Grenze International Journal of Engineering and Technology, June Issue

Virtual Screening of Withnaolides as Potential Drug Candidate for Inhibiting Human Adenovirus 2 Protease: An In-Silico Study

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***Abstract*— Human adenoviruses pose a threat to ophthalmic clinics and are responsible for sporadic, localized respiratory illness across the world. According to recent research, differences in the interferon-mediated regulation of viral replication could contribute to the emergence of persistent infections and reactivation. In addition to being contagious, adenoviral genomes include potent oncogenes promoting the growth of tumors, albeit precision is yet unclear. Adenoviruses infect a wide variety of cells thus used as vectors, allows for the development of innovative treatments for diseases including cancer and heart issues. Intriguingly, *Withania somnifera* (WS) has shown promising antiviral effects against numerous viral infections, including adenovirus, which are contributed by its phytochemicals. *W. somnifera*, often known as Indian ginseng, contain large amounts of a class of pharmacologically active chemicals called withanolides in its roots and leaves. Withanolides, which are C-28 phytochemicals, highly oxygenated steroidal lactones shown to have anticancer, immunomodulatory, and other properties. Four phytochemicals from *Withania somnifera* were examined for their molecular characteristics in this research. The objective was to evaluate their capacity to bind and potentially block the human adenovirus 2 protease protein, which is essential for the reproduction of the adenoviruses, through the use of in-silico work. Using computational methodologies, the drug-likeness characteristics of the phytochemicals were ascertained; followed by molecular docking investigations. These results suggested that out of selected phytochemicals withanolide B is the most promising drug candidate (with binding energy of -8.3kcal/mol) for inhibiting the catalytic activity of human adenovirus 2 protease protein resulting thus preventing infection cycle of adenovirus. The ADME analysis also showed no violation of Lipinski rule of five, no permeation of blood brain barrier, and ames test gave negative value indicating no mutagenic activity by any of the candidate phytocompounds.**

***Index Terms*— Adenoviruses, Interferon-mediated regulation, Withania somnifera (WS), Withanolides**

I. INTRODUCTION

The term "adenovirus" comes from its discovery in 1953 in human adenoid tissue cultured in vitro. Human adenoviruses (HAdVs) continue to provide therapeutic issues for diagnosis and treatment. They are a significant source of infections in both immunocompetent and immunocompromised patients [1]. Human adenoviruses (HAdVs) have been a constant source of difficulty in a range of therapeutic situations since they were originally

Grenze ID: 01.GIJET.10.2.739

isolated from adenoid tissue more than 60 years ago. Adenoviral genomes have been revealed to include strong oncogenes besides their well-known function as infectious agents. Moreover, many mammalian animal models have indicated that certain virus types may cause tumor development [2]. Their potential carcinogenicity in people has remained mysterious despite several research addressing the potential involvement of HAdVs in malignant illness in humans [3], [4]. Adenoviruses' potential to infect a variety of cell types also made it easier to employ them as gene delivery vectors, which produced novel instruments for the inventive treatment of significant diseases including cancer and cardiovascular conditions. As a result, adenoviruses are extremely adaptable creatures with a wide range of therapeutic functions [5], [6], [7].

HAdVs are 70–100 nm diameter, nonenveloped viruses. The primary viral protein, hexon, contributes significantly to the icosahedral symmetry of the virus's outer protein shell, which has There are 12 vertices, 30 edges, and 20 triangular faces. There are 252 capsomeres in the viral capsid, which are made up of 240 exons and 12 pentons. At each of the 12 vertices, five penton base proteins assemble to produce a unique capsomere. A variable-sized trimeric fiber protein extends from the capsid vertex of each capsomere. The tail, shaft, and knob are the three structural domains that make up the fibre protein. The penton base binds to its binding site in the tail domain. The shaft domain varies in length across HAdV types, which affects the fiber's flexibility and how it interacts with integrins in the host cell. The protein's distal, C-terminal, the principal host cell receptor is attached to the virus by the fiber knob domain. [8]. HAdVs are categorized under the genus Mastadenovirus together with other mammalian adenoviruses. They are separated into seven further species, denoted as A through G, with species B being further split into subspecies B1 and B2[9], [10]. Serum neutralization (SN) and hemagglutination inhibition (HI) assays were used in the past to identify, characterize, and classify HAdVs. However, recently, serological methods for the typing of novel viruses have been replaced by genomic and bioinformatics studies of the entire viral genome.[11]. Adenovirus infections are common all over the world and usually result in mild feverish illnesses that go away on their own in young children. By the time they are 10 years old, most persons have numerous serotypes of infection confirmed by serology. The type of virus and its mode of dissemination will determine the incubation time might vary from two days to two weeks [12].

Through the years, substances from nature have been extensively utilized to cure a wide range of viral infections [13]. Phytochemicals present in medicinal plants are known to possess antiviral potential by targeting proteins of human adenovirus. To find out whether phytochemicals or plant metabolites, including unusual chemical compounds, have any antiviral qualities that might be utilized to combat human adenovirus, additional studies are needed to be done on these substances. Naturally occurring products are seen to be the most promising sources of lead compounds because of their fascinating bioactivities. Moreover, a large number of medications that are sold are either chemicals derived from natural sources or directly derived from plant-based sources [14]. The herb *Withania somnifera* is commonly referred to as ashwagandha. Over 3,000 years have seen extensive usage of this significant medicinal herb, which is distributed throughout the Indian subcontinent in traditional Ayurvedic and other systems [15]. *Withania somnifera*'s biological actions are mostly linked to two compounds: withanolide-A and withaferin-A using withanolide-D, and withanone using both withanolide-E and withanolide-F [16].

In-silico study, which uses computational methods and bioinformatics tools, is typically employed by scientists to find and examine novel drug candidates. Identifying target leads is among the key components of the initial drug development process. Lead discovery searches for novel chemicals that bind to specific targets. Target discovery looks for suitable targets to validate for drug development for therapeutic use [17]. In this study, we explored the binding efficacy of human adenovirus with the phytoconstituents of W. somnifera. Also, evaluation of antiviral prospect of several phytocompounds present in *Withania somnifera* using bioinformatics tools and computational methodologies were done. This work highlights the potential of natural compounds in fighting viral infections by studying the molecular docking of the human adenovirus with different phytochemicals.

II. Methods And Methodology

A. Screening for ligands and Data collection

To find antiviral properties in Ashwagandha (Withania somnifera), phytochemicals that have demonstrated potential against human adenovirus were searched. SMILES details of several natural compounds through the databases of PubChem (https://pubchem.ncbi.nlm.nih.gov/) were downloaded. Various factors, including Lipinski, lead similarity, and BBB permeability were looked down. An online web server SwissADME (http://www.swissadme.ch/), was used to screen the compounds that meet the above criteria. Out of all the selected compounds, only four - Withanolide A, Withanolide B, Withanolide C, and Withanolide D – met those criteria and were chosen for molecular docking.

B. Preparation of targeted protein and ligand

a) The protein for this study was obtained from the RCBS protein databases (https://www.rcsb.org/). PyMOL and other visualization tools were used to identify structural issues in the protein. The protein under investigation had a sequence length of 204 amino acids and an A chain, with a PDB ID 4PIE and Uniport ID is P03252 (Human adenovirus C serotype 2). To dock the human adenovirus protease with the chosen chemicals, BIOVIA Discovery Studio was used for preparation. Once the target protein's structure was generated, it was saved in PDB format. To convert the ligand (phytochemicals) SDF structures to PDB format, visualization program BIOVIA Discovery Studio was again used. Polar hydrogens were added and water molecules were eliminated (not involved in interactions) from the targeted protein.

b) As the requisite sets of parameters were satisfied by four ligands, PubChem (https://pubchem.ncbi.nlm.nih.gov/) was used to get all four of the ligands, and they were stored in MOL SDF format. The ligand compounds were visualized by BIOVIA and the SDF files were converted into PDB format by using Openbabel and saved, later on used for molecular docking.

C. Molecular docking using PyRx

In this study, a tool called PyRx integrated with other docking systems like AutoDock and AutoDock Vina was used. The targeted protein and ligand we were uploaded on PyRx version 8.0 (https://pyrx.sourceforge.io/). Adjusted the docking settings, such as the search space, grid box, and docking algorithm, as needed. Once all the settings were in place, docking simulation was started using PyRx, it was also used to predict the binding positions and affinities of the ligands within the protein binding site. Other docking tools such as AutoDock (https://autodock.scripps.edu/), PyRx (https://pyrx.sourceforge.io/downloads), ZDOCK (https://zdock.wenglab.org/) etc. can be used.

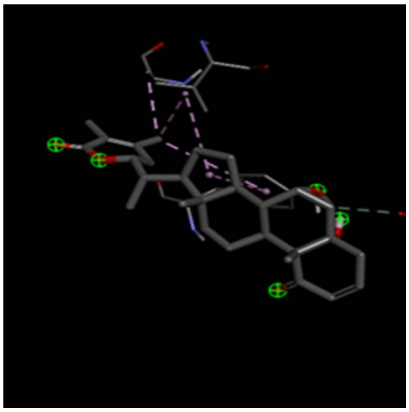

Figure 1. Molecular docking interaction of Withanolide B with human adenovirus 2 protease.

D. Structural analysis of docked protein and ligands

After the docking process was completed, PyMoL (https://pymol.org/2/) was used to visualize the resultant file. It determined which molecules were participated in the interaction. Moreover, 2D and 3D photographs of the outcomes were produced using the Discovery Studio software.

III. Result And Discussion

Molecular docking: The present investigation assessed the binding affinities for human adenovirus 2 protease active sites and Withanolide A, B, C, and D. Withanolide A demonstrated a significant degree of contact with its target molecule, as evidenced by its binding affinity of -8.1 kcal/mol. With an affinity value of -8.3 kcal/mol, withanolide B exhibited the greatest binding affinity of all the examined withanolides, indicating a strong and stable association. On the other hand, withanolide D showed an affinity of -7.9 kcal/mol, whereas withanolide C with a moderate affinity of -7.1 kcal/mol (Table1).

ADME analysis: Consequently, by ADME results, withanolide A (-6.86cm/s) and D (-6.96cm/s) showed moderate skin permeability, withanolide B with a score of -5.76cm/s showed the lowest and withanolide C with comparatively the highest (-8.05cm/s) permeation. Lipinski rule of Five was followed by withanolide A, B, C and D with 0 violation except withanolide C with 1 violation (MW>500). The result given by Ames test also came out to be negative offering their potential use against human adenovirus.

TABLE I. LIST OF HUMAN ADENOVIRUSES 2 PROTEASE INHIBITORS WITH RESPECTIVE BINDING AFFINITY.

Sr. No.	Phytocompound	Hydrophobic bond residues	Hydrogen bond residues	Binding energy(kcal/mol)
1	Withanolide A	VAL53, PHE73, VAL83, VAL83	TYR84, TYR84	-8.1
2	Withanolide B	VAL53, PHE73, VAL83, VAL83	TYR84, TYR84, TYR84	-8.3
3	Withnaolide C	VAL53, PHE73, VAL83, TYR84, TYR84, PRO117, ALA120, LEU158, LEU201	GLY2, SER3, GLY52, TYR84, TYR84	-7.1
4	Withanolide D	VAL53, VAL83, VAL83, TYR84, TYR84, LEU201	VAL53, HIS54, TYR84, TYR84, ASN159	-7.9

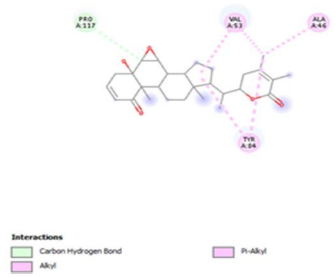

Figure 2. Withanolide A docked with human adenovirus

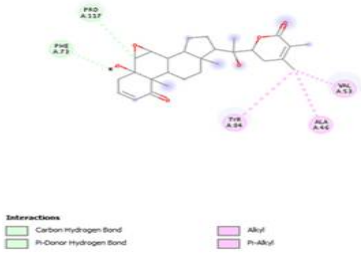

Figure 3. Withanolide B docked with human adenovirus

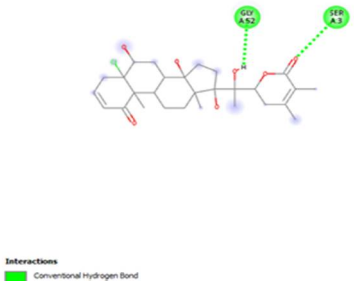

Figure 4. Withanolide C docked with human adenovirus

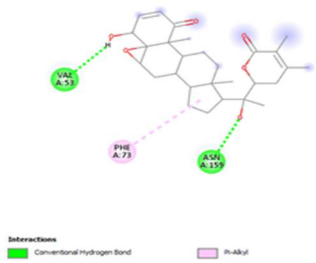

Figure 5. Withanolide D docked with human adenovirus

ADME ANALYSIS

The SwissADME platform was used to assess the ADME (Absorption, Distribution, Metabolism, and Excretion) properties of the withanolides. All four compounds satisfied Lipinski's rule of five, suggesting favorable drug-like characteristics. Withanolide B exhibited the least skin permeability (-5.76 cm/s), indicating favorable bioavailability (table 2). All of the compounds adhered to the major pharmacokinetic parameters, indicating that they are appropriate candidates for further drug development.

TABLE II. ADME RESULT OF ALL THE FOUR COMPOUNDS.

Phytocompound	Skin permeability (cm/s)	Lipinski's Rule violations	Ames Test Result
Withanolide A	-6.89	0	Negative
Withanolide B	-5.76	0	Negative
Withanolide C	-8.05	1 (MW>500)	Negative
Withanolide D	-6.96	0	Negative

The computational analysis has presented convincing evidence that withanolide B is a highly promising candidate for inhibiting human adenovirus 2 protease. The compound's strong binding aptitude and its ADME traits for absorption, distribution, metabolism and excretion of the compound make it a possible lead in antiviral drug development. Robust interactions of binding withanolide B reveal its possible significance in inhibiting the catalytic activity of the protease and thus preventing adenovirus replication. The pharmacokinetic analysis provides further proof on the feasibility of this drug candidate since no significant limitations have been found from ADME's point of view.

IV. Conclusions

The current study outlines a new means of inhibiting the protease of human adenovirus 2 with phytochemicals derived from Withania somnifera. In-silico methods were used to evaluate the approach. This innovative research specifically looks at the binding affinities of four withanolides (A, B, C, and D) from Withania somnifera against adenovirus protease. Among other withanolides, our research findings indicate that withanolide B has the highest binding affinity (8.3 kcal/mol). This means that it has a more effective and stable inhibitory interaction with the target protease molecule among others. Moreover, the potency of this compound as a powerful antiviral agent against human adenovirus is evident from this observation. In addition, the ADME studies have demonstrated that this compound has drug like characteristics which can be ascertained by its compliance to Lipinski rule of five and having pharmacokinetic properties that are highly favourable. This study is noteworthy as it does not just involve identification but investigates extensively how these natural substances may be authenticated using advanced computational methods. Furthermore, such negative results on Ames test strengthen their safety profiles and pave ways for future use in treatment.

Acknowledgment

The authors wish to thank their lab mates and guide. This work was carried out in Delhi Technological University (Department of Biotechnology).

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