresthesia, insomnia

Table 2. Binding energy of bioactive compounds and their corresponding amino acid interactions with spike (S) protein of SARS-CoV-2.

Sr. No.	Bioactive Compounds	Plant Sources	Pub Chem ID	Binding Energy (Kcal/Mol)	No. of HB with Amino Acids	No. Hydrophobic Bond	Common Amino Acid Residue
1	Withanolide B ($C_{28}H_{38}O_5$)	Withania *somnifera* (Ashwagandh)	14236711	**-8.6**	ASN:343, SER:373	LEU:441, PHE:374TRP:436	Phe
2	Withanolide A ($C_{28}H_{38}O_6$)		11294368	**-8.3**	CYS:336, GLY:339,SER:373	LEU:335, PHE:342, TRP:436,PHE:374	
3	Withaferin A ($C_{28}H_{38}O_6$)		16760705	**-8.1**	SER:373, ASN,440	TRP:436, PHE:374LEU:441	
4	Gedunin ($C_{28}H_{34}O_7$)	Azadirachta *indica* (Neem)	12004512	**-7.6**	SER:371 SER373PHE 342	TRP:436	
5	Berberine ($C_{20}H_{18}NO_4^+$)	Tinospora cordifolia (Giloy)	2353	**-7.4**	SER:371, SER:373,CYC 336,GLY 339	PHE:374, TRP:436VAL:367	

The analysis of Dexamethasone molecular interactions with spike protein revealed hydrogen bonds and hydrophobic bonds with specific amino acid residues, like Ser 373, Ser 371, Asp 364, Val:362 and Val:367 (Fig: **2A-1**, **2A-2**). In comparison to the docked free energy scores of drugs against the SARS-CoV-2 spike (S) protein, the molecular docking interactions of the screened bioactive compounds exhibited optimal docked free binding energies. Particularly, bioactive compounds like Withanolide B (CID_14236711), Withanolide A (CID_11294368), and Withaferin A (CID_16760705) derived from the plant Withania *somnifera* exhibited docked binding energies of -8.6 kcal/mol, -8.3 kcal/mol, and -8.1 kcal/mol, respectively. Remarkably, Withanolide B (CID_14236711) showed a high affinity for the active site of the SARS-CoV-2 spike (S) protein, corresponding hydrogen bond and hydrophobic bonds to specific amino acid residues, such as ASN:343, SER:373, LEU:441, PHE:374 and TRP:436 (Figs. **3A-1**, **3A-2**). This binding affinity proved to be higher compared to the affinity of Withanolide A and Withaferin A (see Fig. **4A**).

3.2. Analysis of Physicochemical and Toxicity Properties/parameters of Screened Bioactive Compounds

In silico method to analyze the physicochemical and toxicity properties of the top five bioactive compounds: Withanolide B (CID: 14236711), Withanolide A (CID: 11294368), Withaferin A (CID: 16760705), Gedunin (CID:

12004512), and Berberine (CID: 2353). Subsequently, employing Discovery Studio 4.0, the absorption, distribution, metabolism, excretion, and toxicity (ADMET) properties of these compounds were estimated (see Table **3**). The ADME and toxicity assessments of selected bioactive compounds showed drug-like characteristics that make them suitable for human use. Mutagenicity, CYP2 inhibition activity, blood-brain barrier penetration, and intestinal absorption were included among the variables considered into consideration throughout the evaluation. Notably, Withanolide B (CID: 14236711), Withanolide A (CID: 11294368), Gedunin (CID: 12004512), and Withaferin A (CID: 16760705) exhibited high blood-brain penetration and were non-mutagenic. In contrast, the compound berberine (CID: 2353) exhibited minimum blood-brain penetration, while the selected bioactive compounds demonstrated neither mutagenic nor inhibitory effects on CYP450, with the exception of Berberine. These bioactive compounds were also tested for absorption and mutagenicity, and the results showed that they were highly permeable across cell membranes and exhibited nearly no mutagenicity. These compounds are considered non-carcinogenic and highly suitable for drug development, aligning with the Lipinski rule of five (RO5). The compound Withanolide B (CID: 1423611) possessed an evaluation score of -6.301 for ADMET and solubility potential, while Withanolide A achieved a value of -5.2±8. Importantly, none of the compounds that were evaluated had been found to be considered a risk to human health (Fig. **4A**, **B**).
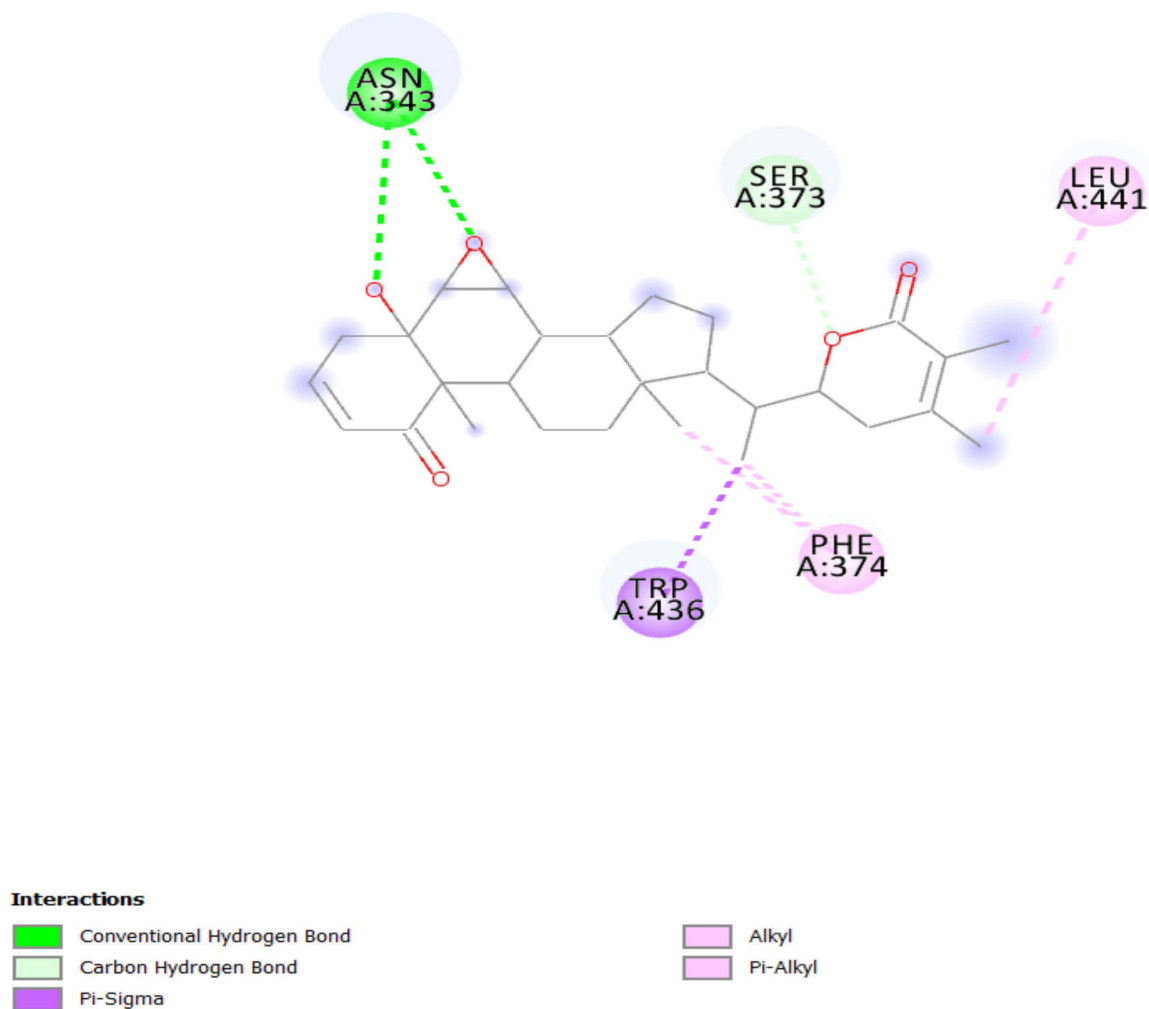

2A-1

Fig. 2 contd.....

2A-2
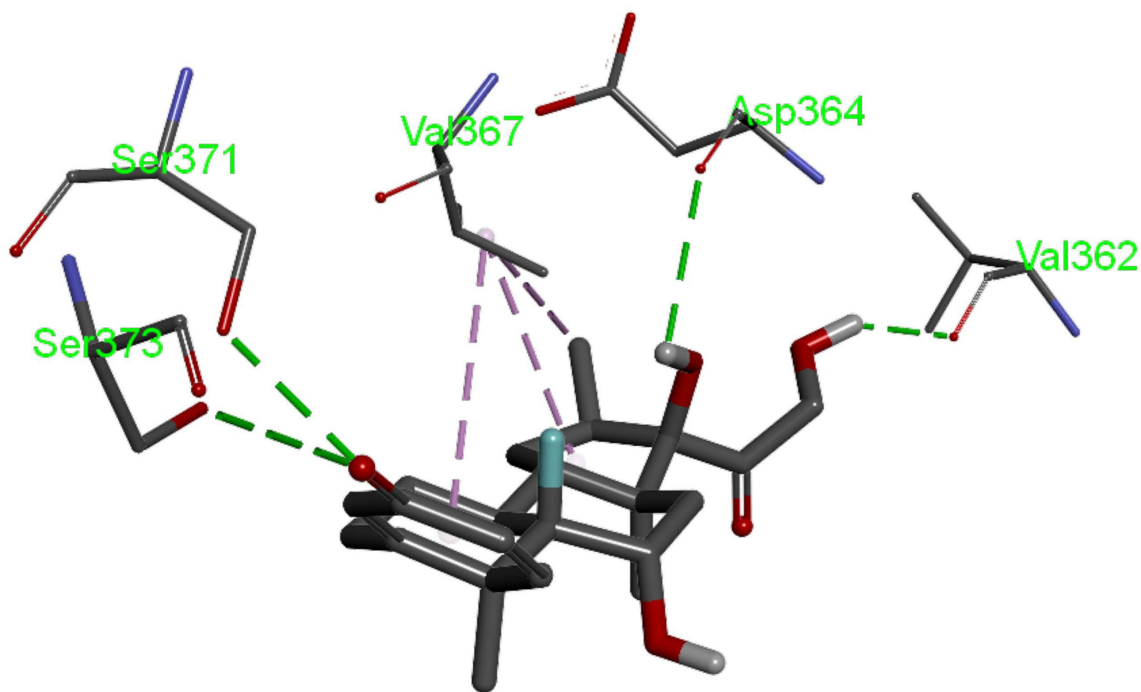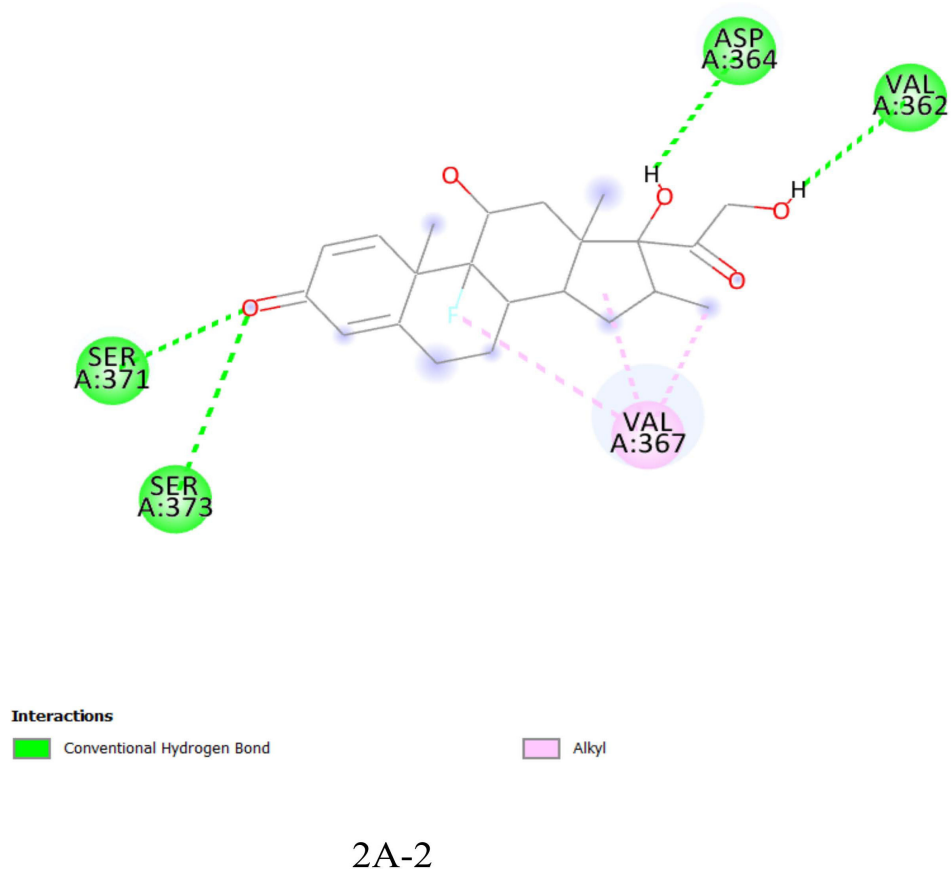
Fig. (2). 3-Dimensional (**2A-1**) and 2-dimensional (**2A-2**) view of Molecular interaction of FDA-approved drugs Dexamethasone against spike (S) protein of SARS-Cov-2.


3A-1
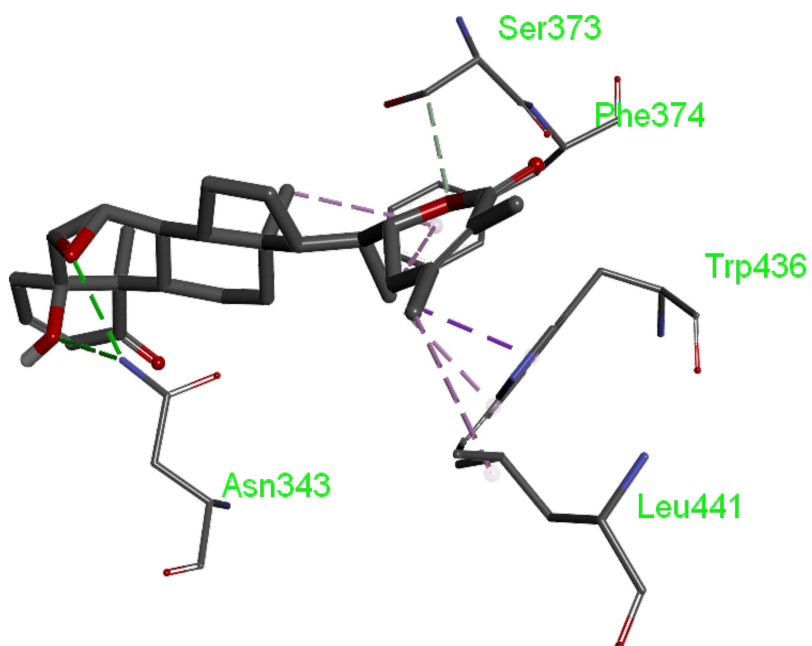
3A-2

Fig. (3). 3-Dimensional and 2-dimensional view of (**3A-1**, **2**) of Molecular interactions of bioactive compounds Withanolide B against spike (S) protein of SARS-Cov-2.

Table 3. ADMET property of bioactive compounds.

Sr. No.	Compound Name and Pubmed ID	ADMET Solubility	Absorption Level	ADMET BBB	BBB Level	CYP2 Inhibitor	PPB	PPB	Alog	PSA2D	Mutagencity
1	Withanolide B CID_1423611	-6.301	0	-0.05	1	False	4.62429	True	4.411	73.277	Non-mutagen
2	Withnolide A CID_11294368	-5.242	0	-0.593	3	False	2.83101	True	3.397	94.092	Non-mutagen
3	Withaferin A CID_16760705	-5.124	0	-0.5.96	3	False	2.68768	True	3.388	94.092	Non-mutagen
4	Gedunin CID_12004512	-5.994	0	-0.563	3	False	-1.29319	True	3.349	91.247	Non-mutagen
5	Berberine CID_2353	-5.541	0	-0.261	1	True	6.79215	True	3.446	41.068	Mutagen
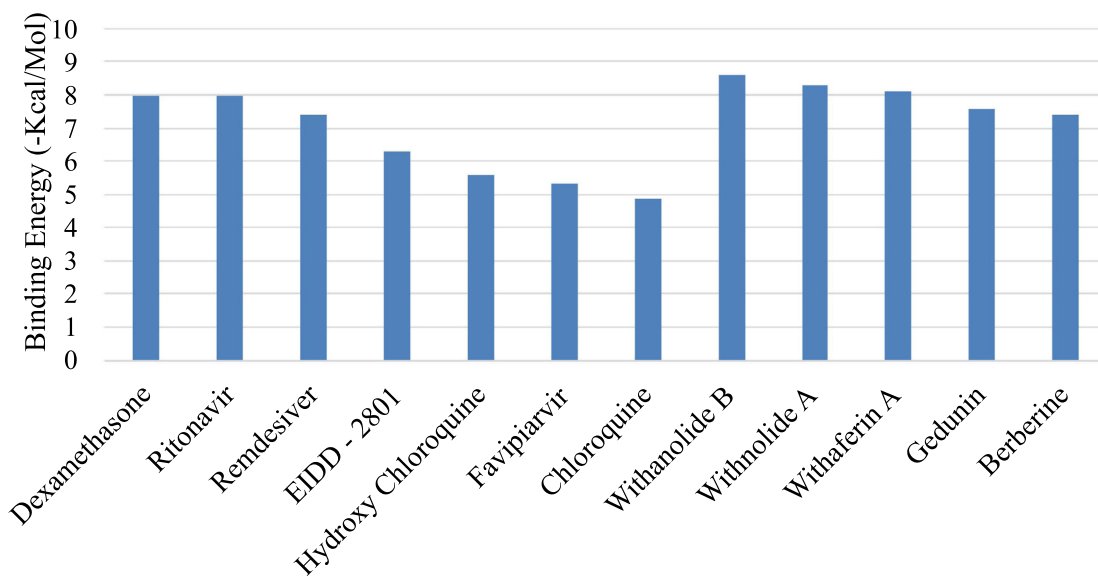
A
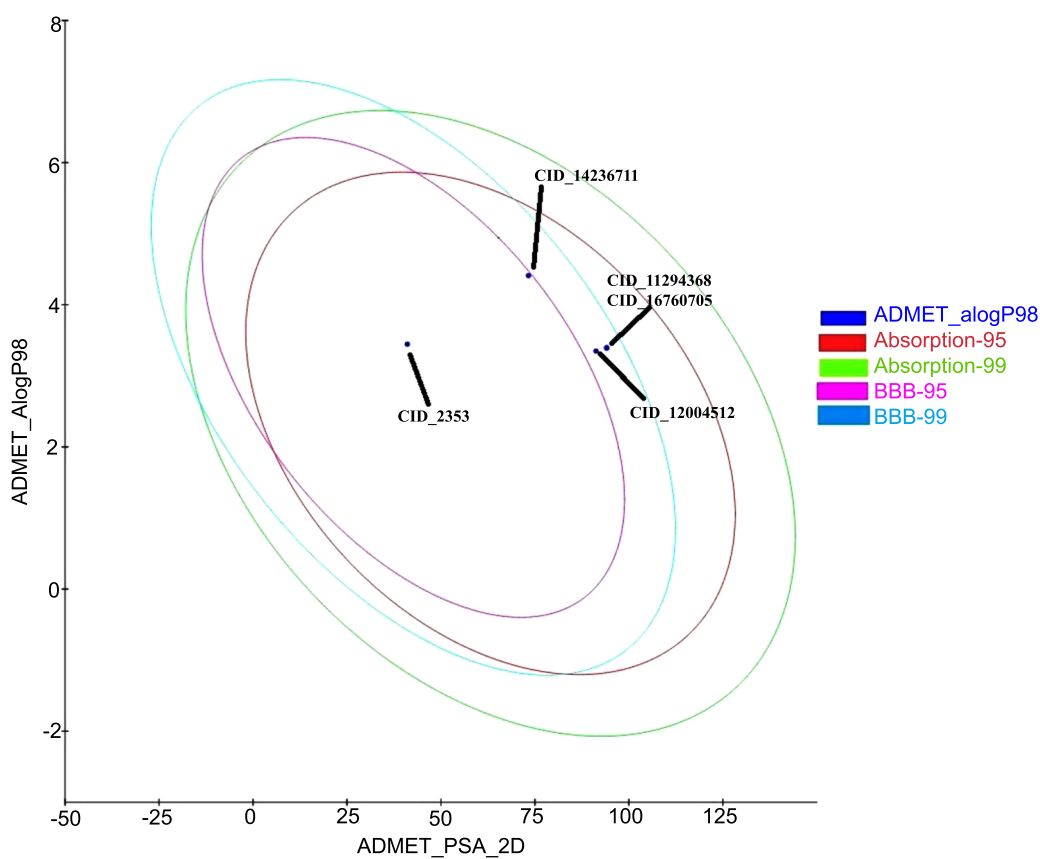
B

Fig. (4). Comparative analysis free binding docked energies of potential bioactive compounds and FDA drugs against spike (S) protein of SARS-Cov-2 (**A**), ADMET bitplot curve of bioactive compounds namely Withanolide B, Withanolide A, Withaferin A, Gedunin and Berberine (**B**).
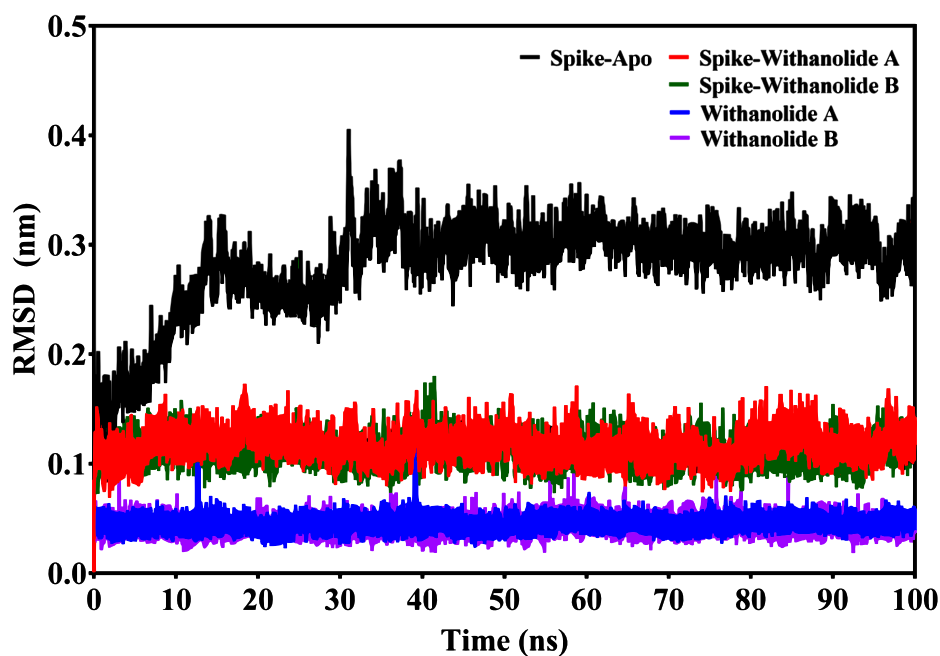
3.3. MD Simulation Analysis of the Docked Structure of Withanolide B, and Withanolide A Compounds with a Spike (S) Protein of SARS-CoV-2

In order to investigate the stability and flexibility of the docked structures of Withanolide B and Withanolide A compounds when combined with the SARS-CoV-2 spike (S) protein, and structural dynamics and the behaviour of protein-ligand complexes were performed MD Simulation [46]. In this study, throughout the simulation process, the docked structures of Withanolide B and Withanolide complexes showed nearly equal affinity binding energy -8.6 kcal/mol and -8.3 kcal/mol, respectively. To assess the stability and rigidity of the docked complexes, we used root-mean-square deviation (RMSD) and root-mean-square fluctuation (RMSF) values obtained during the 100 ns molecular dynamics simulation. The simulation employed a dodecahedron water model (TIP3P). The docked complexes Spike+Withanolide B and Spike+Withanolide A exhibited high stability until the 40 ns time period, while the unbound spike protein of SARS-CoV-2 showed instability with greater variation in the RMSD values and fluctuation (refer to Fig. **5A**). Additionally, the fluctuation and compactness of the Withanolide B compound were observed to be high at the 40 ns trajectory time period (refer to Fig. **5B**). Consequently, to assess the stability and behaviour of the spike (S) protein of SARS-CoV-2 and its complexes with withanolide B and withanolide A by examining a number of parameters, including radius of gyration (Rg) (refer to Fig. **5C**), solvent-accessible surface area (SASA) (refer to Fig. **5F**), root mean square deviation (RMSD), root mean square fluctuation (RMSF), and hydrogen bonds (Fig. **5D** and **5E**) was performed.

4. DISCUSSION

The binding affinities and interaction patterns between the ligands and the receptor protein have been revealed by the molecular docking interactions [47]. The *in-silico* analysis offers valuable insights into the binding interactions between the spike (S) protein and screened ligands, providing potential therapeutic options for combating SARS-CoV-2. The ADMET analysis revealed favourable properties for the selected bioactive compounds, including Withanolide B, Withanolide A, Withaferin A, Gedunin, and Berberine, in terms of absorption, distribution, metabolism, and excretion. These compounds exhibited high blood-brain penetration, suggesting their potential to reach the central nervous system and exert therapeutic effects. Additionally, the non-mutagenicity and inhibitory activity against CYP450 enzymes in most of the selected bioactive compounds indicate their safety and minimum risk of adverse effects. However, it is important to note that Berberine showed lower blood-brain penetration, suggesting a potential limitation in its ability to reach the central nervous system. The assessment of the compounds' absorption and mutagenicity, along with their adherence to the Lipinski rule of five, further reinforces their potential as drug candidates. These compounds displayed high permeability through cell membranes and exhibited low mutagenicity, making them suitable for consumption and indicating their safety for use as novel drugs. In summary, the *in-silico* analysis of the physicochemical and toxicity properties of the selected bioactive compounds highlights their promising characteristics as potential therapeutic agents.
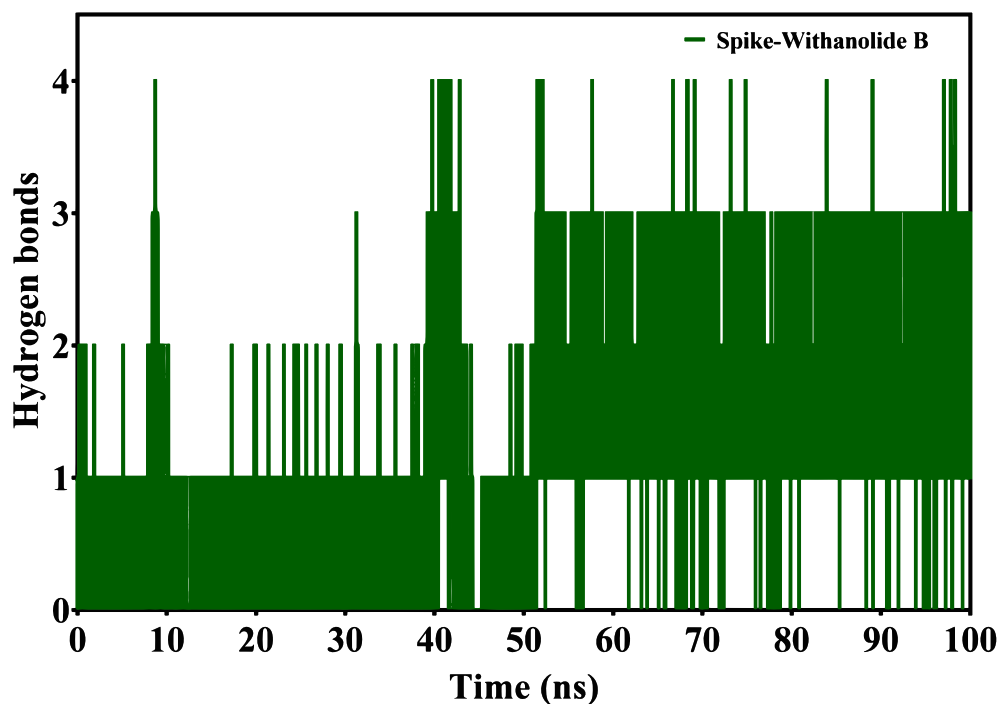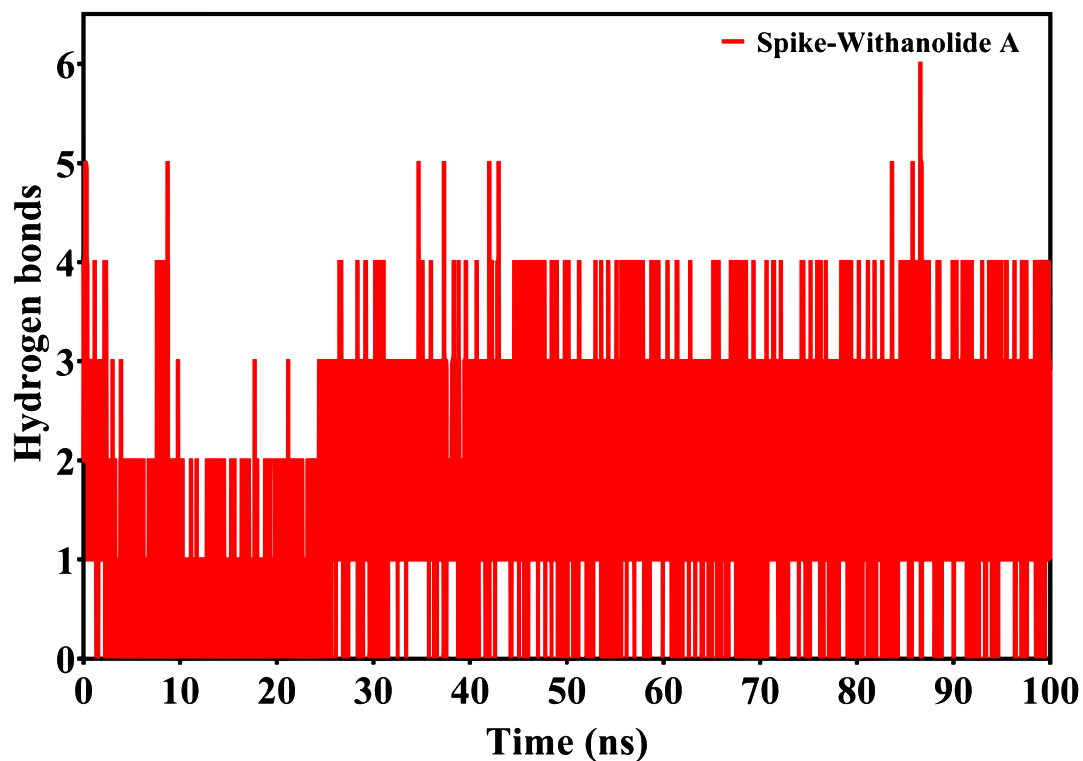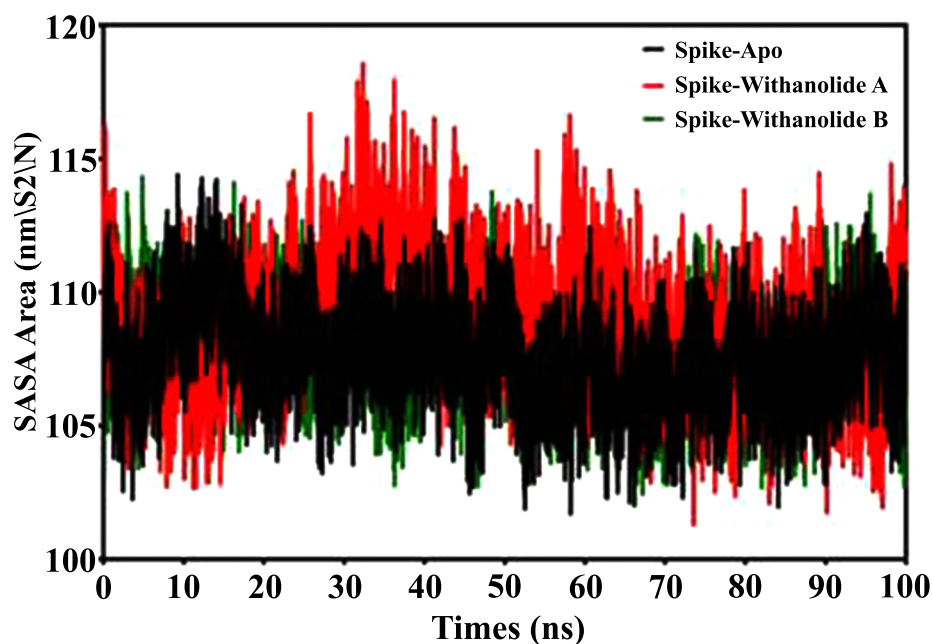

5A
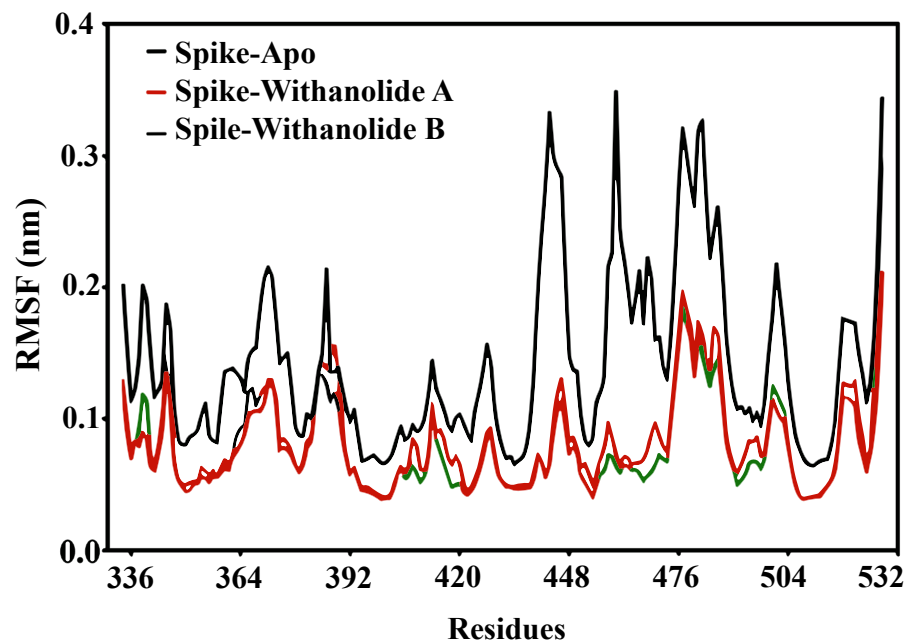
5B
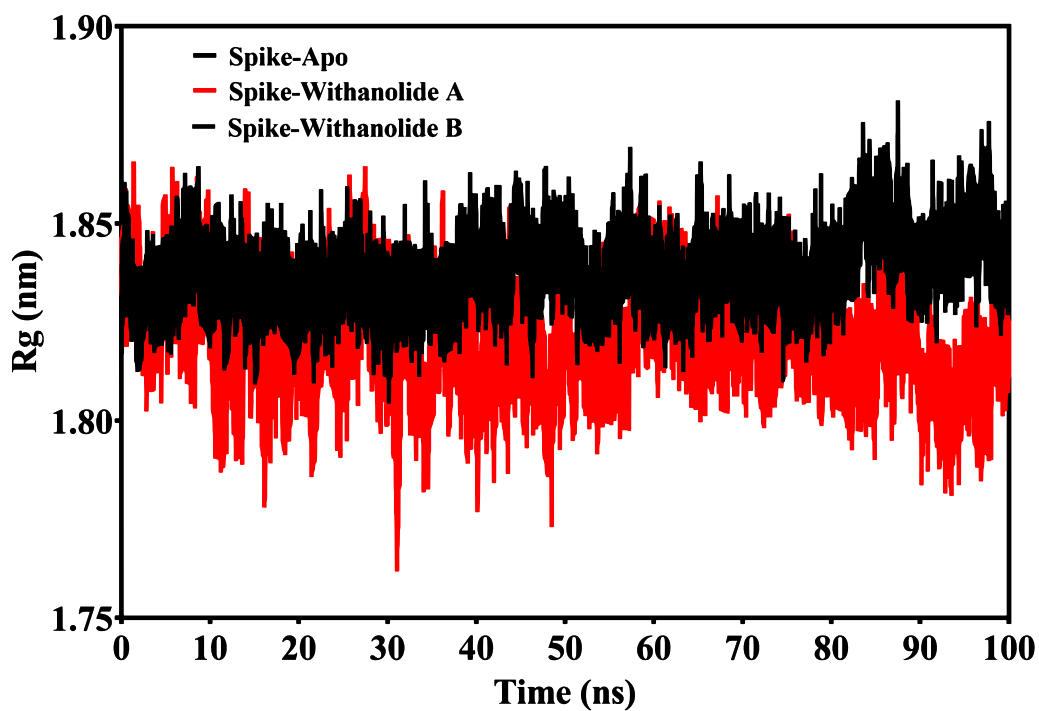
5C

5D

5E

Fig. 7 contd.....

5F

Fig. (5). MDS of Spike (S) protein of SARS-Cov-2 and Bioactive compounds Withanolide B and Withanolide A (**5A**) RMSD (**5B**) RMSF (**5C**) Radiation of Gyration (**5D-1**) Hydrogen Bonds of Withanolide B (**5D-2**) Hydrogen Bonds of Withanolide A (**5E**) SASA (**5F**).

These findings serve as a foundation for further experimental studies to validate their efficacy and safety profiles. While, the MD simulation analysis provided insights into the stability and flexibility of the docked complexes of Withanolide B and Withanolide A with the spike (S) protein of SARS-CoV-2. The calculated free binding energies demonstrated a strong affinity between the bioactive compounds and the spike protein, indicating their potential as effective binders to the target protein. The docked complexes of Spike+Withanolide B and Spike+Withanolide A preserved their structural integrity and stability for a considerable portion of the simulation time frame in accordance with the stability evaluations conducted *via* RMSD values. This consistency correlates with findings from other research [48]. The research reports persistent complex forms between the spike protein and bioactive compounds. On the contrary, the unbound spike protein of SARS-CoV-2 displayed greater variability in RMSD values, indicating its inherent flexibility and conformational changes. This finding is consistent with previous studies that have highlighted the dynamic nature of the spike protein in the absence of ligand binding. Moreover, the analysis of fluctuation and compactness in Withanolide B revealed significant changes at specific time points during the simulation. This dynamic behaviour suggests potential conformational adjustments of the compound, which could have implications for its binding interactions and pharmacological effects. Their results, which showed that the bound complexes had lower RMSD values than the

unbound protein, were in line with our findings and suggested that the ligand had increased stability. The resemblance points to a widespread pattern of spike protein complexes became more stable. Additionally, studies conducted [49, 50] explored the RMSF patterns of different proteins in order to comprehend their flexibility. They determined that proteins with stable secondary structures had lower RMSF values, which is consistent with our findings. In our study, the bound complexes of Withanolide B and Withanolide A displayed significantly lower RMSF values than the unbound spike protein, indicating a more rigid and stable secondary structure. Regarding hydrogen bonding, our analysis revealed that the bound complex of Withanolide B formed four hydrogen bonds, while the bound complex of Withanolide A formed six hydrogen bonds. This insight correlates with the findings of other studies investigating interactions between proteins and ligands as a case study [51-53], which emphasized the importance of hydrogen bonding in stabilizing protein-ligand complexes. As compared to Withanolide A, Withanolide B's bound complex exhibits fewer hydrogen bonds, which might suggest that the first compound is more stable. Subsequently, to assess protein compactness, comparing the Rg values indicated no significant changes in Rg throughout the simulation, similar findings have been reported in other studies investigating protein dynamics, as seen in a study [54]. The analysis of SASA in this study revealed similar values for the unbound spike protein and the bound complexes of Withanolide B and Withanolide A. This suggests that the

binding of these compounds did not significantly impact the folding and unfolding profiles of the protein surface. Therefore, further investigations are necessary to provide a more comprehensive understanding of the impact of ligand binding on protein surface accessibility and thoroughly explore the therapeutic potential of Withanolide B and Withanolide A against the virus.

CONCLUSION

This *in silico* study highlights the anti-viral potential of Withanolide B and Withanolide A and suggests their role in developing therapeutic agents against SARS-CoV-2 and other viral infections. These bioactive compounds demonstrate significant potential in inhibiting the activity of the spike (S) protein and disrupting the molecular interaction between the viral receptor and host recognition. Their scaffolds hold promise for future therapeutic agents against SARS-CoV-2 and other viral infections, emphasizing the importance of exploring alternative treatments, especially natural bioactive compounds, in the context of the COVID-19 pandemic. Additionally, experimental studies are necessary to establish their efficacy and safety compared to commonly used drugs during the COVID-19 pandemic. These bioactive compounds present a promising avenue for developing novel antiviral treatments, holding significant potential in addressing the ongoing challenges posed by SARS-CoV-2 and other viral pathogens.

LIST OF ABBREVIATIONS

CSS = Cytokine storm syndrome

ARDS = Acute respiratory distress syndrome

SASA = Solvent accessible surface area

RMSD = Root mean square deviation

RMSF = Root mean square fluctuation

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

Not applicable.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIAL

All the data and supporting information are provided within the article.

FUNDING

None.

ACKNOWLEDGEMENTS

REFERENCES

[1] Regan JJ, Moulia DL, Gelles LR, *et al.* Use of updated COVID-19 vaccines 2023–2024 formula for persons aged≥ 6 months: Recommendations of the advisory committee on immunization practices—United States, September 2023. MMWR Morb Mortal Wkly Rep 2023; 72(42): 1140-6.
http://dx.doi.org/10.15585/mmwr.mm7242e1 PMID: 37856366

[2] Shereen MA, Khan S, Kazmi A, Bashir N, Siddique R. COVID-19 infection: Emergence, transmission, and characteristics of human coronaviruses. J Adv Res 2020; 24: 91-8.
http://dx.doi.org/10.1016/j.jare.2020.03.005 PMID: 32257431

[3] Patel M, Mazumder R, Kaushik KK, Debnath A, Mishra R, Pal S. A brief description of COVID-19 pulmonary viral infection and repurposing of drugs for its treatment. Int J Appl Pharm 2022; 7(10): 22-31.
http://dx.doi.org/10.22159/ijap.2022v14i5.45168

[4] Tabish M, Khatoon A, Alkahtani S, *et al.* Approaches for prevention and environmental management of novel COVID-19. Environ Sci Pollut Res Int 2021; 28(30): 40311-21.
http://dx.doi.org/10.1007/s11356-020-10640-3 PMID: 32970259

[5] Rotondo JC. Advanced molecular and immunological diagnostic methods to detect SARS-CoV-2 infection. Microorganisms 2022; 10(6): 1193.

[6] Contini C. Advances in SARS-CoV-2 Infection. Microorganisms 2023; 11(4): 1048.

[7] Jain N, Choudhury A, Sharma J, Kumar V, De D, Tiwari R. A review of novel coronavirus infection (Coronavirus Disease-19). Glob J Transfus Med 2020; 5(1): 22.
http://dx.doi.org/10.4103/GJTM.GJTM_24_20

[8] Polatoğlu I, Oncu-Oner T, Dalman I, Ozdogan S. COVID-19 in early 2023: Structure, replication mechanism, variants of SARS-CoV-2, diagnostic tests, and vaccine & drug development studies. MedComm 2023; 4(2): e228.
http://dx.doi.org/10.1002/mco2.228 PMID: 37041762

[9] Zhong NS, Zheng BJ, Li YM, *et al.* Epidemiology and cause of severe acute respiratory syndrome (SARS) in Guangdong, People's Republic of China, in February, 2003. Lancet 2003; 362(9393): 1353-8.
http://dx.doi.org/10.1016/S0140-6736(03)14630-2 PMID: 14585636

[10] Rotondo JC, Martini F, Maritati M. SARS-CoV-2 infection: New molecular, phylogenetic, and pathogenetic insights. Efficacy of current vaccines and the potential risk of variants. Viruses 2021; 13(9): 1687.

[11] Wu T. The socioeconomic and environmental drivers of the COVID-19 pandemic: A review. Ambio 2021; 50(4): 822-33.
http://dx.doi.org/10.1007/s13280-020-01497-4 PMID: 33507498

[12] Zeng ZQ, Chen DH, Tan WP, *et al.* Epidemiology and clinical characteristics of human coronaviruses OC43, 229E, NL63, and HKU1: A study of hospitalized children with acute respiratory tract infection in Guangzhou, China. Eur J Clin Microbiol Infect Dis 2018; 37(2): 363-9.
http://dx.doi.org/10.1007/s10096-017-3144-z PMID: 29214503

[13] Zhu N, Zhang D, Wang W, *et al.* A novel coronavirus from patients with pneumonia in China, 2019. N Engl J Med 2020; 382(8): 727-33.
http://dx.doi.org/10.1056/NEJMoa2001017 PMID: 31978945

[14] Li X, Zai J, Wang X, Li Y. Potential of large "first generation" human-to-human transmission of 2019-nCoV. J Med Virol 2020; 92(4): 448-54.
http://dx.doi.org/10.1002/jmv.25693 PMID: 31997390

[15] Tiwari U, Bano A, Ahmad Khan MK. A review on the COVID-19: Facts and current situation. NeuroPhar J 2021; 6: 180-91.
http://dx.doi.org/10.37881/1.616

[16] Li N, Wang R, Cai M, Jiang W, Feng Y, Xiao L. Outbreak of

cryptosporidiosis due to Cryptosporidium parvum subtype IIdA19G1 in neonatal calves on a dairy farm in China. Int J Parasitol 2019; 49(7): 569-77.
http://dx.doi.org/10.1016/j.ijpara.2019.02.006 PMID: 31071320

[17] Steiner S, Kratzel A, Barut GT, *et al.* SARS-CoV-2 biology and host interactions. Nat Rev Microbiol 2024; 2024: 1-20.
PMID: 38225365

[18] Hui DS, I Azhar E, Madani TA, *et al.* The continuing 2019-nCoV epidemic threat of novel coronaviruses to global health — The latest 2019 novel coronavirus outbreak in Wuhan, China. Int J Infect Dis 2020; 91: 264-6.
http://dx.doi.org/10.1016/j.ijid.2020.01.009 PMID: 31953166

[19] Song W, Gui M, Wang X, Xiang Y. Cryo-EM structure of the SARS coronavirus spike glycoprotein in complex with its host cell receptor ACE2. PLoS Pathog 2018; 14(8): e1007236.
http://dx.doi.org/10.1371/journal.ppat.1007236 PMID: 30102747

[20] Liu Z, Ciais P, Deng Z, *et al.* Author correction: Near-real-time monitoring of global CO2 emissions reveals the effects of the COVID-19 pandemic. Nat Commun 2020; 11(1): 6292.
http://dx.doi.org/10.1038/s41467-020-20254-5 PMID: 31911652

[21] Liu C, Zhou Q, Li Y, *et al.* Research and development on therapeutic agents and vaccines for COVID-19 and related human coronavirus diseases. ACS Cent Sci 2020; 6(3): 315-31.
http://dx.doi.org/10.1021/acscentsci.0c00272 PMID: 32226821

[22] Noreen S, Maqbool I, Madni A. Dexamethasone: Therapeutic potential, risks, and future projection during COVID-19 pandemic. Eur J Pharmacol 2021; 894: 173854.
http://dx.doi.org/10.1016/j.ejphar.2021.173854 PMID: 33428898

[23] Han Y, Wang Z, Ren J, Wei Z, Li J. Potential inhibitors for the novel coronavirus (SARS-CoV-2). Brief Bioinform 2021; 22(2): 1225-31.
http://dx.doi.org/10.1093/bib/bbaa209 PMID: 32942296

[24] Patel SK, Saikumar G, Rana J, *et al.* Dexamethasone: A boon for critically ill COVID-19 patients? Travel Med Infect Dis 2020; 37: 101844.
http://dx.doi.org/10.1016/j.tmaid.2020.101844 PMID: 32791213

[25] Khuroo MS. Chloroquine and hydroxychloroquine in coronavirus disease 2019 (COVID-19). Facts, fiction and the hype: aA critical appraisal. Int J Antimicrob Agents 2020; 56(3): 106101.
http://dx.doi.org/10.1016/j.ijantimicag.2020.106101   PMID: 32687949

[26] Agrawal U, Raju R, Udwadia ZF. Favipiravir: A new and emerging antiviral option in COVID-19. Med J Armed Forces India 2020; 76(4): 370-6.
http://dx.doi.org/10.1016/j.mjafi.2020.08.004 PMID: 32895599

[27] Hashemian SM, Farhadi T, Velayati AA. A review on remdesivir: A possible promising agent for the treatment of COVID-19. Drug Des Devel Ther 2020; 14: 3215-22.
http://dx.doi.org/10.2147/DDDT.S261154 PMID: 32821086

[28] Uzunova K, Filipova E, Pavlova V, Vekov T. Insights into antiviral mechanisms of remdesivir, lopinavir/ritonavir and chloroquine/hydroxychloroquine affecting the new SARS-CoV-2. Biomed Pharmacother 2020; 131: 110668.
http://dx.doi.org/10.1016/j.biopha.2020.110668 PMID: 32861965

[29] Elakovich SD. The neem tree: Source of unique natural products for integrated pest management, medicine, industry and other purposes. General & Introductory Life Sciences. Wiley 1996.

[30] Niranjan PA. Biological activities of turmeric (*Curcuma longa* L.) - A review. J Food Sci Technol 2008; 45(2): 109-16.

[31] Sangwan RS, Chaurasiya ND, Misra LN, *et al.* Phytochemical variability in commercial herbal products and preparations of Withania somnifera (Ashwagandha). Curr Sci 2004; 86(3): 461-5.

[32] Joshi RK. Phytoconstituents, traditional, medicinal and bioactive uses of Tulsi (Ocimum sanctum Linn.): A review. J Pharmacogn Phytochem 2017; 6(2): 261-4.

[33] Gani HMO, Hoq MO, Tamanna T. Ethnomedicinal, phytochemical and pharmacological properties of Piper longum (Linn). Asian J Med Biol Res 2019; 5(1): 1-7.
http://dx.doi.org/10.3329/ajmbr.v5i1.41038

[34] Wu Y, Wang F, Shen C, *et al.* A noncompeting pair of human

neutralizing antibodies block COVID-19 virus binding to its receptor ACE2. Science 2020; 368(6496): 1274-8.
http://dx.doi.org/10.1126/science.abc2241 PMID: 32404477

[35] Rizvi SM, Shakil S, Haneef M. A simple click by click protocol to perform docking: AutoDock 4.2 made easy for non-bioinformaticians. EXCLI J 2013; 12: 831-57.
PMID: 26648810

[36] Saxena VL, Nand Chaubey S, Srivastava S, Eram S, Trivedi P. Computational repositioning of ethno medicine elucidated anti-harper drug target, E6 (1C4Z). MEDT 2018; 4(1): 22.
http://dx.doi.org/10.21767/2572-5394.100022

[37] O'Boyle NM, Banck M, James CA, Morley C, Vandermeersch T, Hutchison GR. Open Babel: An open chemical toolbox. J Cheminform 2011; 3(1): 33.
http://dx.doi.org/10.1186/1758-2946-3-33 PMID: 21982300

[38] Morris GM, Huey R, Lindstrom W, *et al.* AutoDock4 and AutoDockTools4: Automated docking with selective receptor flexibility. J Comput Chem 2009; 30(16): 2785-91.
http://dx.doi.org/10.1002/jcc.21256 PMID: 19399780

[39] Rarey M, Kramer B, Lengauer T, Klebe G. A fast flexible docking method using an incremental construction algorithm. J Mol Biol 1996; 261(3): 470-89.
http://dx.doi.org/10.1006/jmbi.1996.0477 PMID: 8780787

[40] Gupta CL, Babu Khan M, Ampasala DR, Akhtar S, Dwivedi UN, Bajpai P. Pharmacophore-based virtual screening approach for identification of potent natural modulatory compounds of human Toll-like receptor 7. J Biomol Struct Dyn 2019; 37(18): 4721-36.
http://dx.doi.org/10.1080/07391102.2018.1559098   PMID: 30661449

[41] Jayaram B, Singh T, Mukherjee G, Mathur A, Shekhar S, Shekhar V. Sanjeevini: A freely accessible web-server for target directed lead molecule discovery. BMC Bioinformat 2012; 13(S17): S7.
http://dx.doi.org/10.1186/1471-2105-13-S17-S7 PMID: 23282245

[42] Vardhan S, Sahoo SK. *In silico* ADMET and molecular docking study on searching potential inhibitors from limonoids and triterpenoids for COVID-19. Comput Biol Med 2020; 124(124): 103936.
http://dx.doi.org/10.1016/j.compbiomed.2020.103936   PMID: 32738628

[43] Pronk S, Páll S, Schulz R, *et al.* GROMACS 4.5: A high-throughput and highly parallel open source molecular simulation toolkit. Bioinformatics 2013; 29(7): 845-54.
http://dx.doi.org/10.1093/bioinformatics/btt055 PMID: 23407358

[44] Schüttelkopf AW, van Aalten DMF. *PRODRG* : A tool for high-throughput crystallography of protein–ligand complexes. Acta Crystallogr D Biol Crystallogr 2004; 60(8): 1355-63.
http://dx.doi.org/10.1107/S0907444904011679 PMID: 15272157

[45] Pathak RK, Gupta A, Shukla R, Baunthiyal M. Identification of new drug-like compounds from millets as Xanthine oxidoreductase inhibitors for treatment of Hyperuricemia: A molecular docking and simulation study. Comput Biol Chem 2018; 76: 32-41.
http://dx.doi.org/10.1016/j.compbiolchem.2018.05.015   PMID: 29906649

[46] Shah AA, Ahmad S, Yadav MK, Raza K, Kamal MA, Akhtar S. Structure-based virtual screening, molecular docking, molecular dynamics simulation, and metabolic reactivity studies of quinazoline derivatives for their anti-EGFR activity against tumor angiogenesis. Curr Med Chem 2023; 31(5): 595-619.
http://dx.doi.org/10.2174/0929867330666230309143711 PMID: 36892124

[47] Oostenbrink C, Villa A, Mark AE, Van Gunsteren WF. A biomolecular force field based on the free enthalpy of hydration and solvation: The GROMOS force-field parameter sets 53A5 and 53A6. J Comput Chem 2004; 25(13): 1656-76.
http://dx.doi.org/10.1002/jcc.20090 PMID: 15264259

[48] Bharadwaj S, Dubey A, Yadava U, Mishra SK, Kang SG, Dwivedi VD. Exploration of natural compounds with anti-SARS-CoV-2 activity *via* inhibition of SARS-CoV-2 Mpro. Brief Bioinform 2021; 22(2): 1361-77.
http://dx.doi.org/10.1093/bib/bbaa382 PMID: 33406222

[49] Di Muzio E, Toti D, Policelli F. DockingApp: A user friendly interface for facilitated docking simulations with AutoDock Vina. J Comput Aided Mol Des 2017; 31(2): 213-8.
http://dx.doi.org/10.1007/s10822-016-0006-1 PMID: 28063067

[50] Lin LT, Hsu WC, Lin CC. Antiviral natural products and herbal medicines. J Tradit Complement Med 2014; 4(1): 24-35.
http://dx.doi.org/10.4103/2225-4110.124335 PMID: 24872930

[51] Huang CF, Ma L, Sun LJ, *et al*. Immunosuppressive withanolides from Withania coagulans. Chem Biodivers 2009; 6(9): 1415-26.
http://dx.doi.org/10.1002/cbdv.200800211 PMID: 19774603

[52] Daglia M. Polyphenols as antimicrobial agents. Curr Opin Biotechnol 2012; 23(2): 174-81.
http://dx.doi.org/10.1016/j.copbio.2011.08.007 PMID: 21925860

[53] Pal S, Kumar V, Kundu B, *et al*. Ligand-based pharmacophore modeling, virtual screening and molecular docking studies for discovery of potential topoisomerase I inhibitors. Comput Struct Biotechnol J 2019; 17: 291-310.
http://dx.doi.org/10.1016/j.csbj.2019.02.006 PMID: 30867893

[54] Denaro M, Smeriglio A, Barreca D, *et al*. Antiviral activity of plants and their isolated bioactive compounds: An update. Phytother Res 2020; 34(4): 742-68.
http://dx.doi.org/10.1002/ptr.6575 PMID: 31858645