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Original Article

Computational Investigation of Withaferin A as a Potential Inhibitor of Epstein–Barr Virus Nuclear Antigen 1 (EBNA1): Molecular Docking and ADMET Analysis

Mohammed Torki Hammood

Department of Biotechnology, College of Science, University of Anbar, Ramadi, 31001, Iraq

Email: mohammed.torki@uoanbar.edu.iq

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Abstract

Background: Epstein–Barr virus (EBV) is an oncogenic herpesvirus associated with several human cancers. Epstein–Barr nuclear antigen 1 (EBNA1), essential for viral latency and episome maintenance, represents a promising therapeutic target. **Aim:** To evaluate the binding affinity and predicted pharmacokinetic properties of Withaferin A, a natural steroidal lactone from *Withania somnifera*, against EBV EBNA1 using molecular docking and ADMET analysis. **Methods:** Withaferin A was docked against EBNA1 (PDB ID: 6NPP) using SwissDock and compared with the reference ligand KWG. Protein–ligand interactions were analyzed using BIOVIA Discovery Studio and PyMOL, while ADMET-AI was used to predict pharmacokinetic and toxicity properties. **Results:** Withaferin A showed stronger predicted binding affinity toward EBNA1 than the reference ligand KWG, with a minimum binding energy of −6.866 kcal/mol and an average docking score of −5.193 ± 0.727 kcal/mol, compared with −5.494 kcal/mol and −4.465 ± 0.435 kcal/mol for KWG, respectively. Withaferin A formed multiple hydrophobic interactions with key EBNA1 residues, including Phe508, Tyr510, Leu539, Pro540, Met563, Thr596, Cys598, Phe600, and Val604. ADMET analysis indicated favorable drug-likeness, intestinal absorption, oral bioavailability, and safety. Despite moderate CYP3A4 and P-glycoprotein inhibition, Withaferin A showed lower predicted risks of liver injury (0.32 vs 0.97) and carcinogenicity (0.03 vs 0.38) than the reference ligand.

Conclusion: Withaferin A demonstrates promising computational activity against EBNA1, with favorable binding and ADMET properties, suggesting its potential as a lead compound for EBV-associated cancer therapy. Further molecular dynamics, *in vitro*, and *in vivo* studies are required for validation.

Keywords: Epstein–Barr virus; EBNA1; Withaferin A; *Withania somnifera*; Molecular docking; SwissDock.

1. Introduction

Epstein-Barr virus (EBV), or human herpesvirus type 4 (HHV-4), is an enveloped double-stranded DNA virus that is part of the family Herpesviridae. It is one of the most common viruses of humans, infecting more than 90% of the world's adult population. EBV infects B lymphocytes, and after primary infection, which is often asymptomatic, EBV remains latent for life. Viral infection has been linked to the pathogenesis of many malignancies such as nasopharyngeal carcinoma, Burkitt lymphoma, Hodgkin lymphoma, EBV-associated gastric carcinoma, and post-transplant lymphoproliferative disorders [1,2]. Most of the oncogenic properties of EBV are thought to stem from the expression of latent viral proteins that

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stimulate uncontrolled cell proliferation, block apoptosis, and help the infected cells escape host immune surveillance.

Epstein–Barr nuclear antigen 1 (EBNA1) plays a pivotal role in the maintenance of latent infection and the pathogenesis of EBV-associated malignancies. EBNA1 is a constitutively expressed protein in all known EBV-associated tumors, unlike other latent proteins, and is required for episomal genome replication, the faithful segregation of viral DNA during mitosis, and the regulation of the expression of latent viral genes. In addition to these basic viral activities, there is growing evidence that EBNA1 contributes to the instability of the host genome and the limitation of host immune recognition, enabling the transformation of cells to malignancy. The role of EBNA1 in the latent stage of infection has made it an ideal therapeutic target for EBV-associated cancers [3,4].

Existing antiviral therapies are not effective in curing EBV latent infection. Drugs like acyclovir and ganciclovir affect viral DNA synthesis mainly during the lytic phase of infection and are thus less effective against latent viral reservoirs, in which EBNA1 persistently continues to function unabated [5]. This therapeutic obstacle highlights the need for the identification of novel small molecules that are able to selectively inhibit EBNA1 and disrupt its biological function.

The enormous structural diversity and wide pharmacological activity of natural products have always been a treasure trove for the development of novel therapeutic agents. One of these bioactive molecules, Withaferin A, a naturally occurring steroidal lactone extracted from Withania somnifera (Ashwagandha), has been the subject of much scientific interest. Its various biological activities have been described in numerous studies, such as anticancer, antiviral, antioxidant, anti-inflammatory, and immunomodulatory activities. Mechanism-wise, Withaferin A can modulate various signaling pathways associated with cancer spreading, such as NF-κB, STAT3, PI3K/Akt/mTOR, heat shock protein, and oxidative stress pathways; hence, it can act as a multitarget therapeutic drug [6,7].

Thus, computer-aided drug design (CADD) has revolutionized the initial phases of pharmaceutical research, allowing for screening and optimization of candidate molecules with efficiency prior to laboratory testing. Molecular docking has gained immense popularity in recent years as a computational tool for estimating binding affinity between a ligand and a protein and determining the critical interactions between the two. In conjunction with structural visualization and computational pharmacokinetic analysis, docking can give a full picture of the drug-likeness, safety, and biological activity of identified therapeutic candidates, which allows for prioritization of those that are worth experimental study [8,9].

Hence, the present study aimed to investigate the inhibitory effects of Withaferin A on Epstein–Barr nuclear antigen 1 (EBNA1) by performing an in silico molecular docking approach. A highly binding co-crystallized ligand, KWG, was chosen as the reference compound for comparison of the binding performance. Additionally, the patterns of ligand-protein interaction were studied using BIOVIA Discovery Studio Visualizer, and the predicted pharmacokinetic properties and toxicity profiles of the screened compounds were explored using the ADMET-AI platform to assess their potential as therapeutic agents in the fight against EBV-associated diseases.

2. Methodology

2.1. Ligand Preparation

The naturally occurring phytochemical, withaferin A (PubChem CID: 265237), was chosen for investigation, while the co-crystallized ligand KWG (PubChem CID: 118078093) was used as the reference compound for comparison. Both forms of the ligands were obtained as 3D molecular structures in Structure Data File (SDF) format from the PubChem database. The structures downloaded were first imported into BIOVIA Discovery Studio Visualizer 2024 (Dassault Systèmes, San Diego, CA,

USA) for quality control and preprocessing of the ligands. The missing hydrogen atoms were added to the structural molecules, and the molecular geometry was carefully examined for structural integrity and suitability for further molecular docking simulations [10].

2.2. Protein Preparation

Epstein–Barr virus nuclear antigen 1 (EBNA1) was downloaded, along with its native inhibitor, from the Research Collaboratory for Structural Bioinformatics Protein Data Bank (RCSB PDB). Preparation of protein was done before molecular docking in PyMOL version 3.0 (Schrödinger, LLC, New York, NY, USA). In the process, all crystallographic water molecules and the irrelevant heteroatoms were stripped off to maximize the protein structure for the docking procedure, while the co-crystallized ligand KWG was separated and stored for further docking validation. The processed receptor structure was then exported as a Protein Data Bank (PDB) file and was taken as the target protein for all docking simulations [11,12].

2.3. Molecular Docking

Molecular docking studies were carried out using the web platform SwissDock, where the EADock DSS algorithm has been integrated to predict the preferred orientation of docking of the ligand in the receptor and the estimation of the free energy of binding. The three-dimensional structure of EBNA1 (PDB ID: 6NPP) was processed as the receptor, while Withaferin A and the reference ligand KWG were docked separately so that they could be compared to it. Docking simulations were performed in Accurate mode with the computational parameters set by the default configuration offered by the SwissDock server. After the docking procedure, the binding conformations that were generated were ranked based on the predicted Gibbs free energy (ΔG) value. For further structural and interaction analyses, the pose with the lowest binding energy and the best interaction within the active-site cavity was chosen [13,14].

2.4. Visualization of Protein–Ligand Interactions

The highest-scoring docking complexes were all studied in great detail with BIOVIA Discovery Studio Visualizer 2024 (Dassault Systèmes). Two-dimensional (2D) and three-dimensional (3D) representations of the ligand–protein complexes were created to thoroughly characterize the binding patterns in the EBNA1 active site. The interactions between EBNA1 and docked ligands were analysed to determine the most important non-covalent interactions to stabilize the complex, including hydrogen bonding, hydrophobic contacts, π–π stacking, π–alkyl interactions, and van der Waals forces [15].

2.5. ADMET Prediction

The pharmacokinetic parameters and safety of Withaferin A and the reference compound KWG were evaluated using the online prediction platform, ADMET-AI. The SMILES notation for each ligand was given as input to compute important absorption, distribution, metabolism, excretion, and toxicity (ADMET) properties. Physicochemical properties estimation, intestinal absorption, blood–brain barrier penetration, oral bioavailability, cytochrome P450 enzyme interaction, plasma protein binding, clearance in the system, and various toxicity-related endpoints were taken into account. The data were then evaluated to evaluate the drug features, PK profile, and overall therapeutic potential of both compounds [16].

2.6. Statistical Analysis

The molecular docking results were presented by descriptive analysis such as predicted binding free energy (ΔG), ligand–protein interaction pattern, and computational ADMET parameters. The complex with the lowest (most favourable) binding free energy and the highest number of favourable interactions in the EBNA1 binding pocket was determined to be the optimal docking pose. This conformation was then selected for direct comparison of the binding of Withaferin A with the reference ligand (KWG) to predicted pharmacological properties.

3. Results

3.1. Molecular Docking of Withaferin A against EBNA1 (PDB ID: 6NPP)

Molecular docking was performed using Withaferin A on the SwissDock server with Epstein–Barr nuclear antigen 1 (EBNA1; PDB ID: 6NPP) to investigate the binding potential of Withaferin A with Epstein–Barr nuclear antigen 1 (EBNA1). A total of twenty docking poses were obtained, with a range of docking scores between -6.866 and ~ – 4.0 kcal/mol. The mean binding affinity predicted was −5.193 ± 0.727 kcal/mol (SEM = 0.163 kcal/mol), with relatively consistent docking scores for all the docking poses generated.

The highest-ranking docking pose had a binding affinity of -6.866 kcal/mol, which is the most favourable docking mode among the predicted ones. The slight difference in docking scores among the docked structures indicates that the orientation of Withaferin A into the EBNA1 binding pocket is similar in all cases.

The two-dimensional interaction analysis showed that hydrophobic interactions are the major stabilizing interactions for Withaferin A (Figure 1). The ligand interacted with several key amino acid residues through alkyl and π–alkyl interactions, as well as several van der Waals interactions with Thr596 and Val604. In addition, a π–sigma interaction was found with Tyr510, which also contributes to the stabilization of the ligand. No classical hydrogen bonds were observed, suggesting that the predicted ligand–protein complex is mainly driven by non-polar interactions.

Three-dimensional visualization also showed that Withaferin A is well oriented in the hydrophobic binding pocket of EBNA1 (Figure 1). The two-dimensional analysis suggests that the ligand is primarily in contact with hydrophobic residues and that this is the case in the active-site cavity. The docking pose and pattern interaction relates well to the predicted binding mode obtained by docking simulation.

In general, Molecular Docking results indicate that Withaferin A has a good predicted binding affinity and can form a stable hydrophobic interaction network in the active site of EBNA1. These computational results are indicative of the potential use of Withaferin A as a natural scaffold for inhibitor development against EBNA1, but experimental tests are needed to ensure that the molecule is biologically active.

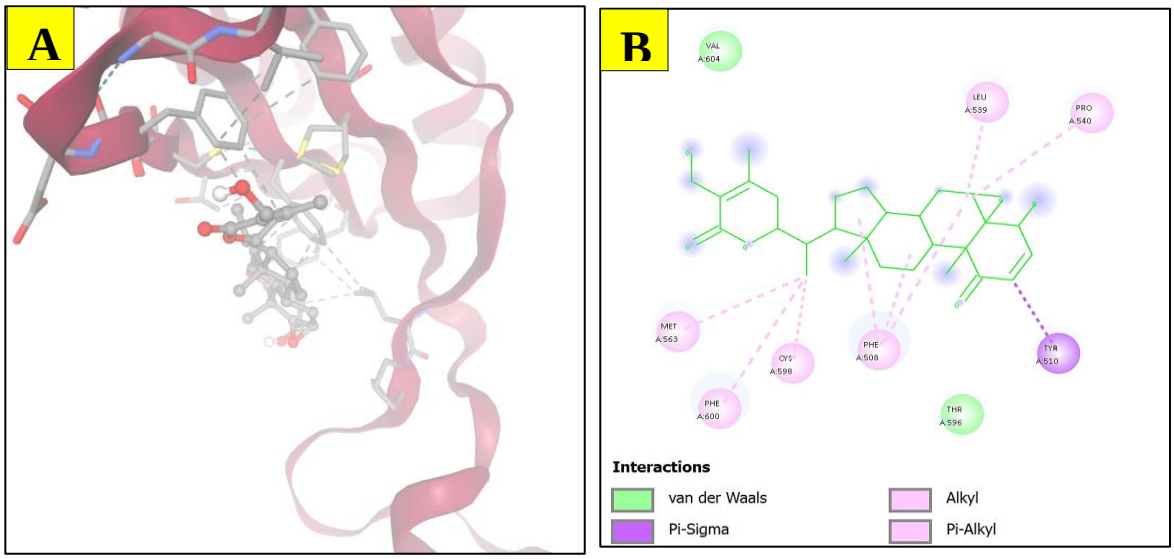

Figure 1. Molecular docking analysis of Withaferin A against EBNA1 (PDB ID: 6NPP). (A) Three-dimensional binding poses of Withaferin A within the EBNA1 active site. (B) Two-dimensional interaction map illustrating the principal amino acid residues and predicted intermolecular interactions responsible for ligand stabilization.

Table 1. Statistical summary of the molecular docking scores of Withaferin A against Epstein–Barr nuclear antigen 1 (EBNA1; PDB ID: 6NPP).

Parameter	Withaferin A	KWG
Number of docking poses	20	20
Best binding affinity (kcal/mol)	**−6.866**	**−5.494**
Mean binding affinity (kcal/mol)	**−5.193 ± 0.727**	**−4.465 ± 0.435**
Standard Error of the Mean (SEM)	**0.163**	**0.097**

3.2. Molecular Docking of the Positive Control (KWG) against EBNA1

The co-crystallised ligand 3-(phenylethynyl)-2-(1H-pyrrol-1-yl) benzoic acid (KWG) was used as the reference compound to test the docking performance of Withaferin A against Epstein–Barr virus nuclear antigen 1 (EBNA1, PDB ID: 6NPP). The average predicted binding free energy was −4.465 ± 0.435 kcal/mol (SEM = 0.097 kcal/mol), indicating that KWG has a moderate affinity for the EBNA1 binding pocket, using the SwissDock platform for molecular docking simulations.

Of all the possible generated docking conformations, the best-scored one had the most favorable binding energy of −5.494 kcal/mol, which corresponds to the most optimal binding geometry of the reference ligand to the target protein. The detailed structural analysis undertaken with BIOVIA Discovery Studio Visualiser showed that most of the interactions involved in the stabilization of the complex were of the aromatic/hydrophobic type. The predominant intermolecular interactions were π–π stacking, π–π T-shaped interactions, π–alkyl, and extensive van der Waals forces.

Several residues were identified as playing important roles in the interaction based on the interaction map (Phe508, Tyr510, Leu539, and Cys598) that contribute via aromatic contact and hydrophobic contact. Van der Waals interactions between Thr596, Met563, and Pro540 were present as additional stabilization. Contrary to this, no conventional hydrogen bonds or ionic interactions were found within the chosen docking complex, suggesting that the KWG binding to EBNA1 is mainly controlled by non-polar aromatic interactions.

Three-dimensional visualization revealed a hydrophobic binding cavity that was occupied by KWG in the same orientation as was compatible with the structure of the active site and the binding cavity occupied by Withaferin A. Thus, the reference ligand served as a well-defined reference to assess the relative binding affinity and interaction properties of the natural compound under study.

3.3. Comparison of the Docking Performance of Withaferin A and the Reference Ligand (KWG)

The re-docking of the co-crystallized ligand 3-(phenylethynyl)-2-(1H-pyrrol-1-yl) benzoic acid (KWG) was used to validate the docking protocol, followed by comparison with Withaferin A. 20 docking poses were generated for KWG with calculated binding affinities between −5.494 and −3.846 kcal/mol. The mean predicted binding affinity was −4.465 ± 0.435 kcal/mol, a moderate binding affinity in the EBNA1 binding pocket.

The highest-ranked docking conformation of KWG showed a calculated binding affinity of –5.494 kcal/mol and was mostly stabilized by π-π stacked, π-π T-shaped, and π-alkyl and van der Waals interactions. A few conventional hydrogen bonds were not detected, and the principal interacting amino acid residues were Phe508, Tyr510, Leu539, Pro540, Cys598, Met563, and Thr596. These interactions suggest that the major binding mode of

the reference ligand in the EBNA1 active site is via hydrophobic contacts.

However, Withaferin A had a better docking profile than KWG, with the best binding affinity being −6.866 kcal/mol vs. −5.494 kcal/mol, and the mean binding affinity being −5.193 ± 0.727 kcal/mol vs. −4.465 ± 0.435 kcal/mol, respectively. They both fit into the same hydrophobic binding pocket and had several common interacting residues such as Phe508, Tyr510, Leu539, Cys598, Met563, and Thr596. Withaferin A also made additional contacts with Val604 and Phe600, however, leading to a wider network of hydrophobic contacts with alkyl, π–alkyl, and van der Waals interactions. The predicted binding energies were lower for the Withaferin A case, but this did not seem to be due to the presence of any conventional hydrogen bonding; it may be due to the docking conditions used.

Table 2. Comparative molecular docking analysis of Withaferin A and the reference ligand (KWG) against EBNA1 (PDB ID: 6NPP).

Parameter	Withaferin A	KWG (Positive Control)
Best binding affinity (kcal/mol)	**−6.866**	**−5.494**
Mean affinity (kcal/mol)	**−5.193 ± 0.727**	**−4.465 ± 0.435**
Main interactions	Alkyl, π-Alkyl, van der Waals	π–π stacked, π–π T-shaped, π-Alkyl, van der Waals
Hydrogen bonds	None	None
Main residues	Phe508, Tyr510, Leu539, Pro540, Cys598, Met563, Val604, Thr596, Phe600	Phe508, Tyr510, Leu539, Pro540, Cys598, Met563, Thr596

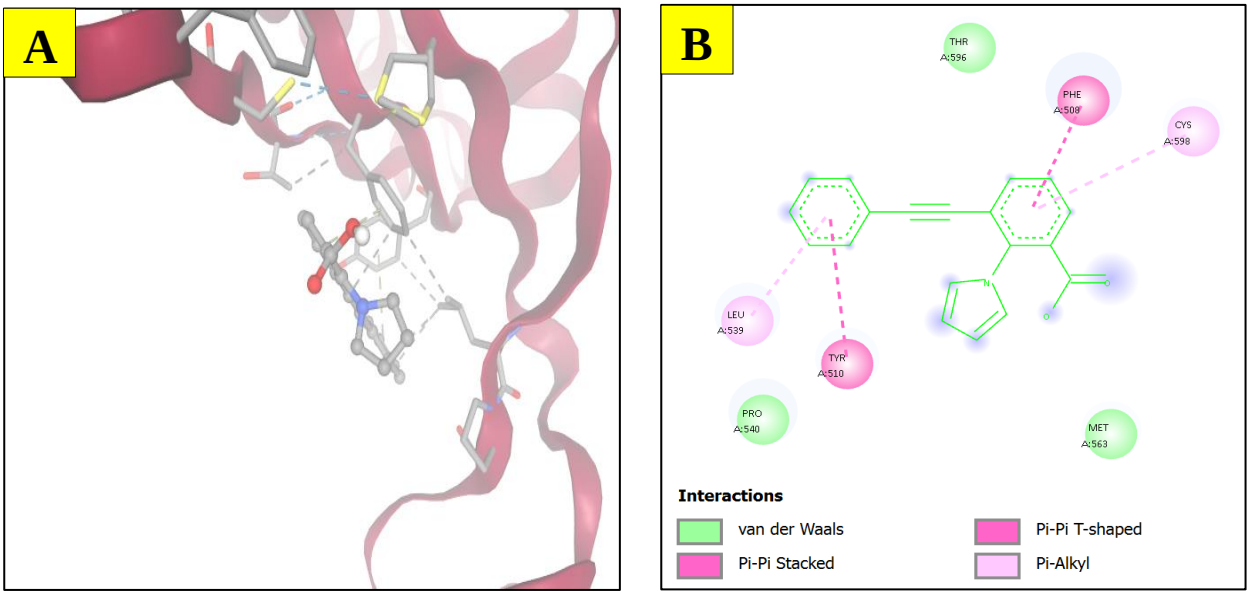

Figure 2. Molecular docking analysis of the reference ligand KWG with EBNA1 (PDB ID: 6NPP): (A) three-dimensional binding pose within the active site and (B) two-dimensional interaction diagram showing the principal amino acid residues involved in ligand stabilization.

3.4. Comparative ADMET Prediction of Withaferin A and KWG

The predicted ADMET profiles of Withaferin A and reference ligand KWG are summarized in Table X, and the general drug-likeness properties are shown with the bioavailability radar plots (Figure X). Both compounds complied with Lipinskis rule of 5, which indicates good drug-like properties. The quantitative estimate of drug-likeness (QED) score of KWG (0.73) was higher than that of Withaferin A (0.49), while both compounds showed excellent predicted human intestinal absorption (HIA = 1.00).

Although slightly lower than KWG (0.75), withaferin A had higher predicted PAMPA permeability (0.91 vs. 0.64) and higher lipophilicity. Moreover, Withaferin A showed a higher probability of P-glycoprotein inhibition (0.75), indicating potential interaction with transporters.

As far as distribution is concerned, both compounds exhibit moderate to moderate-high predicted blood–brain barrier penetration, with Withaferin A exhibiting a slightly higher probability (0.47) than KWG (0.37). Both the ligands showed high plasma protein binding (93.17% and 100%, respectively).

Based on metabolic prediction, Withaferin A exhibited low likelihood of CYP1A2, CYP2C19, CYP2C9, and CYP2D6 inhibition, and a moderate likelihood of CYP3A4 inhibition. KWG, by contrast, had more predicted inhibition of CYP2C9 and a higher probability of being a CYP2C9 substrate.

Withaferin A was also predicted to have a much longer elimination half-life (25.51 h) than KWG, and higher hepatocyte and microsomal clearance values, which reflect its unique elimination profile.

When toxicity was predicted, the probabilities of drug-induced liver injury and carcinogenicity were found to be significantly lower for Withaferin A than for KWG (0.32 vs. 0.97 and 0.03 vs. 0.38, respectively). The probabilities of hERG blockade, mutagenicity, and clinical toxicity were slightly greater for Withaferin A, but were considered to be low. The computational ADMET analysis indicates that Withaferin A has an acceptable pharmacokinetic profile and predicts a better safety profile than the reference ligand KWG, making it a suitable candidate for further studies as an EBNA1 inhibitor

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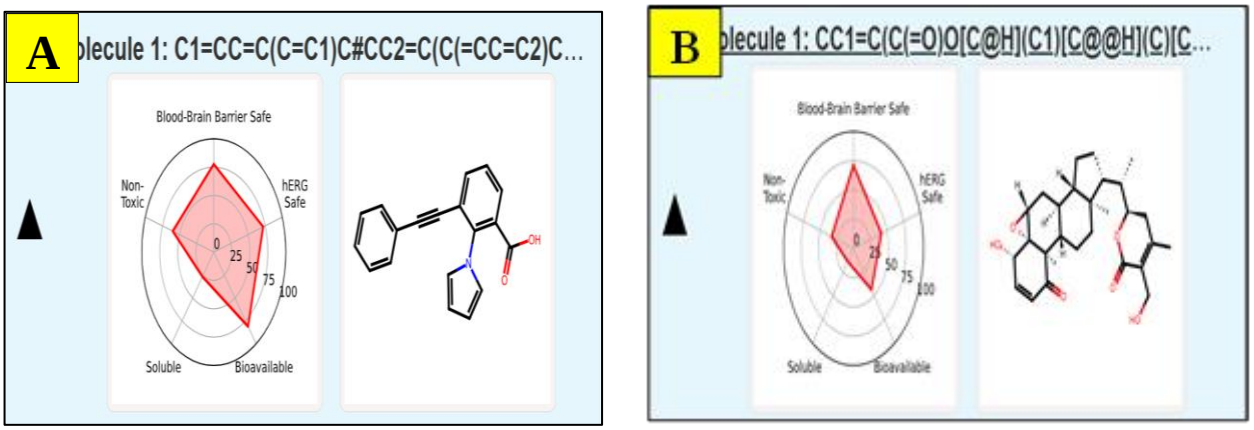

Figure 3. Predicted ADMET bioavailability radar plots generated using ADMET-AI. (A) Positive control (KWG). (B) Withaferin A. The radar plots illustrate the predicted balance between oral bioavailability, aqueous solubility, blood–brain barrier safety, hERG safety, and overall toxicity.

Table 3. Comparative ADMET prediction of Withaferin A and the positive control (KWG) using ADMET-AI.

Category	Property	Withaferin A	KWG (Positive Control)
Physicochemical	Molecular Weight (Da)	470.61	287.32
	LogP	3.35	3.58
	Hydrogen Bond Acceptors	6	2
	Hydrogen Bond Donors	2	1
	Lipinski Rule of Five	4/4	4/4
	QED	0.49	0.73
	Topological Polar Surface Area (Å²)	96.36	42.23
Absorption	Human Intestinal Absorption	1.00	1.00
	Oral Bioavailability	0.75	0.92
	Aqueous Solubility (log mol/L)	−5.02	−4.25
	Lipophilicity	3.12	1.47
	Hydration Free Energy (kcal/mol)	−7.93	−8.17
	Cell Effective Permeability (log10⁻⁶ cm/s)	−5.24	−4.24
	PAMPA Permeability	0.91	0.64
	P-glycoprotein Inhibition	0.75	0.05
Distribution	Blood–Brain Barrier Penetration	0.47	0.37
	Plasma Protein Binding (%)	93.17	100.00
	Volume of Distribution (L/kg)	0.00	3.45
Metabolism	CYP1A2 Inhibition	0.003	0.20
	CYP2C19 Inhibition	0.08	0.36
	CYP2C9 Inhibition	0.05	0.58
	CYP2D6 Inhibition	0.01	0.03
	CYP3A4 Inhibition	0.59	0.10
	CYP2C9 Substrate	0.01	0.52
	CYP2D6 Substrate	0.02	0.02
	CYP3A4 Substrate	0.69	0.20
Excretion	Half-life (h)	25.51	0.00
	Hepatocyte Clearance (µL/min/10⁶ cells)	94.60	30.32
	Microsomal Clearance (µL/min/mg)	28.29	20.77
Toxicity	hERG Blocking	0.39	0.09
	Clinical Toxicity	0.21	0.06
	Mutagenicity	0.31	0.08
	Drug-Induced Liver Injury (DILI)	0.32	0.97
	Carcinogenicity	0.03	0.38
	Acute Toxicity LD_{50} (log(1/mol/kg))	3.61	2.22
	Skin Reaction	0.40	0.40

4. Discussion

EBV nuclear antigen 1 (EBNA1) is essential for maintaining the viral episome, viral genome replication, and episomal viral persistence and is, therefore, a key target for the development of therapeutic agents against EBV-associated malignancies [17,18]. Computational analyses indicated that Withaferin A has good binding properties with EBNA1 in the present study, which indicates its potential as a natural lead compound for development of novel antiviral agents against EBV latency. The molecular docking results showed that the predicted binding affinity of Withaferin A was more favourable than the co-crystallized reference ligand (KWG), suggesting that there was a stronger theoretical binding to the EBNA1 binding pocket.

Binding affinity is regarded as one of the most important measures of the thermodynamic stability of the predicted ligand–protein complex, with lower binding free energy indicating higher thermodynamic stability [19,20]. The best docking score for Withaferin A was lower (−6.866 kcal/mol) than the reference ligand KWG (−5.494 kcal/mol), and the average docking score was also better. These results suggest that Withaferin A can form more stable interactions with EBNA1 than the natural ligand under the same docking conditions. Even if docking scores are not good indicators of biological activity, they can be very useful in the early stages of drug discovery to assess the recognition of the ligand by the target and the selectivity of the target during this process [21,22].

A surprising finding was that the docked ligand–protein complex was mainly stabilized by hydrophobic interactions, not by the traditional hydrogen bonds. In the top-ranked docking position, the hydrophobic interactions between Phe508, Tyr510, Leu539, Pro540, Met563, Cys598, Phe600, and Val604 were always found. Phenylalanine and tyrosine, which contain aromatic amino acids, are often involved in stabilizing the ligand by π–alkyl and π–π interactions, while aliphatic amino acids, such as leucine and methionine, enhance the hydrophobic packing in the active-site cavity [23,24]. It has been previously shown that hydrophobic contacts can be the most important force in a viral DNA-binding protein that recognizes a ligand, especially if the binding pocket does not have many hydrogen-bonding groups [25].

The docking pose was very stable, even without any hydrogen bonding, highlighting that hydrogen bonding is not all that is required to achieve high ligand affinity. Many antiviral inhibitors use van der Waals, hydrophobic, and aromatic stacking interactions as the main forces in the stable protein–ligand complexes [9]. Other natural products with biological activity based on large hydrophobic interactions instead of strong electrostatic interactions have been observed with similar effects [26]. Thus, the interaction profile identified for Withaferin A is in accord with known rules of molecular recognition, and does not diminish its therapeutic potential.

These results are further corroborated by comparison with the co-crystallised ligand. Both ligands fit into the same hydrophobic cavity and have several common interacting amino acid residues, but Withaferin A has extra contacts with amino acid residues like Val604, Phe600, and so on, which intensifies the hydrophobic interaction network. The higher binding affinity of Withaferin A compared to KWG may be due to the presence of extra stabilizing contacts. In many structure-based drug design studies of viral proteins, these similar correlations between enhanced interaction networks and enhanced docking performance have been reported [8,27].

In addition to desirable molecular recognition, the ideal drug candidate will have good pharmacokinetics and toxicity properties. The drugs' potential was predicted to be favorable, with computational ADMET properties fulfilling Lipinski's Rule of Five. Withaferin A had, however, several favourable pharmacokinetic properties; it

was highly predicted to be absorbed in the human intestine, had high permeability in PAMPA, and had acceptable oral bioavailability properties, which are desirable properties of orally administered therapeutic agents. Both ADMET and systemic exposure are parameters that have been observed in early drug development for compounds with satisfactory systemic exposure, and both have been observed in the past [28,29].

In addition, metabolic prediction indicated that the enzymes CYP1A2, CYP2C19, CYP2C9, and CYP2D6 were unlikely to be inhibited, while moderate metabolism prediction indicated CYP3A4 enzyme inhibition. Limited inhibition of the cytochrome P450 enzymes likely precludes clinically relevant drug–drug interactions in general [30,31] since these enzymes have central roles in metabolizing xenobiotics. Furthermore, the expected elimination half-life of 25.5 h may indicate that Withaferin A could be used to obtain a long-lasting systemic effect, thereby permitting less frequent applications if confirmed experimentally.

Another important part of the early drug discovery process is safety prediction. Curiously, the predicted probabilities of liver injury and carcinogenicity of Withaferin A were significantly lower than those of the reference ligand. Slightly elevated hERG and mutagenicity potential were also predicted, but these potentialities are still relatively low and should be tested rather than ruled out. Computational Toxicity Models are useful screening tools that can't replace in vitro or in vivo toxicological studies [32,33].

Withaferin A has been extensively studied in the past for its diverse biological activities, such as antiviral, anti-cancer, anti-inflammatory, antioxidant, and immunomodulatory properties [34,35,36]. The steroidal lactone has been shown in several studies to modulate a number of signaling pathways relevant to cancer progression and viral replication, which is why it is being repositioned as a multifunctional therapeutic agent [35,37]. Thus, the positive docking affinity towards EBNA1 is biologically plausible and also consistent with the known pharmacological signature of Withaferin A.

However, some caveats should be noted. The molecular docking method gives just a snapshot of ligand–protein interaction and cannot describe the conformational flexibility of the receptor, the effect of solvent molecules, or the conformational stability over time [38]. Thus, additional molecular dynamics simulations, MM/PBSA free energy calculations, enzyme inhibition assays, and cell-based antiviral experiments are required to validate the computational results and to verify if these interactions can be translated into measurable biological activity.

In general, the present computational investigation shows that Withaferin A has a higher predicted binding affinity to EBNA1 than the reference ligand and a good pharmacokinetic profile and acceptable predicted safety properties. In total, the results here presented indicate that Withaferin A could be a promising natural platform for antiviral drugs targeting EBNA1, and suggest an extensive experimental evaluation using biochemical and cellular models.

5. Conclusion

The present study shows that the bioactive steroidal lactone withaferin A found in Withania somnifera has a potential inhibitory effect on Epstein – Barr virus nuclear antigen 1 (EBNA1) using an in silico approach. Withaferin A was found to have a more favourable predicted binding affinity compared with the co-crystallised reference ligand KWG through molecular docking, with strong hydrophobic interactions with the key amino acid residues of the EBNA1 binding pocket. In addition, ADMET prediction showed that the drug had good compliance with Lipinski's rule of five, good predicted intestinal absorption, acceptable oral bioavailability, and a more positive safety profile, in terms of lower predicted toxicity of drug-induced liver injury and carcinogenicity. While these results suggest that Withaferin A is a promising lead compound for targeting EBNA1 and developing new

drugs against EBV-related cancers, they are purely computational. Thus, extensive in vitro and in vivo studies, as well as molecular dynamic simulations and binding free-energy calculations, are needed to validate it for use as an antiviral agent, in terms of its pharmacokinetic profile and therapeutic potential.

Conflict of Interest: NIL
Funding: NIL

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