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Original Article

Dual inhibition of COVID-19 spike glycoprotein and main protease 3CLpro by Withanone from *Withania somnifera*

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ABSTRACT

Objective: To identify the safe and effective natural inhibitors of spike glycoprotein and main protease 3CLpro using potential natural antiviral compounds which are studied under various animal models and viral cell lines.

Methods: First, compounds were retrieved from the PubChem database and predicted for their druggability using the MolSoft web server, and compounds having drug-like property were predicted for major adverse drug reactions like cardiotoxicity, hepatotoxicity, arrhythmia, myocardial infarction, and nephrotoxicity using ADVERPred. Docking of nontoxic antiviral compounds with spike glycoprotein and main protease 3CLpro was performed using AutoDock vina by PyRx 0.8 version. The stability of compound-protein interactions was checked by molecular dynamic (MD) simulation using Schrodinger Desmond software.

Results: Based on the druggable and nontoxic profile, nine compounds were selected. Among them, Withanone from *Withania somnifera* showed the highest binding affinity and best fit at active sites 1 of spike glycoprotein (glycosylation site) and main protease 3CLpro via interacting with active site amino acid residues before and after MD simulation at 50 ns. Withanone, which may reduce the glycosylation of SARS-CoV-2 via interacting with Asn343 and inhibit viral replication.

Conclusion: The current study reports Withanone as a non-toxic antiviral against SARS-CoV-2 and serve as a potential lead hit for further experimental validation.

1. Introduction

In the last twenty years, numerous viral epidemics such as SARS (Severe Acute Respiratory Syndrome Coronavirus) in 2002–2003, H1N1 flu in 2009 (Cascella et al., 2020), MERS-CoV (Middle East Coronavirus Respiratory Syndrome) in 2012 (Khan and Sheek-Hussein, 2020) have emerged to cause epidemics. In late December 2019, an outbreak of new coronavirus disease (SARS-CoV2; COVID-19; 2019-nCoV) has been reported (Xu et al., 2020). On January 30, 2020, WHO declared the outbreak of COVID-19 as a global health emergency, and on March 11, 2020, WHO declared COVID-19 as a pandemic (Lai et al., 2020). COVID-19 is an acute respiratory syndrome, primarily affects the respiratory system (Singhal, 2020). The time from onset of COVID-19 symptoms to death ranged from 6 d to 41 d with a median of 14 d (Rothan and Byrareddy, 2020). This period depends on the patient age and state of the immune system. The most common symptoms in the early stages of COVID-19 are fever, dry cough, and fatigue. The symptoms associated with the onset of severity include sputum production, headache, hemoptysis, diarrhea, dyspnea and lymphopenia (Kumar et al., 2020a).

Recognition of host receptors by the virus is the first stage of a viral infection and is a key factor determining the tropism of host cells and tissues (Baranowski et al., 2001). Coronavirus is an enveloped, positive single-stranded RNA virus belongs to the subspecies Orthocoronavirinae, containing "crown-like" spikes on their surfaces (Hageman, 2020). Among the structural elements of CoVs, Spike (S) glycoprotein cleaved by proteases into two subunits i.e. S1 and S2 (Walls et al., 2020). S1 is further divided into SA and SB domains. The SB domain interacts with the angiotensin-converting enzyme 2 (ACE2) to enter cells by acting on receptor binding domain S1 (Walls et al., 2020). Whereas the S2 subunit containing the fusion peptide, transmembrane domain, and cyto-

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plasmic domain are highly conserved (Xia et al., 2020). S2 plays a key role in the fusion of the SARS-CoV-2 S1-ACE2 complex formation with the cell membrane and is highly glycosylated at evolutionarily conserved sites. It is well demonstrated that increased glycosylation of SARS-CoV-2 and ACE2 increases the binding affinity between SARS-CoV-2 and hACE2 which correlates with increased virus transmission and disease severity in human (Brufsky 2020). SARS-CoV-2 glycosylation sites have been identified to affect the host immune response and have been suggested as a possible target for future alterations (Vetrivel et al., 2021). Targeting glycosylation site aid in the early and rapid immune response to neutralize the virion (Vetrivel et al., 2021). By decreasing the level of glycosylation in the lung epithelium could reduce the binding affinity of SARS-CoV-2 viral particles with the ACE2 in the lung (Brufsky 2020). Furthermore, researchers identified that the SARS-CoV-2 spike protein S2 subunit has a sequence identify of 89.8% and sequence similarity of 96.9% compared to earlier SARS-CoV (Ou et al., 2020). To date, there are no approved specific drugs to prevent their infection due to the host receptors for penetration of the virus into the cell, poorly conserved structural proteins, and high rates of mutation and recombination pose became the major challenge in the development of broad-spectrum anti-CoV drugs and vaccines (Tse et al., 2020). Currently, a number of studies were carried out to design lead molecules against SARS-CoV-2 spike glycoprotein-mediated membrane fusion. Attempts are also made to design SARS-CoV S murine polyclonal antibodies to inhibit SARS-CoV-2 S protein mediated entry into human cells through ACE2 receptor (Wang et al., 2020; Walls et al., 2020). Also, FDA approved chloroquine phosphate and hydroxychloroquine sulphate for the management COVID-19 based on the clinical data and significant effect on *in vitro* cell lines (Weston et al, 2020). Hydroxychloroquine showed potent activity against COVID-19 but it causes drug poisoning and adverse drug events in patients with heart disease, hypertension, and diabetes having COVID-19 infection (Singh et al., 2021) and also all trails on hydroxychloroquine sulphate are paused by WHO due to their lack of efficacy, greater risk of experiencing heart arrhythmias and dying in hospital than those COVID-19 patients who did not received hydroxychloroquine sulphate (Mahase, 2020; Tang et al., 2020). Moreover, these agents showed acute respiratory distress syndrome (ARDS) via inhibition of pro-inflammatory cytokines and associated with lysosomal dysfunction which leads to the psychiatric symptoms (Tufan et al., 2020). Retinal toxicity was seen in a patient with acute renal impairment. Administration of hydroxychloroquine with atorvastatin to the diabetic patient caused the severe hypoglycemia (Wondafrash et al., 2020).

Currently, effective treatment against COVID-19 is limited. In order to rapidly identify the therapeutic compounds, scientists screened millions of lead compounds by virtual drug screening, structure-assisted drug design, and high-throughput screening against spike glycoprotein and main protease 3CLpro but none of the compounds showed a significant effect. Main protease 3CLpro plays a crucial role in viral replication and transcription and many researchers identified it as a therapeutic target for antiviral drug design in the management of COVID-19 infection (Jin et al., 2020).

In the current study, an attempt was made to the best use of existing traditional knowledge as a potent resource against COVID-19 and we identified the probable lead molecule "Withanone" from *Withania somnifera* (Linn.) Dunal having a drug-like property, nontoxic profile, best fit and highest affinity with active site residues of the SARS-CoV-2 spike glycoprotein and main protease 3CLpro to prevent viral infection. *W. somnifera* (Ashwagandha) is used for the treatment of asthma, bronchitis, viral infections, colds, coughs, and so on (Verma and Kumar, 2011; Umadevi et al., 2012). Withanolides are the main bioactive components from the root part and are well known for therapeutic properties. Withanone is a steroidal lactone with a withanolide-type A skeleton (Vaishnavi et al., 2012) and possess antiviral activity against SARS-CoV-2 (Kumar et al., 2020a; Kumar et al., 2020b). This small molecule may provide researchers an advantage in developing medications to control or treat COVID-19.

2. Materials and methods

2.1. Selection and preparation of natural antiviral compounds

Bioactive phytocompounds that are claimed to prevent and/or treat different types of viral infections, which are previously studied under various viral cell lines, animal models to inhibit the progression of viral infection were selected from literature/scientific records. Also, the compounds present in the herbs which are utilized to treat viral infection or viral-induced conditions in the Indian traditional medicine system were cross-verified using traditional claims reported by the traditional practitioners. List of phytocompounds with their references for antiviral activity were listed in Table S1. Furthermore, three dimensional (3D) structures of each compound were downloaded in structure data file (.sdf) format from the PubChem chemical database (https://pubchem.ncbi.nlm.nih.gov/) and free binding energy were minimized using the MMFF94 force field and saved in protein data bank (.pdb) format.

2.2. Drug-likeness characteristics and toxicity profile

For the assessment of the pharmaceutically relevant properties of compounds, molecular weight, number of hydrogen bond donors and acceptors, logP value data were retrieved from PubChem chemical database and the drug-likeness score was predicted based on the Lipinski rule of five (Patil et al., 2019). In the current study, canonical SMILES of each compound were quired into Molsoft online server to predict the druggable property based on the structure–activity relationship and with respect to the known approved therapeutic compounds to treat diseases. Further, to identify the compounds having a nontoxic property, we predicted major toxic effects or adverse drug reactions such as cardiotoxicity, hepatotoxicity, arrhythmia, myocardial infraction, and nephrotoxicity was assessed using the ADVERpred online server (Ivanov et al., 2018).

2.3. Identification of targets and their active sites

Three-dimensional X-ray crystallographic structure of spike-host cell receptor or spike glycoprotein having PDB ID: 6LZG with 2.50 Å and main protease having PDB ID: 7BUY with 1.60 Å was selected as therapeutic targets for SARS-CoV-2 viral entry and replication and structures were retrieved from RCSB PDB (www.rcsb.org). Also, in order to clean the binding pocket and make calculations easier so that ligand can form satisfactory interactions with the protein, we removed water molecules, and heteroatoms contained in protein structure using the Discovery studio visualizer 2020 version. The distribution of backbone dihedral Phi and Psi angles of amino acids were analyzed by Ramachandran plot using PROCHECK and the overall quality factor of the protein molecules was checked by the ERRAT web server. The chain was selected based on its completeness of amino acid residue and the presence of the active site domain. The active site amino acid residues of spike glycoprotein and main protease responsible for the host receptor recognition, viral entry into the host cell, and viral replication were retrieved from the PDB structures data and literature (Wang et al., 2020; Jin et al., 2020).

2.4. Docking studies

Molecular docking of selected nontoxic compounds with main protease and spike glycoprotein was performed using AutoDock vina by PyRx 0.8 version (Dallakyan and Olson, 2015; Patil and Khatib, 2020; Patil et al., 2020). The compounds were imported into the tool and converted .pdb to .pdbqt AutoDock vina format. Grid box was generated to the individual protein and compound based on the active amino residues contained in the active site domain of protein molecule. The exhaustiveness of 100 was kept to run the AutoDock vina algorithm. Moreover, the compound having the lowest binding energy (kcal/mol) was selected, and compound-protein best fit at the active site region and pocket, ligand–protein contact distance, hydrogen bond interactions with the active amino acid residues and the orientation of ligand in the binding site of spike glycoprotein and main protease and alignment of ligand molecules based on their states at the beginning of docking simulation and after docking simulation were analyzed and visualized by discovery studio visualizer 2020 academic version.

2.5. Molecular dynamics

Molecular dynamic simulation was carried out to check stability and molecular interactions of ligand with protein molecule using Schrödinger Desmond software (Bowers et al., 2006). Firstly, ligand–protein complex system was solvated, neutralized, and the net charge was balanced using Na^+ and Cl^- ions. Secondly, the energy minimization step was carried out, number of atoms, temperature (310 K) were considered for the relaxation of the system. The molecular dynamic simulation time was set to 50 ns. To calculate the long-range interactions between ligand and protein, Particle mesh Ewald method was used and Lennard-Jones interactions cut-off was set to 10 Å. Finally, the stability of the complex was analyzed by checking the interaction between ligand with active site residues of protein molecule and structural variations of the complex via measuring the root-mean-square deviation (RMSD, Å) of ligand and protein complex at 50 ns.

3. Results

3.1. Selection, drug-likeness and toxicity profile of natural antiviral compounds

Forty-eight potent natural antiviral compounds that are claimed to prevent and/or treat different types of viral infection, which are previously studied under various viral cell lines, animal models to inhibit the progression of viral infection were selected. Among 48 phytocompounds, 23 compounds showed the drug-like property. To identify a potent antiviral compound having a nontoxic property, toxicity of the 23 compounds was predicted by ADVERpred. Among them, nine compounds showed nontoxic i.e. dammarenolic acid, deoxypodophyllotoxin, eugeniin, LPRP-Et-97543, manassantin B, samarangenin B, silvestrol, xanthohumol, and withanone. LPRP-Et-97543 scored the highest drug-likeness score i.e. 0.82 and eugeniin scored the lowest i.e. 0.07. Chemical properties such as MW, HBD, HBA, LogP value, and drug-likeness score of nontoxic antiviral phytocompounds were shown in Table 1. Overall predicted drug-likeness characteristics and toxicity profile of 48 compounds were shown at Tables S1 and S2.

3.2. Identification of targets and their active sites

Based on the available literature, SARS CoV-2 spike glycoprotein and main protease 3CLpro were selected as therapeutic targets. The overall quality factor of spike glycoprotein (PDB ID: 6LZG) and main protease 3CLpro (PDB ID: 7BUY) was found to be 85.311% and 93.836% respectively. Further, one amino acid residue i.e. Lys386 of spike glycoprotein was found in disallowed region and about 82.1% and 17.3% amino residues were found in favoured and additional allowed region. No amino acid residues of main protease protein were found in disallowed region and about 89.8% and 9.1% were found in favoured and additional allowed region. Further, from literature, we identified 31 active site residues of SARS-CoV-2 spike glycoprotein involved in the complex formation with ACE2 i.e. Tyr28, Thr29, Asn61, Asn122, Asn125, Asn234, Thr236, Asn280, Asn282, Asn331, Asn343, Pro579, Gln580, Asn603, Asn616, His655, Asn657, Asn709, Asn717, Asn801, Ser803, Gln804, Leu922, Glu1072, Asn1074, Asn1098, Gly1099, Phe1103, Gly1131, Ile1132, Asn1134 (Ortega et al., 2020) and three amino acid of SARS-CoV-2 spike glycoprotein were present in the active site 1 i.e. Phe338, Phe342, and Asn343. Among these three, one was potentially involved in glycosylation i.e. Asn343 (Sun et al., 2020; Wang et al., 2020). Further, a total of 29 active site residues were identified in the main protease i.e. Gly2, Phe3, Met6, Ala7, Phe8, Gly15, Met17, His41, Gly71, Lys97, Ser123, Gly127, Lys137, Gly138, Phe140, Asn142, Gly143 Ser144 Cys145, His164, His163, Glu166, His172, Arg188, Gly189, Thr190, Gly192, Leu282, Arg298. The amino acid residue distribution within the favorable and non-favorable regions and quality factors of proteins were shown in Fig. 1. Overall characteristics features of protein molecules were shown at Table 2.

3.3. Docking studies

To understand the mechanism of action of antiviral compounds with the SARS CoV-2 spike glycoprotein and main protease 3CLpro active site residues, a molecular docking study was executed to calculate the affinity via calculating binding energy (kcal/mol). Among nine nontoxic antiviral compounds, Withanone showed the highest binding affinity with spike glycoprotein (−8.7 kcal/mol) and main protease 3CLpro (−7.0 kcal/mol). Withanone formed three hydrogen bond interactions i.e. Phe338...O-, Gly339...O-, and Asn343...O- with spike glycoprotein. Among them, one amino acid is involved in glycosylation i.e. Asn343. O group of Withanone interacted with the Asn343 amino acid residue. Further, we analyzed the Withanone and spike glycoprotein amino acid interaction distance, the distance between Phe338...O-, Gly339...O-, and Asn343...O- was found to be 2.63, 2.17, and 2.19 Å (Supplementary Fig. S1A), which indicates the highest affinity of Withanone towards Phe339 and Asn343. Alignment of the Withanone with spike glycoprotein pre- and post-simulation states showed that 7.531% and 30.46% of the change in orientation of movable bonds in steric and electrostatic effect respectively. Similarly, Withanone formed three hydrogen bond interactions with main protease 3CLpro i.e. His163...=O, Glu166...O-, and Gln189...O-. Withanone formed two Alkyl interactions (Asn142, Cys145) and one van der Waals interactions (Pro168). A peer interpretation showed that five amino acid residues i.e. Asn142, Cys145, His163, Glu166, and Gln189 were identified as active site residues and potentially involved in the function of main protease 3CLpro. Further, we analyzed the Withanone and main protease amino acid interaction distance, the distance between His163...=O, Glu166...O-, and Gln189...O- was found to be 2.20, 2.27, and 1.89 Å (Supplementary Fig. 1b), which indicates the highest affinity of Withanone towards Gln189. Alignment of the Withanone with main protease 3CLpro pre- and post-simulation states showed that 5.17% and 26.16% of the change in orientation of movable bonds in steric and electrostatic effect respectively. Binding affinity and hydrogen bond interaction of compounds with spike glycoprotein and main protease 3CLpro were shown in Tables 3 and 4 respectively. Interaction of

Table 1
Natural antiviral compounds having druggable characteristics and nontoxic property.

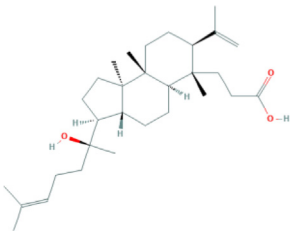
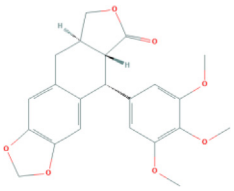
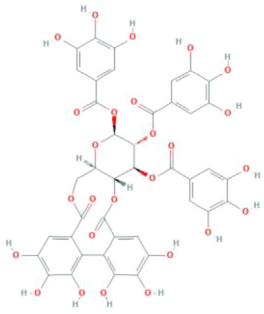
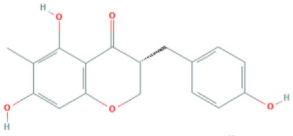
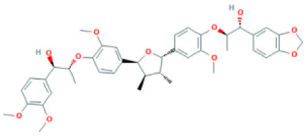
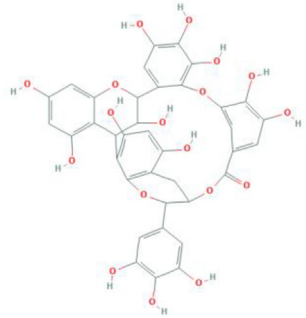
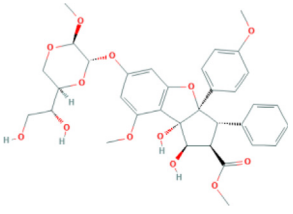
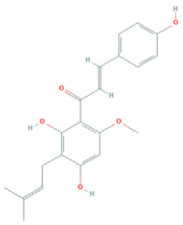
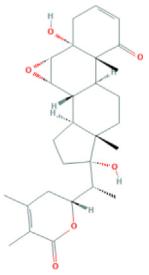
Compounds	PubChem ID	MW	MF	HBD	HBA	LogP	DLS	Chemical structures
Dammarenolic acid	22215841	458.38	C_{30} H_{50} O_3	2	3	7.21	0.54	
Deoxypodophyllotoxin	345501	257.11	C_{15} H_{15} N O_3	1	3	2.68	0.29	
Eugeniin	442679	938.1	C_{41} H_{30} O_{26}	10	26	2.05	0.07	
LPRP-Et-97543	71585044	300.1	C_{17} H_{16} O_5	3	5	3.28	0.82	
Manassantin B	10439828	716.32	C_{41} H_{48} O_{11}	2	11	5.29	0.06	
Samarangenin B	85131379	760.13	C_{37} H_{28} O_{18}	13	18	2.75	0.24	
Silvestrol	11787114	654.23	C_{34} H_{38} O_{13}	4	13	1.47	0.32	

Table 1 (*continued*)

Compounds	PubChem ID	MW	MF	HBD	HBA	LogP	DLS	Chemical structures
Xanthohumol	639665	354.15	C_{21} H_{22} O_5	3	5	4.87	0.58	
Withanone	21679027	470.27	C_{28} H_{38} O_6	2	6	3.55	0.45	

MW: Molecular weight; MF: Molecular formula; HBD: Hydrogen bond donor; DLS: Druglikeness score

Withanone with spike glycoprotein and main protease 3CLpro active site and substrate-binding pocket were shown in Fig. 2 and Fig. 3 respectively. The orientation of Withanone in the binding site of spike glycoprotein and main protease 3CLpro and alignment of Withanone based on its states at the beginning of the simulation and after simulation were shown in Fig. 4 and Fig. 5 respectively.

3.4. Molecular dynamics

The dynamic simulation of Withanone with spike glycoprotein and main protease exhibited very stable root-mean-square deviation (RMSD) for both C(alpha) (Protein RMSD Å) and Lig-fit (Ligand RMSD Å) at 50 ns. Withanone and spike glycoprotein complex showed Lig-fit RMSD fluctuation between 0 and 5 ns and found to be stable throughout simulation from 5 to 50 ns at 9 Å, while C(alpha) was stable throughout the simulation with slight fluctuations between 2.4 and 4.2 Å. The contact between Withanone and spike glycoprotein were found to be stable before and after MD simulation. Withanone formed hydrogen bond interactions with Phe338, Gly339, and Asn343 before MD simulations and formed hydrogen bonds with Phe338, Gly339, Phe342, Asn343, Asn370, Ser371, and Ser373 of spike glycoprotein after MD simulation to form a stable complex. Similarly, Withanone and main protease 3CLpro complex showed C(alpha) and Lig-fit RMSD fluctuation between 0 and 4 ns and found to be stable throughout the simulation i.e. from 5 to 50 ns. Ligand RMSD was observed and the results reflected Withanone was fit into the cavity and its Lig-fit-Prot RMSD was found to be stable with Protein C(alpha) RMSD from 4 to 50 ns. When we analyzed contacts between Withanone with main protease 3CLpro before and after the MD simulation, Withanone formed three hydrogen bond interactions with main protease 3CLpro i.e. His163, Glu166, Gln189 and formed three hydrophobic bonds with Asn142, Cys145, Pro168 before MD simulation and exhibited a high degree of hydrogen and hydrophobic bonds with Asn142, Cys145 (17%), His163 (14%), Glu166 (49%), Gln189 (14%), Thr190 (18%), Gln192 after 50 ns simulation. RMSD fluctuation, ligand–protein contacts, and protein RMSF of Withanone with spike glycoprotein and main protease complex are represented in Figs. 6 and 7 respectively.

4. Discussion

To date, COVID-19 viral infection continues to increase worldwide and no satisfactory treatment is available. Recently, the National Health Commission of the People's Republic of China recommended two potent anti-malarial compounds i.e. chloroquine phosphate and hydroxychloroquine sulphate to reduce the complications associated with disease but these compounds cannot block viral infection, associated with toxic effects (Singh et al., 2020). Identification of new drug candidates is not only the key against disease, but considering the potentially favorable benefit-risk balance also plays a crucial role. Identification of therapeutic compounds with high benefits and low risk would be the desirable treatment for current pandemic COVID-19 (Martinez, 2020). In the current study, we have focused only on searching potent anti-viral pure compounds from a plant source that can inhibit the action of COVID-19 spike glycoprotein and main protease 3CLpro. Based on existing literature, we identified 48 natural antiviral compounds. These compounds were already studied under various viral cell lines and animal models. First, we screened selected compounds for their druggable characteristics using the MolSoft web server which predicts drug-likeness score based on the Lipinski rule of five and already existed known drug molecules for the treatment of various diseases. Compounds having positive drug-like properties were further screened for cardiotoxicity, hepatotoxicity, arrhythmia, myocardial infraction, and nephrotoxicity using ADVERpred web server which predicts Probable activity (Pa) to show toxic effect in term of Pa value and Probable inactivity for the drug to show nontoxic effect by Pi value. Among 48 compounds, nine compounds showed a nontoxic effect and selected them for docking study with spike glycoprotein and main protease 3CLpro.

In silico docking study results revealed Withanone as a potent dual inhibitor of spike glycoprotein and main protease 3CLpro. Withanone (Withanolide) is a main active principle from *W. somnifera* (Linn.) Dunal (Ashwagandha) roots. Ashwagandha is an Ayurvedic medicinal herb, used to treat cold and cough, bronchitis, asthma, viral infections, etc (Verma and Kumar, 2011; Umadevi et al., 2012). Roots of Ashwagandha are utilized to treat asthma, dyspepsia, hypertension, arthritis, rheumatism, and syphilis. The extract of Ashwagandha roots showed potent anti-inflammatory, antidiabetic, tumor cell proliferation inhibitory,

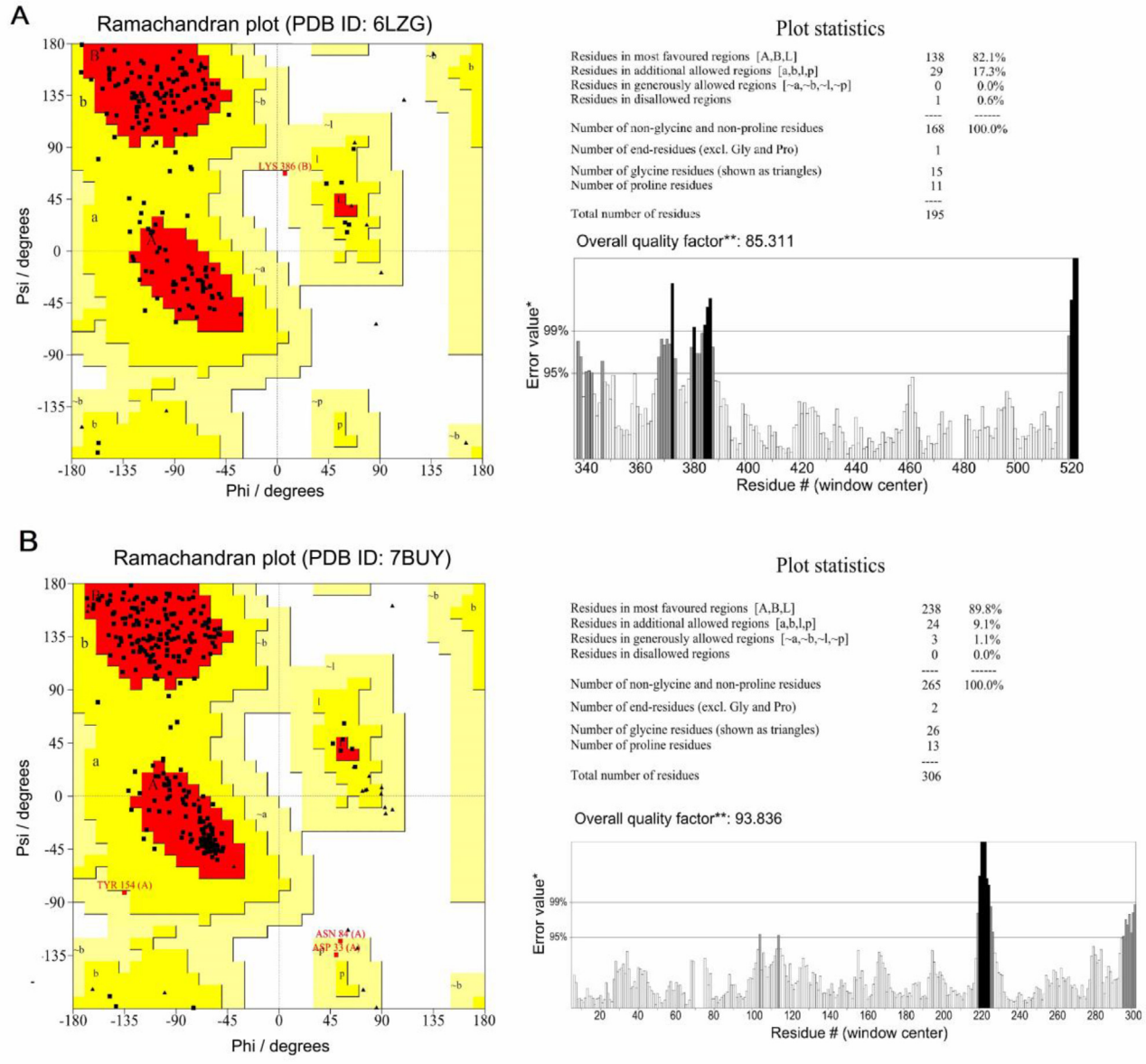


Fig. 1. Amino acid distribution and quality factor of (A) Spike glycoprotein (PDB ID: 6LZG) and (B) main protease (PDB ID: 7BUY).

Table 2
Characteristics of spike glycoprotein (PDB ID: 6VYB) and main protease (PDB ID: 7BUY).

Protein Characteristics	Therapeutic targets of SARS-CoV-2 Spike glycoprotein (PDB ID: 6LZG)	Main protease (PDB ID: 7BUY)
Resolution	2.50 Å	1.60 Å
Residues in most favored regions	82.1%	89.8%
Residues in additional allowed regions	17.3%	9.1%
Residues in generously allowed regions	0.0%	1.1%
Residues in disallowed regions	0.6%	0%
Total number of residues	195	306
Overall quality factor	85.31%	93.83%
Active site amino acid residues (Ortega et al., 2020; Wang et al., 2020)	SARS-CoV-2 - ACE2 binding residues: Arg403, Gly404, Asp405, Glu406, Arg408, Gln409, Lys417, Ile418, Tyr421, Val445, Tyr449, Tyr453, Leu455, Phe456, Tyr473, Gln474, Ala475, Gly485, Phe486, Asn487, Cys488, Tyr489, Phe490, Leu492, Gln493, Ser494, Tyr495, Gly496, Pro499, Thr500, Asn501, Gly502, Val503, Gly504, Tyr 505 Residues involved in Active site 1 and Glycosylation: Phe338, Phe342, Asn343	Gly2, Phe3, Met6, Ala7, Phe8, Gly15, Met17, His41, Gly71, Lys97, Ser123, Gly127, Lys137, Gly138, Phe140, Asn142, Gly143 Ser144 Cys145, His164, His163, Glu166, His172, Arg188, Gly189, Thr190, Gly192, Leu282, Arg298

Table 3
Binding affinity and hydrogen bond interactions of antiviral phytocompounds spike glycoprotein (PDB ID: 6LZG).

Compounds	Compound type	PubChem ID	BE/ (kcal·mol^{-1})	HBI between ligand...amino acid
Dammarenolic acid	Triterpenoid	22215841	−7.3	Arg355...=O, Arg355...OH
Deoxypodophyllotoxin	Flavolignan	345501	−8.3	Gln498...OH, Asn501...OH, Tyr505...OH
Eugeniin	Tannin	442679	−8.7	Arg403...=O, Gly496...OH, Gly498...OH, Asn501...OH, Gly502...O-, Tyr505...OH
LPRP-Et-97543	Polyphenol	71585044	−7.3	Asn343...O-, Val362...OH Asp364...OH
Manassantin B	Lignan	10439828	−7.3	Gly496...OH, Asn501...OH,
Samarangenin B	Terpenoid	85131379	−7	Tyr453...=O, Gln493...O-, Gly496...OH, Gly498...OH, Tyr505...OH
Silvestrol	Benzofuran	11787114	−6.4	Gln493...=O, Gly496...O-, Gly498...OH, Asn501...O-
Xanthohumol	Chalcone	639665	−6.9	Gly401...O-, Gly496...OH
Withanone	Steroid (Anthraquinone Glycoside)	21679027	−8.7	Phe338...O-, Gly339...O-, Asn343...O-

BE: Binding energy; HBI: Hydrogen bond interactions

Table 4
Binding affinity and hydrogen bond interactions of antiviral phytocompounds with main protease (PDB ID: 7BUY).

Compounds	Compound type	PubChem ID	BE/(kcal·mol^{-1})	HBI between ligand...amino acid
Dammarenolic acid	Triterpenoid	22215841	−5	Gln127...=O, Lys5...OH, Lys5...O-
Deoxypodophyllotoxin	Flavolignan	345501	−4.8	Val125...OH, Glu288...OH
Eugeniin	Tannin	442679	−3.5	Ser139...=O, Val125...OH, Lys5...OH, Gln...127...OH, Thr169...OH
LPRP-Et-97543	Polyphenol	71585044	−5.6	Lys5...OH, Lys5...=O, Glu290...OH
Manassantin B	Lignan	10439828	−5.5	Gly195...O-
Samarangenin B	Terpenoid	85131379	−5	Lys5...OH, Gln127...=O, Glu290...OH
Silvestrol	Benzofuran	11787114	−4.8	Nil
Xanthohumol	Chalcone	639665	−5.5	Nil
Withanone	Steroids (Anthraquinone Glycoside)	21679027	−7.0	His163...=O, Glu166...O-, Gln189...O-

BE: Binding energy; HBI: Hydrogen bond interactions

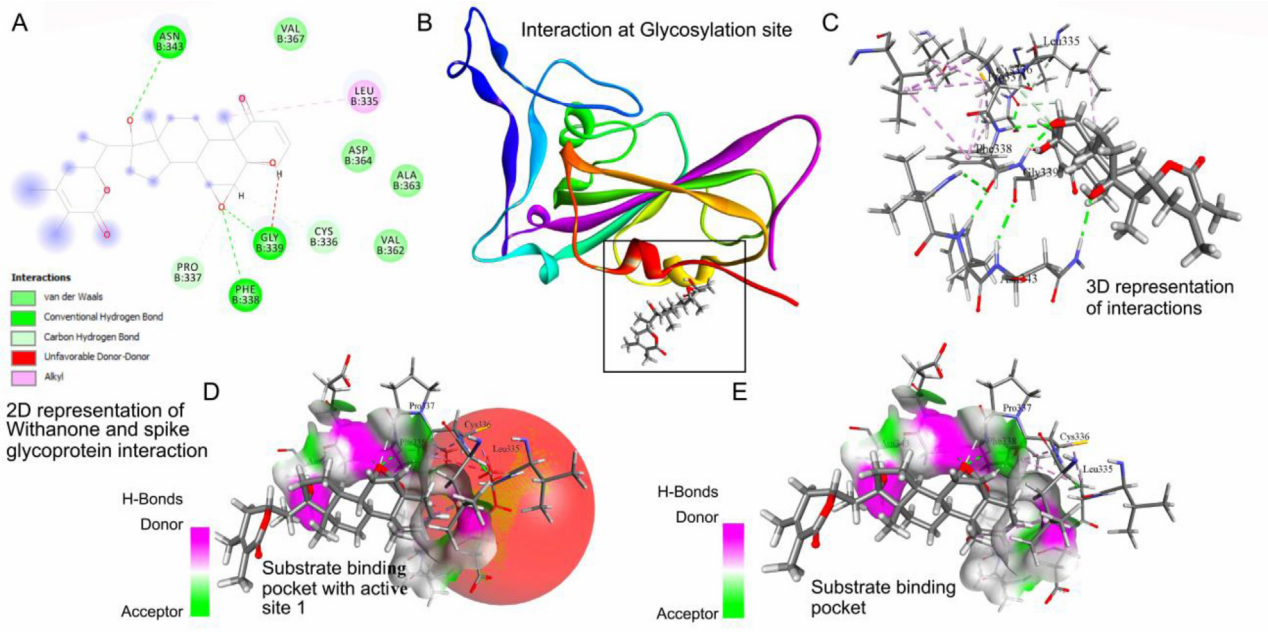


Fig. 2. Interaction of Withanone with Spike glycoprotein. (A) 2D representation; (B) Withanone fit at glycosylation site; (C) 3D representation; (D) Withanone within binding pocket and (E) active site 1 region.

antioxidant, immunomodulatory potency, and many researchers demonstrated that the therapeutic effect of *W. somnifera* roots is due to the presence of withanolides (Subbaraju et al., 2006; Anwer et al., 2008; Kushwaha et al., 2012). In the current study, Withanone formed two hydrogen bond interactions with active site 1 region amino acid of spike glycoprotein i.e. Phe338, Asn343, and one hydrogen bond with non-active site residue i.e. Gly339. Asn343 is potentially involved in the glycosylation of the SARS-CoV-2 enzyme. In SARS-CoV-2 spike glycoprotein – ACE2 complex structure, spike glycoprotein contains 195 consecutive density traceable residues spanning Tyr333 to Pro527 together with N-linked glycosylation at Asn343 (Wang et al., 2020). It is well described that SARS-CoV-2 utilizes an extensively glycosylated spike glycoprotein for host-cell entry via ACE2 (Watanabe et al., 2020). However, compounds that inhibit glycosylation may blunt the severity of COVID-19. In the current study, the interaction distance between Withanone and Asn343 (2.19 Å) was very less than Phe338 (2.63 Å), which indicates the highest affinity towards

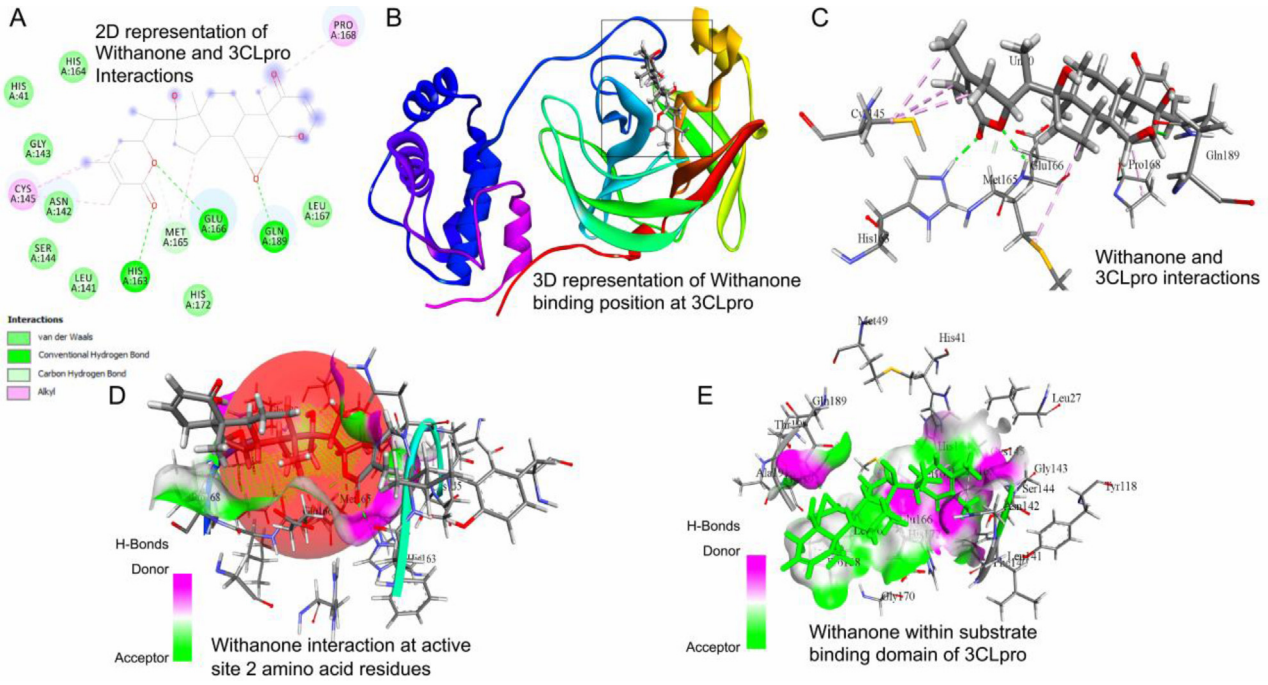


Fig. 3. Interaction of Withanone with main protease 3CLpro. (A) 2D representation; (B) Withanone fit ligand binding site; (C) 3D representation; (D) Withanone within binding pocket and E) active site 2 region.

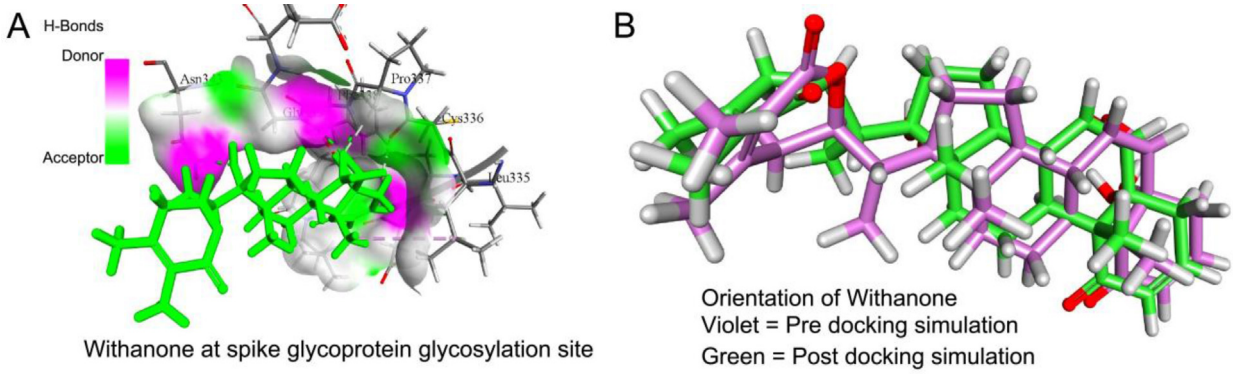


Fig. 4. Orientation of Withanone in glycosylation site of spike glycoprotein (A) and ligand state alignment (B) pre-simulation (violet) and post-simulation (green).

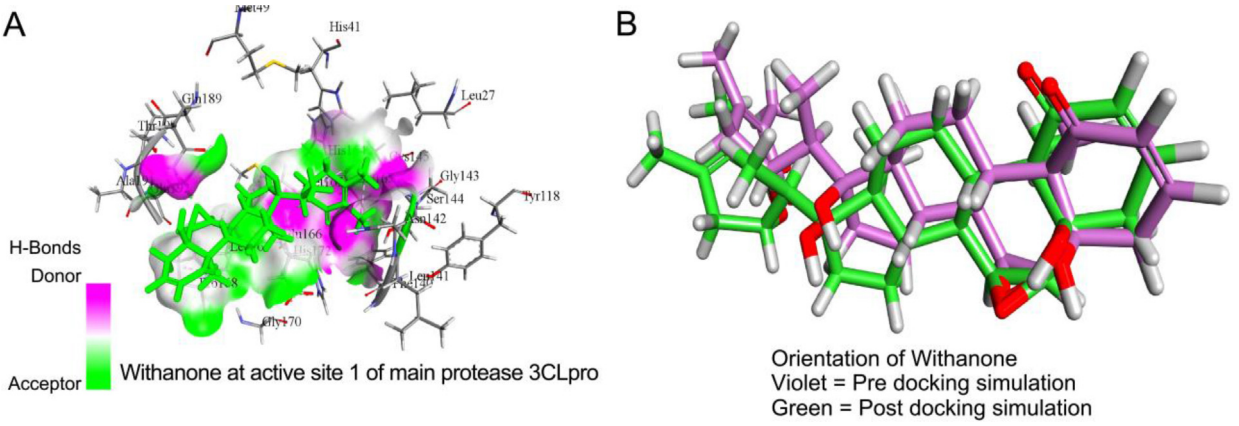


Fig. 5. Orientation of Withanone in binding site of Main protease 3CLpro (A) and ligand state alignment (B) pre-simulation (violet) and post-simulation (green).

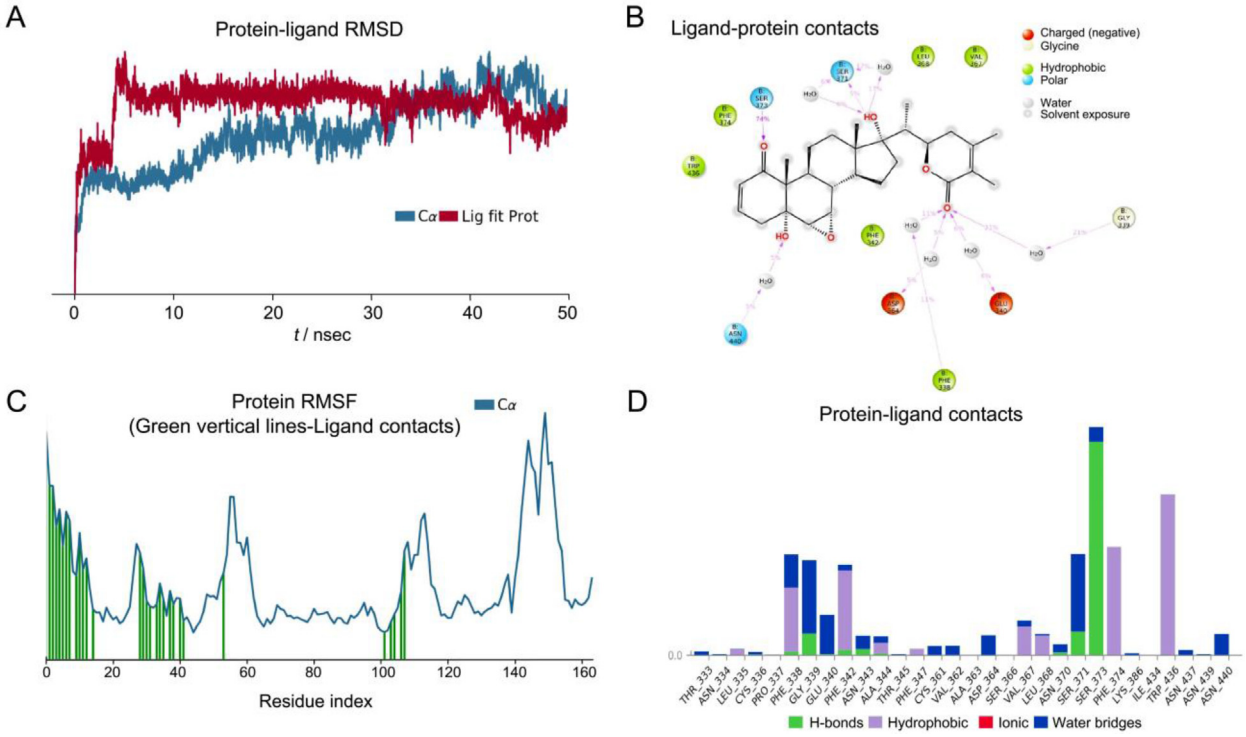


Fig. 6. Molecular dynamic simulation of Withanone - spike glycoprotein complex at 50 ns. RMSD (A), contacts (B), RMSF with contacts (C), and hydrogen, hydrophobic, ionic bonds, and water bridges (D).

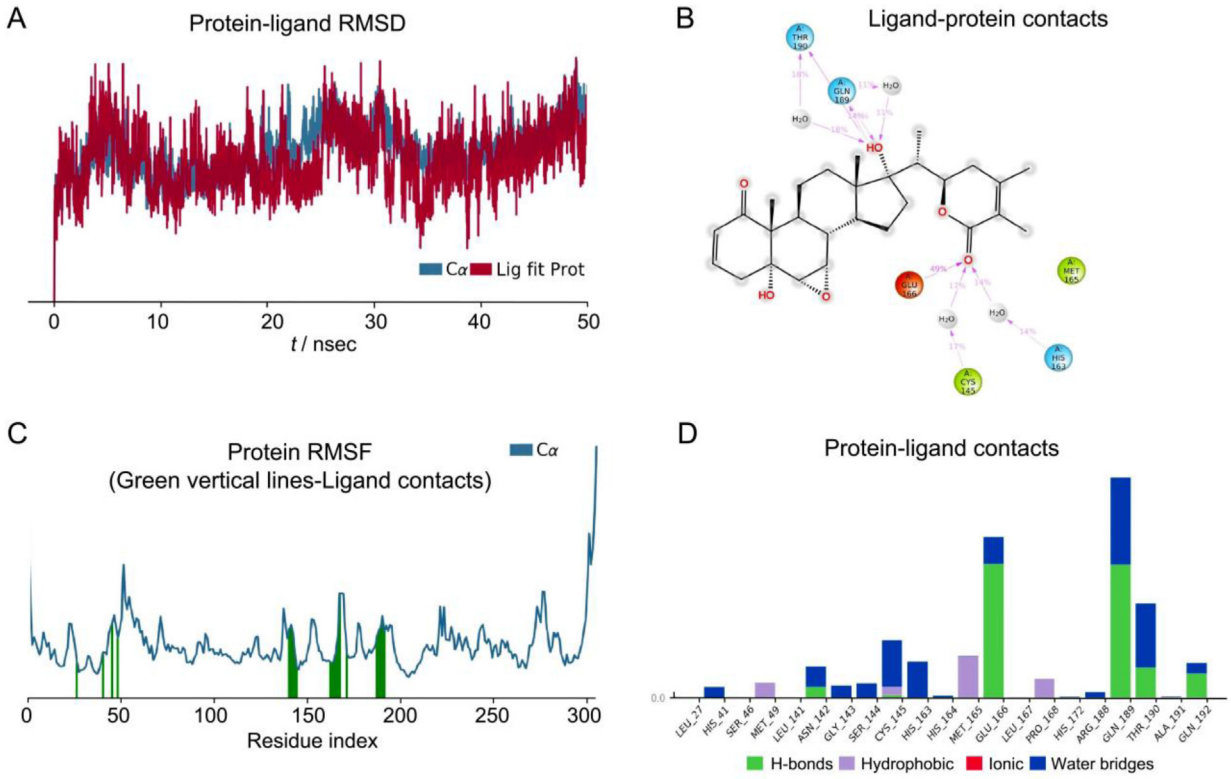


Fig. 7. Molecular dynamic simulation of Withanone – Main protease 3CLpro complex at 50 ns. RMSD (A), contacts (B), RMSF with contacts (C), and hydrogen, hydrophobic, ionic bonds, and water bridges (D).

Asn343. Moreover, Withanone formed one hydrogen bond with non-active site residue and one non-covalent bond interaction with spike glycoprotein to form a stable complex. Herein, Withanone could reduce the amount of glycosylated viral binding sites in the lung, and hence probably reduce the inflammation and symptoms associated with COVID-19 viral infection.

Recently in May 2020, an *in silico* study performed by Kumar et al. identified Withanone as a potent inhibitor of COVID-19 main protease 3CLpro (Kumar et al., 2020a). Furthermore, Withanone showed down-regulation of TMPRSS2 mRNA in treated cells and predicted to have dual action to block SARS-CoV-2 entry to the host cells (Kumar et al., 2020a). In the current study, among nine antiviral phytocompounds, Withanone showed the highest binding affinity (−7.0 kcal/mol) with main protease 3CLpro via forming three hydrogen bonds with active site 1 residues i.e. His163, Glu166, and Gln189. However, it also formed three non-conventional bonds with active site residues i.e. Asn142, Cys145, and Pro168 to form a stable complex. Withanone showed drug-like property (0.45), obey the rule of five (MW < 500, HBD < 5, HBA < 10, and LogP < 5) and characterized under small molecules.

5. Conclusion

Glycosylated spike glycoprotein on the surface of SARS-CoV-2 is a determinant for viral invasion and host immune response and main protease 3CLpro controls the activities of the coronavirus replication complex. In the current study, Withanone is predicted to interfere with glycosylation residue Asn343 of the SARS-CoV-2 spike glycoprotein and main protease 3CLpro active site 1 amino acid residues. Hence, Withanone could be a potent non-toxic lead molecule to suppress the progression of COVID-19 infection via inhibiting the function of spike glycoprotein and main protease 3CLpro and weigh the benefit-risk balance during therapy and beneficial for the patient with diabetes and hypertension. Our findings call for *in vitro* and *in vivo* studies for laboratory validation.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.chmed.2021.06.002.

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