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Computational studies evidenced the potential of steroidal lactone to disrupt surface interaction of SARS-CoV-2 spike protein and hACE2

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ABSTRACT

The critical event in severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pathogenesis is recognition of host cells by the virus, which is facilitated by protein-protein interaction (PPI) of viral Spike-Receptor Binding Domain (S-RBD) and Human Angiotensin Converting Enzyme 2-Receptor (hACE2-R). Thus, disrupting the interaction between S-RBD and hACE2-R is widely accepted as a primary strategy for managing COVID-19. The purpose of this study is to assess the ability of three steroidal lactones (SL) (4-Dehydrowithaferin A, Withaferin A, and Withalongolide A) derived from plants to disrupt the PPI of S-RBD and hACE2-R under two conditions (CON–I and CON–II) using in-silico methods. Under CON–I, 4-Dehydrowithaferin A destabilizing the interactions between S-RBD and hACE2-R, as indicated by an increased binding energy (BE) from −1028.5 kJ/mol (control) to −896.12 kJ/mol 4-Dehydrowithaferin A exhibited a strong interaction with S-RBD GLY496 with a hydrogen bond occupancy (HBO) of 37.33%. Under CON–II, Withalongolide A was capable of disrupting all types of PPI, as evidenced by an increased BE from −913 kJ/mol (control) to −133.69 kJ/mol and an increased distance (>3.55 nm) between selected AAR combinations of S-RBD and hACE2-R. Withalongolide A formed a hydrogen bond with TYR453 (97%, HBO) of S-RBD, which is required for interaction with hACE2-R’s HIS34. Our studies demonstrated that SL molecules have the potential to disrupt the S-RBD and hACE2-R interaction, thereby preventing SARS-CoV-2 from recognizing host cells. The SL molecules can be considered for additional in-vitro and in-vivo studies with this research evidence.

1. Introduction

Coronaviruses (CoV; order Nidovirales, family Coronaviridae, subfamily Coronavirinae) have been implicated in transmitting infectious diseases in both humans and animals [1]. However, a global outbreak of a respiratory illness [Coronavirus disease (COVID-19)] caused by newly discovered coronavirus variants, SARS-CoV-2, threatened human existence by claiming 54.76 lakhs lives until January 5, 2022 [2]. The WHO designated Alpha, Beta, Gamma, Delta, and Omicron as SARS-CoV-2 variants of concern [3].

The genetic material of SARS-CoV-2 is a positive-sense single-stranded RNA virus (approximately 30,000 bases) that codes for both structural (spike, nucleocapsid, membrane, and envelope) and non-structural (proteases and RNA dependent RNA polymerases) proteins [4,5]. Researchers worldwide have delineated the molecular mechanism of SARS-CoV-2 pathogenesis using a polyphasic approach. Crown-like spikes cover the virus’s outer surface, composed of heterotrimeric transmembrane glycoproteins (S glycoprotein), which is critical for early detection and facilitates viral entry into the host cell. The hACE2-R attached to the host cell’s outer surface serves as the actual viral recognition site, facilitating the interaction of the SARS-CoV-2 S-RBD and thus initiating the pathogenesis process [6].

The increased virulence of SARS-CoV-2 over SARS-CoV can be attributed to its increased affinity for hACE2-R due to variation in the amino acid sequence of S-RBD [7–10]. Several attempts have been made to block the S-RBD using small molecules, peptides, ACE2 fractions, convalescent serum from individuals recovered from COVID-19 infection, and polyclonal/monoclonal antibodies, among others [11,12] in order to decrease the affinity between S-RBD and hACE2-R. However, success with small molecules is limited due to the involvement of large interacting surface between S-RBD and hACE2-R in PPI. Additionally, the increased transmissibility and virulence of the SARS-CoV-2 variants of concern, namely Alpha, Beta, Gamma, Delta, and Omicron, are primarily due to a favourable spike protein mutation exacerbates the

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situation. As a result, it is assumed that these variants will overcome the effect of currently available spike protein blocking vaccines or peptides.

With the rapid global spread of SARS-CoV-2, numerous antiviral drugs and therapies became available to alleviate the additional burden of COVID-19. Remdesivir (inhibitor of viral RNA-dependent RNA polymerase) was the only drug approved by US Food and Drug Administration for COVID-19 treatment [13]. Monoclonal antibodies (REGEN-COV, Bamlanivimab, Tocilizumab, and Etsevimab), convalescent plasma therapy, renal replacement therapies, and immune modulators are reported as effective [14]. Nevertheless, following the successful development of vaccines (Covishield, Covaxin, COVOXAV, mRNA-1273, Sputnik V, Ad26.COV2.S, and others) and the implementation of a global vaccination program, morbidity and mortality decreased significantly [15]. However, the threat remains, as the new SARS-CoV-2 “Omicron” variant of concern (B.1.1.529) spreads faster than the wild type and other variants.

Due to the diversity of secondary metabolites at the structural and functional levels, plants are considered a critical source of drugs. Natural herbs have recently gained increased importance as potential antiviral agents due to their minimal adverse effects [16–19]. In 12 randomized controlled trials, traditional Chinese medicine demonstrated significant improvements in clinical symptoms while lowering COVID-19 mortality and recurrence rate [20,21]. Similarly, other clinical trials conducted on improvements in clinical symptoms while lowering COVID-19 mortality.

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### 2. Materials and methods

#### 2.1. Preparation of ligands and proteins

3D structure data file (SDF files) and canonical SMILES of three SL molecules (4-Dehydrowithaferin A (PubChem Id: 165541), Withaferin A (PubChem Id: 265237) and, Withalongolide A (PubChem Id: 56649343)) were retrieved from the PubChem database (https://pubchem.ncbi.nlm.nih.gov). The crystal structure of SARS-CoV-2 S-RBD bound to hACE2-R (PDB ID: 6M0J) (2.45 Å) was downloaded from Research Collaborator for Structural Bioinformatics (RCSB) Protein Data Bank (PDB) (RCSB, http://www.rcsb.org). CHIMERA 1.13.1 [47] was used to clean the protein complex, and stereo-chemical quality checks and energy minimizations were done using a Swiss-Pdb viewer to get the optimal 3D structure [48].

#### 2.2. Molecular dynamics simulations (MDS)

Ensemble docking (8 clusters) [49] was performed against S-RBD, and the best pose with least BE was chosen for MDS (Sup. Table 1). Molecular dynamic simulation studies were used to determine the SL’s PPI disruption strength, as well as protein’s stability and flexibility in the presence/absence of SL. Two conditions were used to subject the three SL molecules to all-atom MDS. The crystal structure PPI between S-RBD and hACE2-R was retained in CON-I, and the test molecule was introduced based on the docking pose with the least BE on S-RBD. The molecules ability to disrupt previously established AAR interactions between S-RBD and hACE2-R was assessed. Under CON-II, the distance between S-RBD and hACE2-R was increased using CHIMERA 1.13.1 tools, and the test molecule was introduced based on the docking pose that contained least BE on S-RBD.

Additionally, the complex was allowed to interact with hACE2-R. The MDS analysis was carried out using the latest Gromacs-5.0.7 suite [50–52]. The CHARMM36 all-atom force field was chosen for protein [53]. To obtain ligand topology, the Swiss-PARM modelling web server was used [54].

In the absence and presence of ligand, the prepared S-RBD and hACE2-R complex were solvated in TIP3P water model in a cubic box with 1.2 nm distance between the protein surface and the box boundary [55]. Energy minimization was done by the steepest descent minimization algorithm in the aqueous phase. Verlet cutoff scheme [56] and Particle mesh Ewald (PME) were incorporated to control the non-bonded and the long-range electrostatic interaction [50,57].

Equilibration was achieved in two stages: NVT first, then NPT. The LINCS algorithm was incorporated to retain all H-bond constraints [58,59]. System pressure 1 bar and temperature 300K were regulated using the Parrinello-Rahman pressure coupling method and Berendsen temperature coupling, respectively [60,61]. All the MDS was carried for the 50 ns in triplicates, and trajectory was saved at every two fs using the Parrinello-Rahman pressure coupling method and Berendsen temperature coupling, respectively [60,61]. All the MDS was carried for the 50 ns in triplicates, and trajectory was saved at every two fs using.
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High-Performance Computing Facility (HPC), Indian Institute of Technology Delhi, India. The workflow of MDS and data analysis is provided as Sup. Fig. 1.

2.3. Free energy of binding calculation (MMPBSA)

The free BE of the S-RBD and hACE2-R complex in the absence and presence of ligands under CON-I and CON-II was calculated by using the g.mmpbsa toolkit [62,63]. The last 10 ns converged snapshots at every 100 ps were incorporated to analyze binding free energy, polar solvation energy, van der Waals energy, Solvent accessible surface area (SASA) energy, and electrostatic energy.

2.4. RMSD, RMSF, Rg, SASA, and HBN

Using g_rms tool (least-square fit method), the root means square deviation (RMSD) of the protein backbone was calculated. The root mean square fluctuation (RMSF) of the amino acid residues was calculated by using g_rmsf tool. The radius of gyration (Rg) and total SASA were conducted for 50 ns under two conditions. The trajectories were examined using VMD tools [65] and it was determined that none of the molecules found in many Solanaceae plants. Withaferin A is the first member of the ergostane type withanolides. 4-Dehydrowithaferin A and Withanolide A are derivatives of Withaferin A where oxidation at C-4 and C-27 positions leads to the formation of 4-Dehydrowithaferin A, and addition of hydroxyl group at 19 position leads to Withanolide A (Sup. Fig. 2) [68]. Withanolides, the active ingredients of W. somnifera, have displayed promising results in managing COVID-19. The significant biological action of W. somnifera is rendered by Withanolide D, Withaferin-A, Withanoside X, and Withanoside I-VII [69]. Withaferin A has been reported to have anticanicancer, angiogenesis inhibitor, anti-inflammatory, antistress, immunomodulatory, antioxidant and anti-ageing properties [70,71]. Whereas 4-Dehydrowithaferin A and Withanolide A have primarily been investigated for their anticanicancer activity [66,72,73]. The antiviral activity of 4-Dehydrowithaferin A and Withanolide A, on the other hand, has not been evaluated.

Computational studies can be used to identify protein-protein interaction disrupting the small molecules by performing MDS and monitoring the position of S-RBD and hACE2-R by measuring the distance between selected amino acids, analyzing the H-bond and other types of interactions, and free BE calculations. As previously stated, MDS were conducted for 50 ns under two conditions. The trajectories were examined using VMD tools [65] and it was determined that none of the molecule was expelled from the protein-protein interface during the simulation period.

Under CON-I, the equilibrium reached in 10 ns without ligand and was maintained throughout the simulation period (Fig. 2). Under the same conditions, it is expected that when a ligand is introduced between two proteins with previously established AAR interactions between AAR of S-RBD and hACE2-R and form new interactions with either/both proteins. As a result, more deviations in the RMSD values were observed in the presence of ligands (Fig. 2). However, initially, both the proteins were physically separated under CON-II to facilitate the ligand to interact with S-RBD protein in the early simulation period. Initially, the control reactions showed greater fluctuations in RMSD values up to 10 ns. Following that, the proteins achieved equilibrium and remained so throughout the simulation period (Fig. 2). The average RMSD values for control under CON-I and CON-II were 0.260 (nm) and 0.405 (nm), respectively (Sup. Table 3). Withaferin A reached equilibrium faster than control and was maintained for 50 ns under both test conditions, with an average RMSD value of 0.458 nm under CON-I and 0.320 nm under CON-II (Sup. Table 3). Whereas 4-Dehydrowithaferin A and Withanolide A induced significant conformational changes in S-RBD and hACE2-R, as evidenced by increased RMSD values; the same was maintained until the simulation ended without reaching equilibrium. In comparison to CON-I, CON-II resulted in a significant increase in RMSD. This RMSD pattern indicates that 4-Dehydrowithaferin A and Withanolide A create unfavourable conditions for the interaction of S-RBD and hACE2-R (Fig. 2; Sup. Table 3).
A similar trend was observed with respect to Rg values in control and SL molecules under CON–I, with average Rg values of 3.19 nm, 3.12 nm, 3.10 nm and 3.15 nm for control, 4-Dehydrowithaferin A, Withaferin A, and Withalongolide A, respectively (Fig. 2; Sup. Table 3). However, with Withalongolide A (average Rg 3.29 nm), increased Rg values and high fluctuation was observed under CON-II (Fig. 2; Sup. Table 3). During the simulation, these properties cause significant changes in the structural conformation of the protein.

Under CON–I, the SASA values (nm$^2$) ranged between 345 and 390 for control and tested protein-SL complex. The SASA values remained constant under CON-II for the first 20 ns before decreasing with control. The protein-Withalolongolide A complex increased to a value of >390 in the final 10 ns of the MDS (Fig. 2; Sup. Table 3). The root mean square fluctuation (RMSF) of AAR was analyzed to determine whether the ligands affect the dynamic behaviour of AAR of S-RBD and hACE2-R. The C1, C2, and C3 regions of the S-RBD (Fig. 1A) are primarily composed of loops and interact with hACE2-R. During the MDS, the high fluctuation in C1, C2, and C3 regions of S-RBD was also observed. Compared to CON–I, the RMSF values of AAR belonging to both proteins were higher under CON-II. S-RBD exhibited the highest RMSF in the presence of Withalolongolide A, indicating its instability. Whereas Withaferin A demonstrated a decreased RMSF in comparison to its respective controls, implying that it stabilizes S-RBD (Fig. 3).

3.1. Analysis of distance between selected pair of AAR between S-RBD and hACE2-R

The introduction of ligands under CON–I increased the distance between seven selected AAR combinations. Whereas, the distance between ASP405-ALA387 decreased in the presence of withaferin A and 4-Dehydrowithaferin A, indicating that the spike protein’s position may have been shifted, reducing the distance between this amino acid combination (Fig. 5A and B). Whereas, under CON-II, with Withalongolide A and 4-Dehydrowithaferin A, a clear separation of proteins was observed, as
evidenced by the increased distance between all AAR combinations studied. In both cases, the distance between the majority of amino acids studied remains unchanged (Fig. 5B). The distance between all the selected amino acid combinations was >3.55 nm and >1.18 nm in the presence of Withalongolide A and 4-Dehydrowithaferin A molecules, respectively, indicating that PPI via H-bond and other types of interactions is likely. The distance between these amino acid combinations was varied between 0.40 and 1.08 nm in control (Fig. 5B). The results obtained demonstrated that Withalongolide A and 4-Dehydrowithaferin A are capable of disrupting the PPI between S-RBD and hACE2-R.

3.2. Analysis of HBO and MMPBSA under CON

Throughout the simulation period, the AAR interactions (H-bonds) between S-RBD and hACE2-R were found to be consistent (Fig. 6A). Without the ligand, the total number of H-bond between S-RBD and hACE2-R varied between 2 and 17 (Average 8.57) over 50 ns simulation period (Sup. Fig. 3). During the final 10 ns of MDS, 11 AAR interactions between S-RBD and hACE2-R were observed in the form of an H-bond (Fig. 6A). TYR505-GLU37 had the highest HBO of 124%, followed by ASN487-TYR83, THR500-ASP355, LYS417-ASP30, and GLY502-LYS353 recorded >70% HBO (Fig. 6A). Whereas, in the presence of 4-Dehydrowithaferin A, Withaferin A, and Withalongolide A, the total number of H-bond (Average of 50 ns) between S-RBD and hACE2-R was reduced to 2.41, 4.98 and 2.78, respectively (Sup. Fig. 3). With all three test molecules, a slight bend in the spike protein position was observed as early as 10 ns in the presence of ligands. After 20 ns, the orientation of S-RBD shifted significantly and continued to do so until the end (Fig. 4B, C, 4D). When different simulations frames were analyzed, it was discovered that all the molecules were parallel to S-RBD. SL had ergostane framework facing the C1 and the lactone ring facing the C3 of S-RBD. While all three ligands appear to be visually disrupting the S-RBD and hACE2-R interactions, they were unable to dissociate the two proteins. To substantiate this, the BE was found to have increased from $-1028.5 \text{ kJ/mol (control)}$ to $-896.12 \text{ kJ/mol}$, $-845.26 \text{ kJ/mol}$ and $-1000.65 \text{ kJ/mol}$ in 4-Dehydrowithaferin A, Withaferin A and Withalongolide A, respectively (Table 1).

Total AAR interactions were reduced to six in the presence of Withalongolide A. Additionally, HBO of TYR505-GLU37 was reduced to 70%. HBO of LYS417-ASP30, GLY502-LYS353 and TYR453-HIS34, on the other hand, was found to be nearly equivalent to control (Fig. 6A). Six AAR interactions (ASN487-GLN24, ASN501-TYR41, ALA475-SER19,
THR500-TYR41, THR500-ASP355 and ASN487-TYR83) which are crucial in the formation of the S-RBD and hACE2-R complex, were completely disrupted (Fig. 6A). Additionally, Withalongolide A disrupts the interaction between S-RBD and hACE2-R by forming H-bond with TYR453 (10% HBO), GLN493 (13% HBO), SER494 (16% HBO) and TYR505 (9% HBO) of S-RBD and, LYS31 (11% HBO) and LYS353 (12% HBO) of hACE2-R (Fig. 6B). Total AAR interactions were reduced to six in the presence of 4-Dehydrowithaferin A. Five AAR interactions were completely disrupted by 0% HBO (TYR505-GLU37, THR500-ASP355, THR500-TYR41, GLYS502-LYS353, ASN501-TYR41 and ASN487-GLN29). However, a new interaction between TYR505-LYS553 with HBO >70% was observed, which was not observed in control (Fig. 6A). 4-Dehydrowithaferin A formed four H-bond with S-RBD [GLN493 (32% HBO), GLU484 (18% HBO), GLY493 (35% HBO), and SER494 (8% HBO)] and one with hACE2-R [LYS353 (10% HBO)]. Though these AAR did not appear in the H-bond pattern of control, by occupying them, 4-Dehydrowithaferin A may disrupt other adjacent AAR interactions (Fig. 6B), thereby decreasing the affinity of S-RBD for hACE2-R. Withaferin A was found to reduce the number of AAR interactions to six, with TYR505-GLU37, LYS417-ASP30 and GLYS502-LYS353 exhibiting HBO >70%. Withaferin A is also participates in the formation of H-bonds, interacting with five AAR of S-RBD and one AAR of hACE2-R. The highest of 92% and 70% HBO was observed with GLY496 and GLU464 of S-RBD, respectively (Fig. 6B).

3.3. Analysis of HBO and MMPBSA under CON-II

Under CON-II, in control, although both proteins were initially
separated, as the simulation progressed, the interaction between them became stronger due to an increased number of H-bond and other types of molecular interactions (Sup. Fig. 3; Fig. 4A). While the total number of H-bonds (average of 50 ns) between S-RBD and hACE2-R was reduced to 2.0, 3.66, and 1.78 in the presence of 4-Dehydrowithaferin A, Withaferin A and Withalongolide A, respectively, during the simulation period (Sup. Fig. 3). The PPI stabilized with 12 H-bonds near the end of the MDS (last 10 ns) (Fig. 6A). Nine of the restored H-bonds were comparable to the CON-I control (Fig. 6A). When different frames of simulations were analyzed, it was discovered that the alignment of Withalongolide A and 4-Dehydrowithaferin A molecule was parallel to S-RBD, with the ergostane framework was facing towards the C1 of S-RBD and lactone ring is facing towards C3 of S-RBD. At the same time, Withaferin A moved from its original position to C1 and also rotated itself 90°. Interestingly, Withaferin A was found to stabilize the PPI, which was evidenced by decreased free BE from $-913.66$ kJ/mol.
(control) to $-1116.55 \text{ kJ/mol}$ (Table 1). Further increase in free BE from $-913.66 \text{ kJ/mol}$ (control) to $-113.69 \text{ kJ/mol}$ and $-769.62 \text{ kJ/mol}$ with Withalongolide A and 4-Dehydrowithaferin, respectively, indicated their potential to reduce S-RBB and hACE2-R affinity. Up to 30 ns, Withalongolide A renders the protein-protein complex highly unstable. Following that, the two proteins were physically separated. Throughout the simulation period, Withalongolide A was found to be strongly bound to the S-RDB, preventing its reunion with hACE2-R (Fig. 4D; Sup. Video 1). Additionally, Withalongolide A formed H-bonds with TYR453 (97% HBO), TYR495 (8% HBO) and GLN493 (8% HBO) of S-RBD. TYR453 of S-RBD is required for binding to HIS34 of hACE2-R, by blocking this AAR; Withalongolide A decreased S-RBD’s efficiency, preventing it from attaching to hACE2-R.

Similarly, 4-Dehydrowithaferin A was discovered to destabilize the...
S-RBD and hACE2-R complex, as indicated by S-RBD shifting throughout the simulation. It was immediately apparent that the interaction between S-RBD and hACE2-R had been disrupted. However, both the proteins remained attached via a variety of AAR interactions that may not be involved in normal S-RBD and hACE2-R interactions. Only two H-bonds were observed here, with an HBO percentage of 57% (ALA475-GLN24) and 9% (LYS417-HIS34) (Fig. 6A). Additionally, 4-Dehydrowithaferin A formed two H-bonds [LYS417 (18% HBO) and ARG403 (13% HBO)] with S-RBD and one [LYS31 (23% HBO)] H-bond with hACE2-R (Fig. 6B). Withaferin A was the least capable of disrupting S-RBD and hACE2-R interactions of the three SL studied. Five H-bonds were identified here, with GLY502-LYS353, THR500-ASP355 and TYR505-GLU37 exhibiting >80% HBO (Fig. 6A). Withaferin A itself formed an H-bond with TYR473 (6% HBO) of S-RBD and GLN76 (10% HBO) of hACE2-R, respectively (Fig. 6B).

Under both CON-I and CON-II, the interaction of ligand with protein

![Fig. 4C. Snapshots of MDS at the intervals of 10 ns in the presence of Withaferin A under CON-I and CON-II. In each frame to the left, S-RBD (magenta color) and hACE2-R (cyan color) and their interacting positions are shown. Close up of protein-protein or protein-ligand interactions are shown on the right side of each frame. Amino acid residues involved in hydrogen bond formation are represented as sticks. Hydrogen bonds are represented as dashed lines in black color, and all other interactions are represented as yellow dash lines.](image)
resulted in the formation of primarily H-bonds between the oxygen and hydroxyl groups on the cyclohexane A ring on the ergostane frame and the unsaturated lactone ring. Though all three selected SL molecules are structurally similar (Sup. Fig. 2), the minor difference in the oxygen atom and hydroxyl group distribution on the cyclohexane A and B rings appears to play a role in their differential ability to interact with S-RBD and hACE2-R. The current study demonstrated the ability of two PSM (Withalongolide A and 4-Dehydrowithaferin A) to disrupt S-RBD and hACE2-R interactions, thereby preventing a virus’s early recognition of host cell.

Additionally, the three SL studies met Lipinski’s rule of drug-likeness, with a bioavailability score of 0.55. All SL exhibited low water solubility high intestinal absorption, and could not cross the blood-brain barrier (BBB). While SLs were identified as a p-glycoprotein I and II inhibitors and were negative for CYP1A2, CYP2C19, CYP2C9, CYP2D6 and CYP3AY inhibition. Only Withalongolide A was found to be
In a similar attempt, Garcia et al. [11] reported the PPI disrupting the ability of plicamycin in SARS-CoV-2 pathogenesis. The distance between selected protein points increased from 44 Å to 49 Å in the presence of plicamycin. Additionally, the destabilization of the PPI was demonstrated by a decrease in the free energy profile from 3 kcal/mol (native complex) to 2.1 kcal/mol (plicamycin complex) and a decrease in the number of H-bond formations between the proteins. However, several small molecules have previously been shown to disrupt the interaction between S-RBD and hACE2-R. They were limited to MD, and their capabilities were not evaluated through MDS. Our approach of MDS in this study instilled more confidence in the SL, allowing them to be considered for additional in-vitro and in-vivo studies. Balakrishna et al. [38] conducted a similar computational study and discovered that SL (Withanone) molecules have the potential to disrupt the S-RBD and hACE2-R interaction. Further, an in-vitro study found extracts rich in withanone from Withania somnifera, reduced the human-like pathological responses induced in humanized zebrafish by SARS-CoV-2 recombinant spike protein. In a similar attempt, DYGAVENTK, a peptide derived from fruit bromelain, was observed to inhibit the interaction between S-RBD derived from different variants of SARS-CoV-2 and hACE2. The peptide interacted with the critical amino acid involved in the attachment of S-RBD and hACE2. The peptide interacted with the critical amino acid involved in the attachment of S-RBD and hACE2. The peptide interacted with the critical amino acid involved in the attachment of S-RBD and hACE2.
from $-779.8$ kcal/mol (control) to $-677.6$ kcal/mol. 4-Dehydrowithaferin A destabilized the PPI under CON–I. While Withalongolide A completely separates two proteins under CON-II, and ligand remains attached to the S-RBD throughout the simulation. 4-Dehydrowithaferin A and Withalongolide A exhibit strong interactions with S-RBD, indicating that they have a negligible effect on the structural integrity and function of the host protein – hACE2. Also, the present study established for the first time, to our knowledge and that of the literature, the ability of SLs to disrupt PPI of S-RBD-hACE2-R complex via MDS. As a result, these molecules can be further investigated to their full potential through in-vitro, in-vivo, and clinical studies. Due to the fact that these molecules are abundant in Withania somnifera, a well-known medicinal plant used in the Indian Ayurveda system, they can be introduced into the medicinal system with minimal effort to manage COVID-19. While our study focused on wild type SARS-CoV-2, the same approach could be used to screen the aforementioned molecules or secondary metabolites from other sources against emerging variants with increased transmissibility and virulence.

Author contributions

Ajay Yadav and Monu Dinesh Ojha-investigation, writing, review, validation, visualization, data curation.

Prof. Hariprasad P.-conceptualization, resources, supervision, review, data curation.

Declaration of competing interest

All authors declares no conflict of interest.

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Appendix A. Supplementary data

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