

In silico elucidation of plausible anti-obesity activity by Withaferin-A compound targeting alpha-amylase

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Abstract. – OBJECTIVE: The study aimed to evaluate the Withaferin-A against the drug target α -amylase, revealing its plausible mode of action and molecular-level interactions essential for this specific target inhibitory potential computational approach.

MATERIALS AND METHODS: In this scenario, we used computational methods, including docking, molecular dynamics simulation, and model-building simulations, to elucidate the atomic-level details responsible for the inhibitory potential of Withaferin-A derived from *W. somnifera*. The studio visualizer software was used for the visualization of ligands, structures of the receptor, bond length, and rendering of the image. Absorption, distribution, metabolism, excretion, and toxicity (ADMET) characteristics of phytochemicals were investigated. Crystal structure of protein receptors and ligands were generated. Semi-flexible docking was done using Autodock software. Docking was performed using the Lamarckian Genetic Algorithm (LGA). Molecular descriptors were evaluated, and the pharmacological properties of the phytochemicals were explored. Molecular dynamic simulations were analyzed at the atomic level. All the simulations were conducted under the same temperature, pressure, and volume circumstances over the simulated time scale.

RESULTS: Withaferin-A has shown a strong binding affinity towards α -amylase as demon-

strated with -9.79 Kcal/mol with 66.61 estimated nanomolecular IC₅₀ value for plausible anti-obesity activity. Molecular-level relationships and knowledge obtained from this study indicate solid interactions with TYR59, ASP197, and HIS299 residues which are of high importance for future works related to computational screening of target-specific α -amylase inhibitors. The results from the analysis have revealed potential molecular-level interactions useful for further designing/discovering novel α -amylase inhibitors.

CONCLUSIONS: The framework of the studied phytochemicals enables the rapid development of subsequent modifications that could result in more lead-like compounds with better inhibitory efficacy and selectivity for α -amylase.

Key Words:

α -amylase inhibitors, Anti-obesity, Docking, Molecular modeling, Molecular dynamic simulations, Withaferin-A, *Withania somnifera*.

Introduction

Obesity is a metabolic condition characterized by excessive fat accumulation in fatty tissues¹. α -Amylase is one of the key enzymes that catalyze carbohydrates' breakdown into monosaccharides

via hydrolysis of α -(1,4)-D-glycosidic linkages^{2,3}. This study deals with the identification of novel therapeutic options for the management of obesity. Ashwagandha (*W. somnifera*), belonging to the family *Solanaceae*, is an Ayurvedic herb demonstrating various potential medicinal properties in ayurvedic medicine⁴. Extracts from *W. somnifera* were shown to be having antibiotic⁵, immunomodulatory⁶, anti-inflammatory⁷, anti-ageing⁸, anti-stress/adaptogenic⁹, musculotropic¹⁰, anti-convulsant¹¹, neuropharmacological¹², anti-oxidant¹³, anti-hyperglycemic¹⁴, hepatoprotective¹⁵, macrophage-activating¹⁶, antitumour⁶ effects.

Several phytochemicals such as steroidal lactones (withaferins, withanolides), alkaloids (isopelletierine, anaferine), saponins (sitoindoside VII and VIII), and withanolides (sitoindoside IX and X) were identified from this herb. Multiple reports^{17,18} recently demonstrated the potential anti-obesity activity of *W. somnifera* plant extracts, especially concerning the Withaferin-A compound. In this scenario, this study aims to evaluate the Withaferin-A, as mentioned earlier, against the α -amylase drug target, revealing its plausible mode of action and molecular-level interactions, essential for this specific target inhibitory potential computational approach¹⁸.

Materials and Methods

Computational Methods

Software and program

The discovery studio visualizer (Studio, 2008) was utilized to visualize ligand and receptor structures, H-bonding networks, bond length calculations, and image renderings. To get the phytochemical structure, we looked it up in PubChem¹⁹. For semi-flexible docking, this research primarily uses Autodock 4.0²⁰ (available at: <https://autodock.scripps.edu>). For this study, we used Auto-Dock Tools version 1.5.6 to produce a pdbqt file containing the ligands and their associated protein receptors and to determine the size of the grid box. The ADMET characteristics of phytochemicals were investigated using the Molinspiration, Orisis property explorer tool^{21,22} (available at: <https://www.molinspiration.com>). Schrodinger's Maestro v. 9.5 (available at: <https://www.schrodinger.com>) was utilized to visualize the screened compounds' pharmacophore features and molecular dynamics simulation results.

Preparation of Protein Receptor and Ligand

Data from the Protein Data Bank (PDB) were used to generate the crystal structure of amylase (PDB: 4W93)²⁰. Many atoms in the crystal structure were absent, but the repair instructions module in Autodock replaced them. In preparation for docking, we cleaned the protein crystal structure by eliminating the water molecules. H-atoms were added to the target proteins to keep the ionization and tautomerization states of the amino acid residues unaltered. The resulting redesigned structure was employed to perform the semi-flexible dockings. To minimize the energy of the ligand molecule and its receptors in the Accelrys Discovery Studio's Steepest Descent and Conjugate Gradient procedures were used. The CHARMM (available at: <https://www.charmm.org/archive/charmm/resources/charmm-force-fields/>) force field²³ was used in the minimization procedures.

Semi-Flexible Docking

Phytochemical binding posture and associated energy prediction with the therapeutic target amylase are predicted using Autodock Version 4.0, which was also used for docking studies, to allow the prediction of the binding pose, IC₅₀ values, and binding energies. To summarize, default atomic solvation settings were used, and the energy scoring grid box was set to 90, 90, and 90 (x, y, and z), with the center at X= -3.16, Y= 5.98, and Z= -18.98. The amylase was placed in the middle of a three-dimensional grid box. Docking was performed using the Lamarckian Genetic Algorithm (LGA)²⁰, with all defaults left. The best docking solution for each docked complex is provided by Autodock, based on cluster analysis, once each LGA run is completed. Dispersion/repulsion, electrostatic interactions, hydrogen bonding, divergence from covalent geometry, desolvation effects, and internal ligand torsion limitations are the six energy components used to determine the binding Gibbs free energy (G). Using LGA cluster analysis, we compared the energies of 10 possible docking modes and picked the one with the lowest energy to be used in our simulations. Each substance could contain rotatable active linkages to increase its adaptability¹⁷.

Pharmacological Properties of the Phytochemicals

To ensure the pharmacological properties of the therapeutic candidates, we deployed an internet server in conjunction with data warrior

software²¹(Allschwil, Switzerland). We evaluated the molecular descriptors, the logarithm of P, the number of H-bond acceptors, the number of H-bond donors, and the molecular mass of phytochemicals in conjunction with the compounds' toxicological profile.

Molecular Dynamic (MD) Simulations

Using Desmond's (Schrödinger, Inc. New York, USA) default protocol of the OPLS 2005 force field²⁴, molecular dynamic simulations were run to understand the binding interactions at the molecular level and analyze those interactions at the atomic level. TIP3P water model simulations have been conducted with these parameters²⁵ at neutral pH conditions. Using periodic boundary conditions, we could pinpoint the exact dimensions and contours of the water box, which was buffered at ten distances. The volume of the simulation's box was determined to be 505,000 cubics. Long-range electrostatic interactions were estimated using the Particle Mesh Ewald method²⁶, while Van der Waals and short-range electrostatic interactions were capped at nine during the equilibration process. We employed a RESPA integrator²⁷ for long-range electrostatics with a 2-fs time step at 6-fs intervals. Approximately 50,438 atoms were used in the apo condition, and 50,494 atoms were pre-set in the Withaferin-A complex condition by the simulation system. The Nose-Hoover chain relaxation thermostat method and the Martyna-Tobias-Klein relaxation barostat methodology with isotropic coupling style were used to bring Schrodinger's Desmond to equilibrium at 300 K, the isothermal-isobaric (NTP) ensemble tempera-

ture, and 1 bar on one pico-seconds (ps) and two ps time scales, respectively. To ensure consistency in the results, we ran all simulations under the same temperature, pressure, and volume circumstances over the simulated time scale. According to the simulation quality research, the average total energy of the simulated systems remained close to -127,500 Kcal/mol for apo and complexed with Withaferin-A.

Results

Molecular Docking of the Withaferin-A from *W. Somnifera* with α -Amylase

We have carried out the molecular docking for the Withaferin-A (Figure 1) with the α -amylase, the chemical interactions, and binding energies relevant to this target's inhibitory effect. Molecular docking has been performed targeting α -amylase binding site core residues. As per the molecular docking experiment, the Withaferin-A compound showed binding energy of -9.79 Kcal/mol with 66.61 nanomolar of predicted IC₅₀.

Prediction of Pharmacological Properties

We used Osiris Property Explorer to estimate the phytochemicals' pharmacological properties by Lipinski's Rule of Five²⁸ and Oral Bioavailability. Table I displays the expected pharmacological properties of the compound. Lipinski's Five-Parameter Rule was applied to the investigated Withaferin-A, which followed the predicted values.

Table I. Predicted ADME and toxicology profile of the Withaferin-A compound from *W. somnifera*.

Attribute	Withaferin-A
Molecular Formula	C28H38O6
Molecular Weight	470.6
H-Donor	2
H-Acceptor	6
No. of rotatable bonds	3
TPSA	96.36
LogP	2.49
Toxicology profile	
Mutagenic	None
Tumorigenic	None
Reproductive effect	Low
Eye Irritant	None

TPSA-Total prostate-specific antigen. H-Acceptor: Hydrogen acceptor. H-Donor: Hydrogen donor.

MD Simulations Events Analysis of α -Amylase in its Complex with Withaferin-A Compound

To further understand and validate the molecular level interactions, binding ability, and influence of Withaferin-A in complex with α -amylase, we have performed 100 nanoseconds of molecular dynamic simulations. Protein RMSD (root mean square deviation) and RMSD of the ligand and individuals' root means square fluctuations (RMSF) residues were initially analyzed to understand the impact of compound binding on the protein conformation changes (Figure 2a-c).

To further validate the Withaferin-A compound's ability to inhibit the α -amylase, we have analyzed its energy change throughout the simulated timescale. The analysis revealed that the

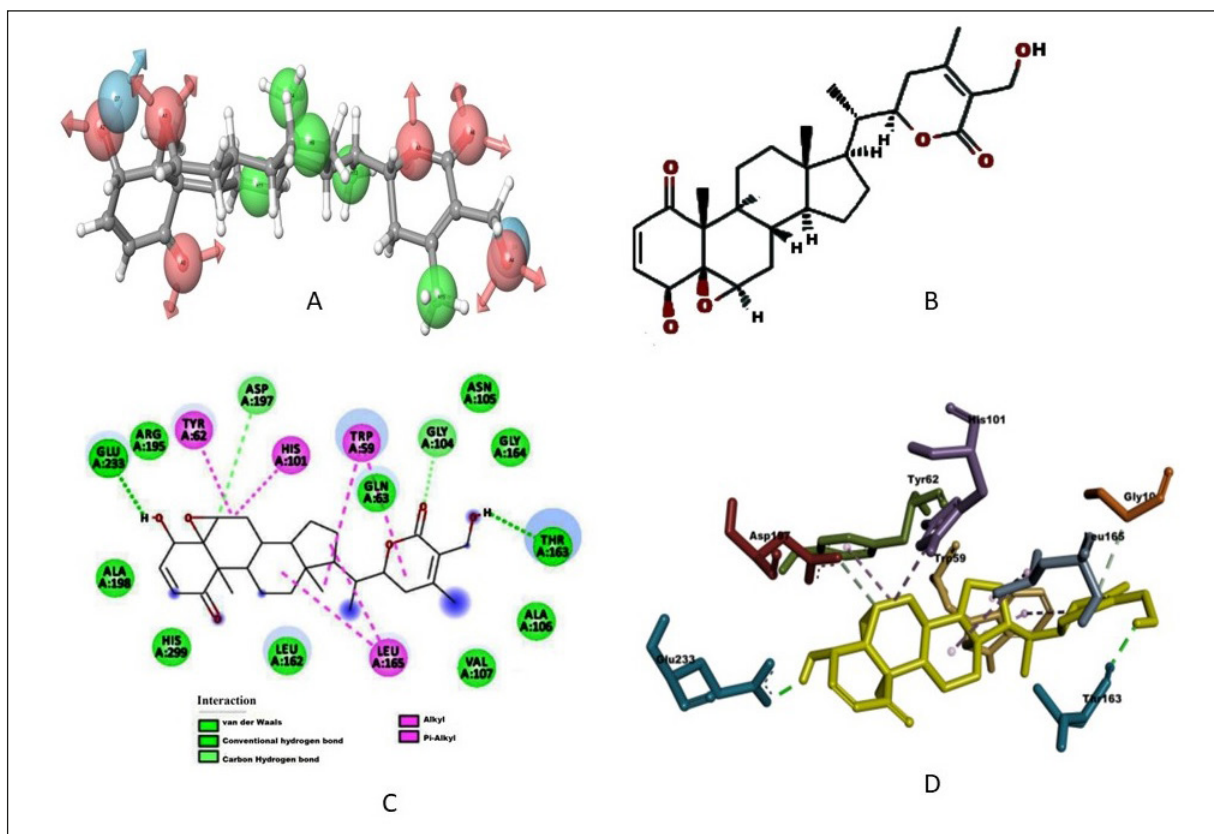


Figure 1. A, 2D structural representation of the Withaferin-A (compound ID: 265237) from *W. somnifera* used in this study. B, Pharmacophore features of the Withaferin-A compound. Red color spheres represent hydrogen acceptors; Cyan color spheres represent hydrogen donors, and green spheres represent hydrophobic regions. C-D, Molecular interactions observed between the Withaferin-A compound with α -amylase from the docking analysis.

energy of amylase in complex with Withaferin-A held an average of approximately -11,250 Kcal/mol (Figure 3a). An independent analysis of Withaferin-A's energy level revealed that it was relatively stable at around -60 Kcal/mol (Figure 3b).

Molecular Interactions of Withaferin-A in Complex with α -Amylase Observed during Molecular Dynamic Simulations

Schrodinger's Desmond software was used to interpret molecular interactions of the α -amylase and Withaferin-A complex in detail. Thirty-two contacts between Withaferin-A and α -amylase were found, out of which nine contacts were involved in hydrogen bonds, 16 contacts played a role in hydrophobic interactions, and 21 contacts in connections involving water bridging were observed, respectively. Details are shown in Figure 4, which shows the molecular interaction profile of Withaferin-A with α -amylase.

Discussion

Obesity is a metabolic condition that affects the body's major systems and is considered a root cause of many diseases and disorders²⁹⁻³¹. The molecular docking study revealed that the anti-obesity lead compound Withaferin-A compound showed binding energy of -9.79 Kcal/mol with 66.61 nanomolar of predicted IC_{50} . When the best confirmation docking study results of the Withaferin's constituents were analyzed, Withaferin-A formed conventional hydrogen bonds with GLU233 and THR163; carbon-hydrogen bonds with ASP197 and GLY104; pi-alkyl bonds with TYR62; HIS101; TRP59 and LEU165 residues of α -amylase. Other studies in literature also show that the molecular weight of Withaferin-A was 470.6 Da, and molecular docking analysis shows hydrogen bond interaction with amino acid residues Glu233 and Val235. Withanolide inhibitor recognition was also found^{26,28} with Gly237, Gln238, and Ser239 *via* hydrogen bonding.

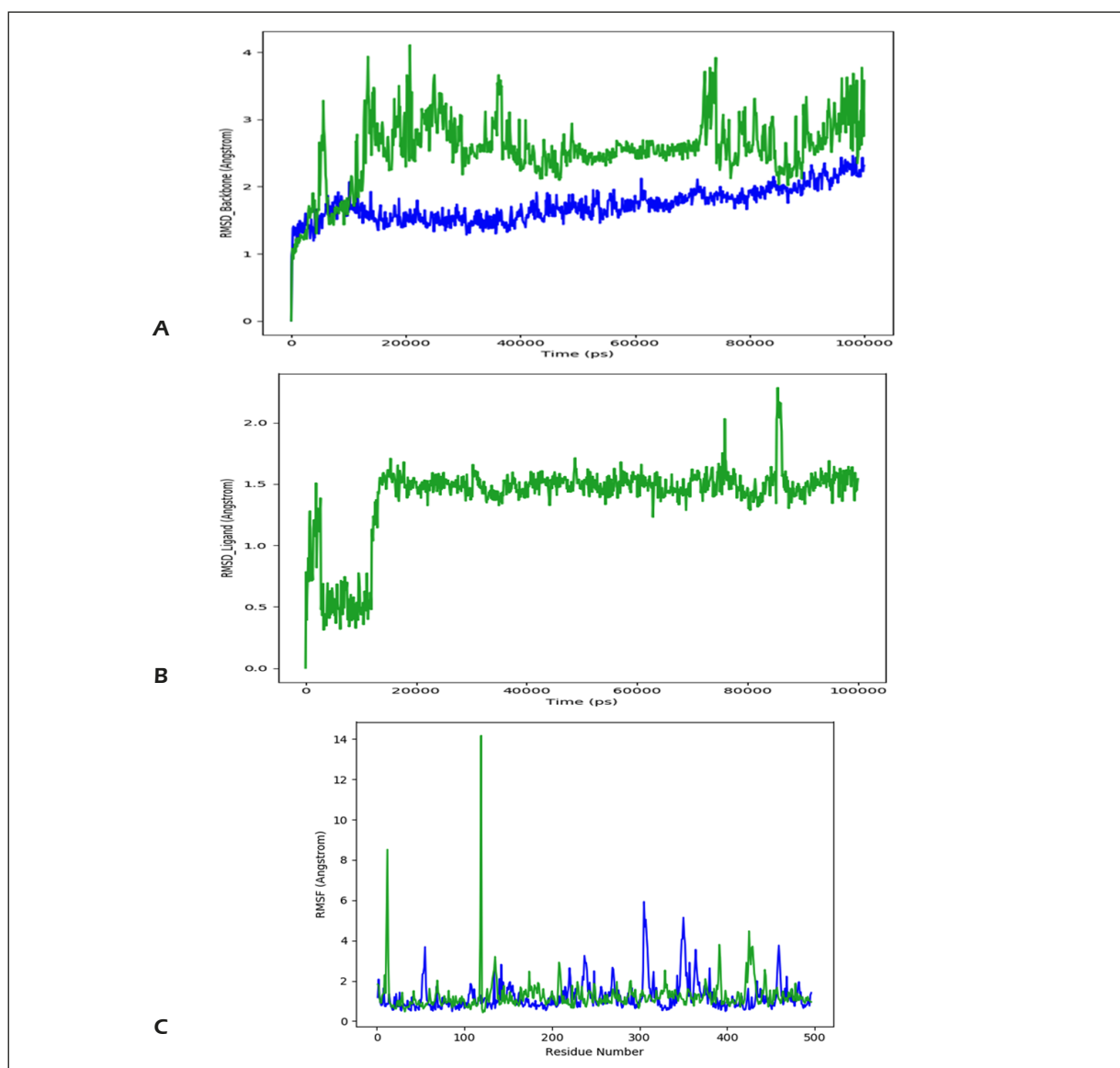


Figure 2. A-B, RMSD of the α -amylase backbone in its apo state and complex with Withaferin-A. C, RMSF of the apo state of the α -amylase backbone with Withaferin-A.

The phytochemicals' pharmacological properties were estimated by Lipinski's Rule of Five²⁸ and Oral Bioavailability. Lipinski's Five-Parameter Rule was applied to the investigated Withaferin-A, which followed the predicted values. Lipinski's rule parameters are the following:

- The maximum molecular weight allowed is 500 Da.
- The logP value and donor hydrogen count must be <5.
- There must be at most ten hydrogen acceptors.
- The molar range of refractivity must be between 40 and 130.

The Withaferin-A compound's toxicity analysis from *W. somnifera* is shown in the results section. High indicates a high tendency of toxicity; Low indicates mid-core, and none suggests a low toxic tendency. Table I shows that the Withaferin-A compound has no toxicity in terms of mutagenicity, tumorigenicity, and eye irritation. However, it may have a few common reproductive effects.

The bioavailability of oral cavity compounds can be quantified using Veber's rule³². Oral bioavailability was defined by low molecular weight (<500 Da), a small number of rotatable bonds (<10), and a small number of H-bond donors and acceptors (<12). The

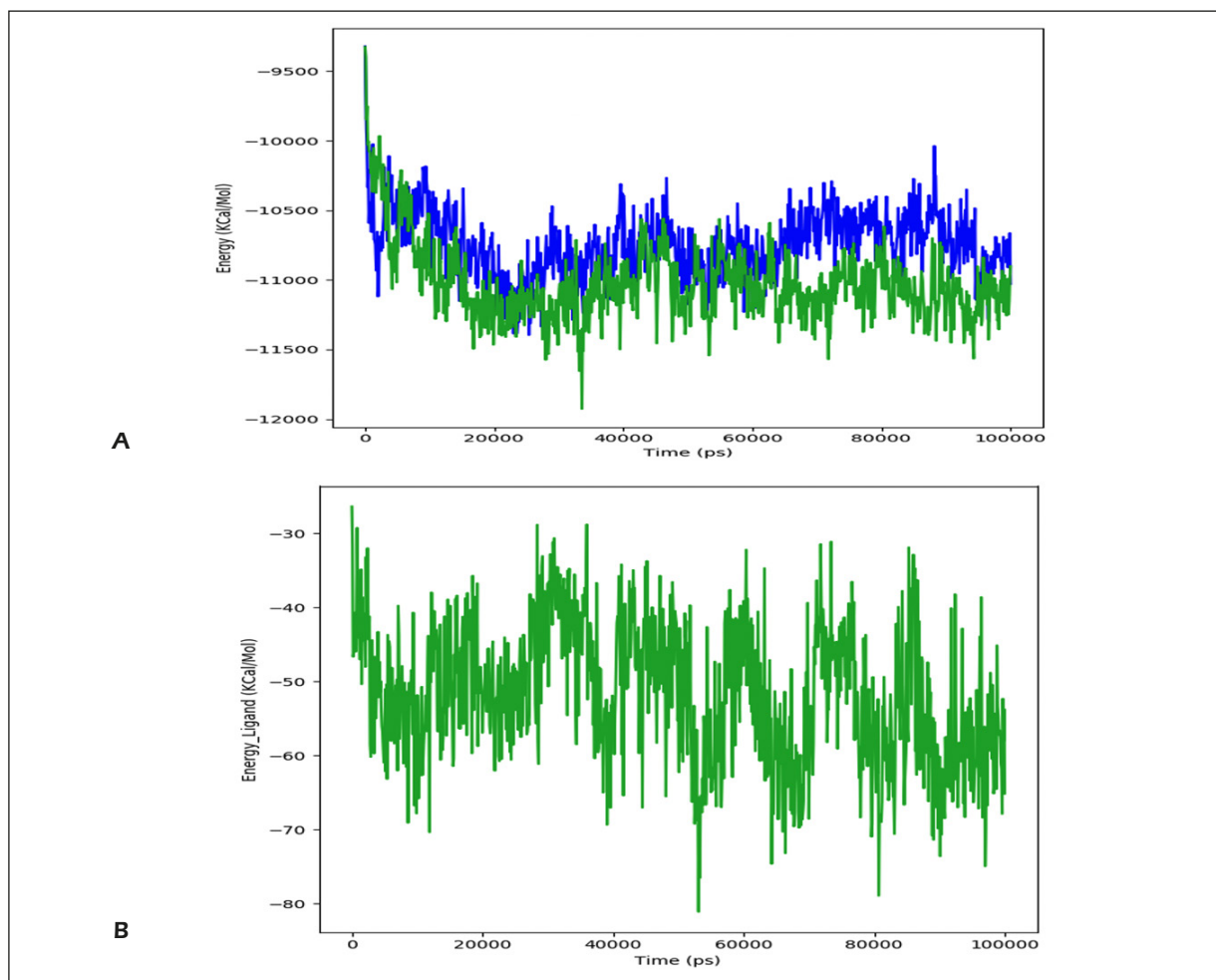


Figure 3. A-B, Calculated energy of Withaferin-A compounds inside the active binding site of α -amylase.

total polar surface area had to be <140. According to Table I, a Withaferin-A molecule has a favorable oral bioavailability. Oral bioavailability has been cited as suitable by other studies^{18,20,23-25} as well.

Based on the data obtained from MD simulations events analysis of α -amylase and its complex with Withaferin-A compound, we could know that the α -amylase backbone's RMSD was observed to be maintaining an approximate average of 3 Å up to 4 Å in complex with Withaferin-A. While the Withaferin-A compound RMSD, regarding its initial structure coordinates, was initially found to be 0.5 Å until the initial ten nanoseconds of simulated timescale and then stabilized at 1.5 Å average. When individual variations in residues were analyzed *via* RMSF, most residues active in apostate were much minimized when complexed with the Withaferin-A compound²⁶. It was also noticed that minimal fluctuations occurred comparatively, except for the starting ten residues and

around 110-115 residues when α -amylase is complexed with the Withaferin-A compound, indicating that the residues are involved in the structural re-arrangement of the protein to accommodate Withaferin-A. These observed movements in the RMSD backbone and at individual residual levels indicate that Withaferin-A has induced conformational changes in α -amylase at the residual level, leading to its inhibitory potential^{26,27}.

The inhibition of α -amylase by Withaferin-A was analyzed by its energy change during the timescale of stimulation. The analysis revealed that the energy of amylase in complex with Withaferin-A held an average of approximately -11,250 Kcal/mol. An independent analysis of Withaferin-A's energy level revealed that it was relatively stable at around -60 Kcal/mol. This much-minimized energy during the entire simulated timescale of about 100 ns is substantial proof that Withaferin-A has a high potential to stabilize its bind-

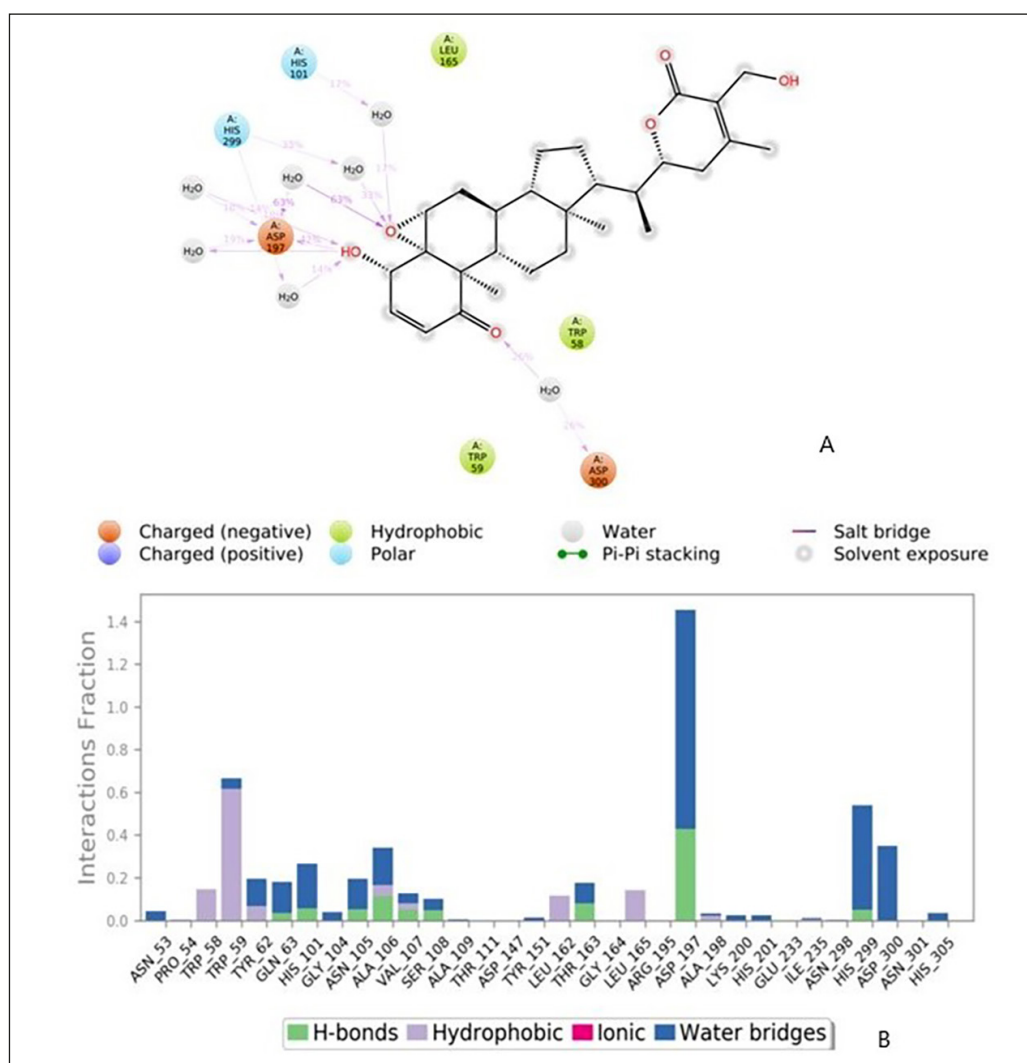


Figure 4. Molecular interactions observed between α -amylase residues in complex with Withaferin-A compound during the 100 ns MD simulation timescale.

ing at the active site of the α -amylase leading to its inhibitory potential. Similar conclusions have been made by past works^{22,24,27}.

In detail, the interpretation of molecular interactions of the α -amylase and Withaferin-A complex reveals Hydrogen bonds with GLN63; HIS101; ASN105; ALA106; VAL107; SER108; THR163; ASP197 and HIS299. Hydrophobic interactions with PRO54; TRP58; TRP59; TYR62; ALA106; VAL107; THR111; ASP147; LEU162; GLY164; LEU165; ARG195; ALA198; GLU233; ILE235 and ASN301. Water bridging interactions with ASN53; TYR62; GLN63; HIS101; GLY104; ASN105; ALA106; VAL107; SER108; ALA109; TYR151; THR163; ASP197; ALA198; LYS200; HIS201; ILE235; ASN298; HIS299; ASP300 and HIS305 were observed.

Throughout the simulated timescale, an average of 8 contacts was maintained from the above-mentioned interactions, among which TYR59, ASP197, and HIS299 were the top 3 most stable.

Conclusions

Our Molecular Dynamic simulations demonstrated the ability of *W. somnifera* phytochemical Withaferin-A as a valuable small ligand molecule against an α -amylase drug target. The Withaferin-A compound from *W. somnifera* has shown a strong binding affinity towards α -amylase as demonstrated with -9.79 Kcal/mol with 66.61 estimated nanomolecular IC₅₀ value for plausible anti-obesity activity. Some of the molecular interac-

tions found with residues subsume active catalytic sites, increasing their combination's thermodynamic stability and raising the significance of this compound's inhibitory potential. Molecular-level relationships and knowledge obtained from this study indicate solid interactions with TYR59, ASP197, and HIS299 residues which are of high value for future works related to computational screening of target-specific α -amylase inhibitors. The presently examined phytochemical has a potential ADMET drug-like profile. The Withaferin-A compound strongly supports the need to evaluate further this phytochemical's ability to control obesity by targeting α -amylase activity. The framework of the studied phytochemicals enables the rapid development of subsequent modifications that could result in more lead-like compounds with better inhibitory efficacy and selectivity for α -amylase.

Conflicts of Interest

The authors declare no conflicts of interest.

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Informed Consent

Not applicable.

Ethics Approval

Not applicable.

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