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Inhibitor-Protein Interactions in Japanese Encephalitis Virus: A Computational Approach to Antiviral Discovery

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Japanese Encephalitis Virus (JEV) is a mosquito-borne virus that poses a significant health threat, causing severe neurological complications. To explore potential therapeutic interventions, this study utilized computational techniques, including molecular docking and molecular dynamics simulations, to investigate the interaction of Withaferin A, a natural compound derived from Ashwagandha, with specific viral proteins (4HDH and 4K6M).

The results indicate strong binding affinity and favorable interactions between Withaferin A and the target proteins, suggesting its potential to disrupt viral processes. The compound's adherence to Lipinski's Rule of Five and low toxicity profile further support its suitability for drug development. Additionally, Withaferin A exhibits immunomodulatory and cytotoxic properties, which could contribute to its antiviral activity. The study employed tools such as PyRx, Discovery Studio, Chimera, and GROMACS to conduct molecular docking and dynamics simulations, providing valuable insights into the potential mechanism of action of Withaferin A. While additional studies, including clinical validation, are necessary, these results position Withaferin A as a promising candidate for therapeutic intervention against JEV. This research contributes to the ongoing efforts to expand the arsenal of strategies to mitigate the impact of this menacing virus and protect vulnerable populations from its severe neurological effects.

Keywords: Japanese Encephalitis Virus (JEV); computational docking; simulation method; neurological afflictions.

ABBREVATIONS

JEV : Japanese Encephalitis Virus
PDB : Protein Data bank
GRAS : Generally Recognized as Safe
SDF : Structured Data File
RMSD : Root Mean Square Deviation
RMSF : Root Mean Square Fluctuations
RG : Radius of Gyration
SASAs : Solvent Accessible Surface Areas
MM-GBSA : Molecular Mechanics Generalized Born Surface Area
ADME : Absorption Distribution Metabolism Elimination
TMPRSS2 : Transmembrane Protease, Serine 2
SARS-CoV-2 : Severe Acute Respiratory Syndrome Coronavirus 2;
Wi-A : Withaferin A
Ns : nanoseconds
Fs : femtoseconds

1. INTRODUCTION

Japanese encephalitis is a significant health concern caused by the Japanese encephalitis virus (JEV), which belongs to the Flavivirus group. This virus primarily induces inflammation in the central nervous system and is primarily transmitted through mosquito vectors, particularly during warmer months. While its impact is most strongly felt in specific regions of Asia, it has managed to expand its geographical reach, posing global health concerns. The genetic makeup of JEV is characterized by a single-stranded positive-sense RNA genome, spanning approximately 11 kilobases in length. This RNA serves as the blueprint for the synthesis of a polyprotein, a precursor molecule that undergoes subsequent processing by both viral and host enzymes to generate essential structural and non-structural protein (Modrow et al. 2013). Among the various proteins produced during this process, the envelope proteins hold particular significance. They play a crucial role in enabling the virus to interact with host cells and gain entry into them. The envelope protein, specifically the E protein, is of particular interest due to its multifaceted functions and structural complexity.

The E protein consists of three distinct parts or domains, each contributing to its essential roles in the virus life cycle. One of its primary functions is to facilitate the fusion of the viral membrane

with the host cell membrane, a crucial step that allows the virus to enter the host cell. This fusion process is critical for the virus's ability to infect and replicate within the host organism. Additionally, the E protein is involved in other vital functions during the virus life cycle. It plays a key role in viral attachment to host cell receptors, which is the initial step in the infection process. This protein also participates in the assembly and maturation of the virus particles, contributing to the production of infectious virions. Understanding the structural and functional intricacies of the E protein within the envelope proteins of JEV is essential for developing strategies to combat Japanese encephalitis. Research efforts aimed at targeting this protein may lead to the development of vaccines or antiviral drugs that can mitigate the impact of this disease, especially in regions where it poses a significant public health threat (Gupta et al. 2013).

Japanese encephalitis (JEV) manifests as a diverse range of symptoms in individuals who become infected with the virus (Yun& Lee 2014). In severe cases, patients often experience a constellation of symptoms, including high fever, severe headaches, and a host of neurological issues, such as seizures. These neurological complications can escalate to life-threatening levels, underscoring the gravity of the disease. Indeed, JE has the potential to result in fatal outcomes, particularly in cases where timely intervention and treatment are lacking. While vaccines against JE do exist, it's important to note that these vaccines primarily offer protection against specific strains of the Japanese encephalitis virus (JEV). This presents a notable limitation in the overall effort to safeguard populations from this viral threat. The variability of JEV strains in different regions has led to gaps in the effectiveness of existing vaccines, as they may not provide comprehensive coverage against all circulating strains.

In response to these limitations, researchers have embarked on a quest to explore innovative and alternative strategies to combat the virus effectively. The urgency of addressing Japanese encephalitis and the need for broader protection has spurred a growing interest in developing novel approaches to prevent and treat the disease. These endeavours encompass a wide range of research, from exploring new vaccine candidates to investigating antiviral drugs and therapeutic interventions that can mitigate the severity of the disease. The multifaceted nature of Japanese encephalitis, with its spectrum of symptoms and potential for severe neurological complications, underscores the importance of continued research and innovation in the field. Scientists and healthcare professionals are committed to finding ways to enhance our defenses against JE, ensuring that individuals in affected regions receive the most effective protection and treatment possible. Ultimately, these efforts are critical in reducing the impact of JE and preventing the loss of lives due to this formidable viral disease (Chen et al. 2019).

This research study is primarily centred around the application of computational methods, specifically molecular docking and molecular dynamics simulations, as powerful tools for the identification of potentially beneficial small molecules derived from plants. The primary focus of these methods is to disrupt the entry process of the Japanese encephalitis virus (JEV) into host cells. The overarching goal of this study is to pinpoint plant-derived compounds that exhibit a high potential for binding to JEV's envelope protein, a critical step that could potentially lead to the development of effective antiviral drugs (Serhan et al. 2019). The central objective of this research is to leverage computational techniques to screen a wide range of plant-derived compounds and identify those with the strongest affinity for JEV's envelope protein. These compounds are of particular interest because they have the potential to interfere with the virus's ability to enter and infect host cells. This strategy represents a promising new avenue for the development of antiviral drugs targeting JEV, which is increasingly becoming a global health concern due to encephalitis-related illnesses (Jha et al. 2023).

Given the rising global concerns associated with JEV and the illnesses it causes, this research seeks to contribute significantly to our understanding of the virus and to explore innovative strategies to combat it. The integration of advanced computational tools with biomedical knowledge is a key feature of this study. By doing so, researchers aim to elucidate the intricate interactions between JEV and the potential drug candidates derived from plant sources. The goal of this research is to identify compounds that can effectively disrupt the virus's entry into host cells, thus mitigating its pathogenicity. By harnessing the power of computational methods and merging them with a deep understanding of JEV biology, this study endeavours to shed light on critical aspects of the virus-host interaction. This

knowledge, in turn, has the potential to pave the way for the development of novel antiviral therapeutics, which could be instrumental in countering the global threat posed by JEV.

2. MATERIALS AND METHODS

2.1 Target Protein Retrieval

Six potent proteins were selected mentioned in (Table 1), and their interaction with the drugs was assessed through docking. These proteins belonged to different functional classes and play important roles in various cellular processes. The docking procedure involves computationally predicting the binding strength between a ligand and a protein target. PDB IDs of these proteins were obtained from the Protein Data Bank (PDB), and their respective PDB format files were downloaded (Singh et al.). Using the BIOVIA Discovery Studio Visualizer https://discover.3ds.com/discovery studio-visualiser-download), a 3D molecular visualization software, the team eliminated pre-bound ligand groups, heteroatoms, water molecules, and any unwanted components from the protein structures, ensuring they were refined and free from duplications.

Table 1. List of protein targets for docking analysis

PDB ID	Protein description	Protein description	Resolution (A°)
4K6M	Crystal Structure of the full-length Japanese encephalitis virus NS5	The full-length crystal structure of the Japanese encephalitis virus NS5 has been determined using X-ray crystallography. NS5 is a viral protein that plays a crucial role in viral replication, including the synthesis of RNA and viral proteins (consortium 2019).	2.60
5WSN	Structure of Japanese encephalitis virus Protein function Protein	The structure of Japanese encephalitis virus (JEV) consists of a lipid envelope containing E and M glycoproteins, surrounding a nucleocapsid core with the viral RNA genome encoding structural and non-structural proteins. The E protein facilitates viral entry and fusion, while M and prM proteins are involved in assembly. Non-structural proteins play critical roles in replication and immune evasion, with NS5 containing important domains for viral RNA synthesis. Understanding the structure and function of JEV is crucial for developing antiviral drugs and vaccines (consortium 2019).	4.30
5MV1	Crystal structure of the E protein of the Japanese encephalitis virulent virus	Crystal structure of the E protein of the Japanese encephalitis virulent virus Protein function Protein - The crystal structure of the E protein of the Japanese encephalitis virus (JEV) provides insights into its function in viral entry and fusion. The E protein is a type II viral fusion protein consisting of three domains: domain I, II, and III. The fusion loop in domain I inserts into the host cell membrane during viral fusion. The dimerization of the E protein is mediated by interactions between domain II of each monomer. The receptor binding site in domain III interacts with host receptors during viral entry, leading to conformational changes that expose the fusion loop and facilitate fusion. Understanding the function of the JEV E	2.25

PDB ID	Protein description	Protein description	Resolution (A°)
		protein is important for developing antiviral drugs and vaccines (consortium 2019).	
3P54	Crystal Structure of the Japanese Encephalitis Virus Envelope Protein, strain SA-14-14-2.	The crystal structure of the Japanese encephalitis virus (JEV) envelope protein, strain SA-14-14-2, provides insights into its function in viral entry and fusion. The envelope protein is a type I viral fusion protein consisting of three domains: domain I, II, and III and the crystal structure also reveals potential antigenic sites that can be targeted for vaccine development. Understanding the function of the JEV envelope protein is crucial for developing antiviral drugs and vaccines against this virus (consortium 2019).	2.10
5YWO	Structure of JEV-2F2 Fab complex	- The crystal structure of the JEV-2F2 Fab complex provides insights into the molecular interactions between the viral envelope protein and a potent neutralizing antibody. The study shows that the 2F2 antibody binds to a conserved epitope on the E protein of the virus, which is essential for virus entry into host cells. The binding of the antibody induces conformational changes in the E protein that prevent the virus from undergoing the necessary conformational changes required for membrane fusion and entry into host cells. This understanding of the molecular mechanisms of neutralization could aid in the development of antiviral therapeutics and vaccines against Japanese encephalitis virus (consortium 2019).	4.70
4HDH	Crystal Structure of viral RdRp in complex with ATP	The binding of ATP to the RdRp can induce conformational changes in the enzyme, which are important for its function. For example, the binding of ATP can trigger the closure of the active site, allowing the enzyme to catalyze the formation of phosphodiester bonds between the nucleotides and act as catalysis of RNA synthesis, energy source of RNA synthesis and Regulation of RdRp activity (consortium 2019).	2.28

2.2 Drug Preparation

In this study, Dr Duke's Phytochemical and Ethnobotanical Databases was used to retrieve a total of 287 potential phytochemical drugs that might exhibit anticancer activity against Japanese Encephalitis Virus (JEV). The database consists of phytochemical which constituents of GRAS (Generally Recognized as Safe) herbs and other economic plants. Further, the phytochemicals were categorized based on their class, with most being flavonoids, alkaloids, terpenoids, and organic compounds, and only a few linked to Steroids, Saponins, Quinones, Phenylpropanoids, and others. (Fig. 1). PubChem database was used to obtain the 2D structure SDF (Structured Data File) format for each drug https://www.ncbi.nlm.nih.gov. The obtained 2D structure SDF files were then used in the molecular docking process to determine the binding affinity of each drug with the selected protein targets (Gupta et al. 2013).

2.3 Energy Minimization of Docking Entities

The proteins were then subjected to energy minimization using the UCSF Chimera software (Pettersen et al. 2004). with the help of DockPrep, a surface/binding analysis tool and the hydrogen bonds and charges obtained from Dunbrack Library were added at the appropriate locations. The ligands were procured from PubChem in SDF format and then converted to pdbqt format using the Open Babel Tab provided in Pyrx virtual screening tool software.

2.4 Docking Methodology

In the molecular docking process, it is extremely important to remove any superfluous interactions between the drug and receptor molecules with other molecules that are already present in order to help drug binding at competitive position on the receptor. A standard docking protocol of energy-minimizing each target protein and was using it as a macromolecule was followed. Even ligands were appropriately energy minimized to initiate the docking procedure using the PyRx Virtual Screening Tool Software's Vina Wizard Control, with the Vina Search Space set to maximum and the Vina Run exhaustiveness set to eight. Binding affinity (kcal/mol) and Root Mean Square Deviation (RMSD) lower and upper bound values were recorded for each run, up to the set run value (Dallakyan & Olson, 2015).

2.5 Visualization and Analysis of Docking Results

Only the top scores were selected out of all the RMSD values. Further the ten ligands with the greatest binding affinity ratings for each protein were chosen. The drug-ligand complexes in their 2D form were visualised with the help of BIOVIA Discovery Studio Visualizer. The structure of the ligand and its corresponding target-protein complex showed various types of interactions such as Van der Waals, conventional H-bond, π-anion, π-cation, amide π-stacked, alkyl and π-alkyl, π-sigma, π-π T shaped, Carbon H-bond, π-Sulfur, π-π Stacked, Unfavourable Donor-Donor, and Unfavourable Acceptor-Acceptor interactions (Jha et al. 2023).

2.6 Bioavailability Radar and Pharmacokinetic (SWISS ADME) Profile Analysis

The bioavailability radar is a tool that helps to assess the drug-likeness of a molecule based on six physicochemical properties. The pharmacokinetic analysis report obtained from the SWISS ADME web tool (http://www.swissadme.ch/), evaluates the drug-likeness of a compound based on its bioavailability. The tool is part of the web tools group provided by the Swiss Drug Design platform, which is supported by the Swiss Institute of Bioinformatics. The report provides an assessment of the compound's pharmacokinetic properties, including its ability to be absorbed, distributed, metabolized, and excreted in the body. A compound with high drug- similarity is more likely to be developed into a successful drug, as it has properties that are desirable for pharmacological activity and can be easily absorbed and distributed in the body (Udugade et al., 2019).

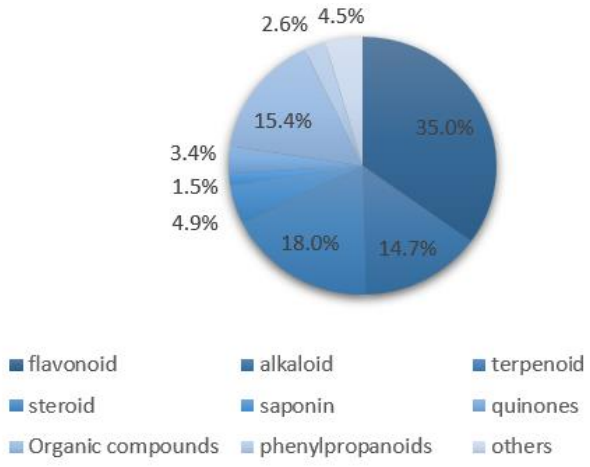


Fig. 1. Phytochemicals classes

2.7 Computational Toxicity Prediction

The ligands that met Lipinski's rule of five were analyzed to predict their potential toxicity using web-based tool, Protox II. It is a virtual laboratory that uses in silico methods to predict the toxicity of small molecules. The canonical smiles of each ligand were used to analyze their toxicity profiles (Banerjee, et al. 2018). These tools can help to identify potential toxicity issues with drug candidates, allowing to make informed decisions about which compounds to prioritize for further testing (Modrow et al. 2013).

2.8 Molecular Dynamics Simulations

After performing docking on the 6 viral proteins, the top ligand showing the best binding affinity was selected for performing a 100ns (nano seconds) long simulation. The software used for these simulations was GROMACS 2023 (GROMACS Documentation Release 2023) on HP-Z4-G4-LLEBSEN workstation with 8 Intel Xeon W-2245(16 threads) and NVIDIA RTX A4000 GPU. The Simulations were performed under AMBER99SB-ILDN force field which are extremely compatible for proteins and their side chains and the ligand was prepared using acpype 2023 (Silva et al. 2012) which were solvated in SCP216 (single point charge) water molecules and TIP3P (transferable intermolecular potential with 3points) water model in a dodecahedron cell of 1nm dimensions from the complex.

2.9 Energy Minimization

After solvation of the complexes, Na^+ Cl^- counter ions were introduced to neutralize the charges and later energy minimization was performed. The first energy minimization included a steepest decent method which was followed by a second energy minimization in conjugate gradient method to ensure that the complex is relax and has flexible interactions with the solvent molecules. Each energy minimization process had about 50000 steps (each step of 2fs) until it reached maximum force of 10 kJ/mol, which allowed us to eliminate any bad contacts present in the complex. Later, these complexes were equilibrated with NVT and NPT simulation process at 300K temperature and 1 bar of pressure for 100 ps. We used V-rescale, a modified Berendsen thermostat and Berendsen barostat along with Partial Mesh Ewald (PME) algorithm for long-range electrostatics and Verlet cut-off for VDW interactions for the equilibrium phases. For the equilibiration the VDW (rvdW) cut-off distance was fixed at 1.2 nm as well as coulomb cut-off (rcoulomb) and neighbourilist (rlist) were fixed to 1.2. Finally, we performed MD production for all the top ranked ligand complexes with the viral proteins for a time period of 100ns which included steps of about 50000000 steps (each step were 2fs). All the trajectories were analysed for their Root Mean Square Deviations (RMSDs), Root Mean Square Fluctuations RMSFs (RMSFs), Rg (Radius of Gyration), Solvent accessible surface areas (SASAs) and the hydrogen bond between protein-ligand complexes.

2.10 MD-Based Binding Energy Calculations

Molecular Dynamics based Binding energy involves studying the interactions energies between protein and ligand. MM-GBSA (Molecular Mechanics- Generalized Born Surface Area) energy methods calculate the end state free energy of the complex. This method calculates the energy difference between the bound and unbound state of the protein-ligand complex or difference between two different solvation states of the same complex. Thus, it analyses the rate of change in the Gibbs Free Energy(ΔG) of the complex. In this study, we performed MM-GBSA calculations to determine the end-state binding free energy of the protein-ligand complexes. In general terms, the binding free energy of the protein with ligand in the solvent can be expressed as:

$$\Delta G_{binding} = G_{complex} - (G_{protein} + G_{ligand})$$

where, ΔG binding represents the binding free energy, G complex represents the free energy of the protein-ligand complex and G protein and G ligand are the independent free energies for the protein and ligand in their isolated solvated state. These calculations are calculated in implicit solvation model, which involve polar and nonpolar solvation energies. For determining the values of the protein complexes, we used the package gmx_MMPBSA, which allowed us to use gromacs trajectories on AMBER MMPBSA method along with providing visualizations on the energies obtained throughout the simulation. This method allows us to predict the binding energies from an ensemble average of the protein-ligand complex trajectories. Solvent Accessible Surface (SASA) model was used to estimate the non-polar solvation energy (Eisenberg et al. 1986, Eisenhaber et al. 1995,

Bondi,1964) term and Coulomb and Lennard-Jones (LJ) potentials were used to extrapolate the (Enon-bonded) include both electrostatic (Eelec) and VDW (EvdW) interactions. Meanwhile, hydrogen bonds also play crucial role in stable binding between the protein-ligands (Hubbard & Kamran 2010). Further to identify which residues are responsible for the binding and stabilization of the complex we performed decomposition analysis. This allowed us to map the exact residues responsible for the energetics of complex formation.

3. RESULTS AND DISCUSSION

Japanese encephalitis is a viral disease transmitted by mosquitoes in rural Asian areas. It leads to brain inflammation and symptoms like fever, headache, and paralysis. While some people don't show symptoms, it can be severe or fatal. Vaccination is crucial for prevention in affected areas. A study focused on how natural chemicals bind to proteins linked to the virus. They used computer models to predict these interactions. They picked 287 chemicals with antiviral properties from plant databases. These compounds, ranging from fats to amino acids, have potential health benefits like reducing inflammation and fighting viruses. The computer predictions revealed which chemicals strongly attach to the virus-related proteins. This offers insight into potential treatments. The study lays groundwork for exploring natural compounds as therapeutic options against Japanese encephalitis, aiding the development of new drugs for this dangerous viral disease (Barrett et al. 2008).

3.1 Molecular Docking

Molecular docking is a computational technique that explores the interactions between proteins and ligands to identify potential drug candidates. This study focused on six specific JEV proteins and 287 bioavailable ligands, analyzing their interactions to rank ligands based on binding energy values (Table 2). Ligands with higher binding affinity scores exhibited strong interactions with the protein binding cavities, primarily through robust hydrogen bonds, favorable electrostatic forces, and π-cation or π-sigma interactions. Among these, Withaferin A emerged as the most potent candidate, demonstrating superior binding affinity and stability across multiple JEV target proteins, including 4K6M, 3P54, and 4HDH. Withaferin A's strong performance was attributed to its precise fit into the protein binding pockets, forming stable hydrogen bonds and π-sigma interactions, alongside favorable electrostatic interactions with key amino acid residues (Fig 2). While other ligands, such as Sanguinarine with 5WSN and Dihydrophicitine with 5MV1, showed moderate interactions, they lacked the stability and interaction strength of Withaferin A. Similarly, Gluacarubolone with 5YWO displayed weaker binding due to limited hydrogen bonds and weaker π-interactions. These findings position Withaferin A as a standout candidate for combating JEV, highlighting the importance of molecular docking in identifying promising therapeutic agents for viral infections. Overall, molecular docking is a powerful technique that can help identify potential drug candidates by predicting the interaction between proteins and ligands. In this study, the researchers used this technique to identify the most promising ligands for inhibiting the proteins associated with Japanese encephalitis virus.

3.2 Evaluation of Pharmacokinetic and Pharmacological Properties

To determine how much the studied phytochemicals' data resembled pharmaceuticals, the Lipinski's rule of five was used. Lipinski's "rule of five," consists of a set of rules, that can be used to predict drug-likeness. According to this, molecules with molecular weights greater than 500, log P greater than 5, hydrogen bond donors greater than 5, and hydrogen bond acceptors greater than 10 have poor absorption or penetration. This rule only explains the molecular features of substances that are connected to their pharmacokinetics. ADME refers to the absorption, distribution, metabolism, and excretion of bioactive substances in a higher organism (Mishra et al. 2009). Therefore, the hydrogen donor-acceptor bond content, molecular weight, and lipophilicity of the medicine are used to determine the rule's application. The drug-likeness of the 287 phytochemicals that showed a greater binding affinity to the selected proteins was assessed using Lipinski's rule of five. Chemicals that didn't follow one or more of Lipinski's rules were eliminated from the study, and the remaining substances went through further testing to find out how dangerous they were. The penetration, metabolism, and absorption of chemicals depend on hydrogen bonding, hence they are also crucial for drug preparation (Neidle 2012).

Table 2. Top ten ligands showcasing highest binding affinity scores against each protein candidate

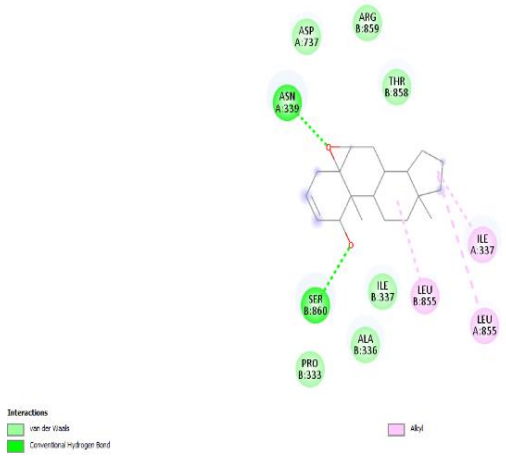
Protein	Ligand Chemical Name	Binding affinity (-kcal/mol)	Grid box parameters
4K6M	Withaferin-A	-9.7	Centre: X:19.35 Y: -46.07 Z:44.45 Dimensions (A°): X:132.69 Y:90.32 Z:94.18
	Castelanone	-9.6	
	Silymarin	-9.4	
	Atalaphillinine	-9.2	
	Hainanolide	-9.1	
	Isolicoflavonol	-9.1	
	Chaparrinone	-9.0	
	Sanguinarine	-8.9	
	Scillarenin	-8.9	
	Glabranin	-8.8	
5WSN	Sanguinarine	-9.2	Centre: X:301.09 Y:223.231 Z:415.37 Dimensions (A°): X: 95.96 Y:72.83 Z:167.92
	Withaferin-a	-9.1	
	Silymarin	-8.9	
	Hainanolide	-8.8	
	Chelerythrine	-8.8	
	Scillarenin	-8.4	
	Glaucarubolone	-8.4	
	Chaparrinone	-8.2	
	Glabranin	-8.2	
	Glycycoumarin	-8.2	
3P54	Withaferin-A	-9.0	Centre: X: 12.54 Y: -16.93 Z:-23.90 Dimensions (A°): X: 44.62 Y:50.16 Z:129.9359
	Sanguinarine	-7.9	
	Scillarenin	-7.8	
	Atalaphillinine	-7.7	
	Isolicoflavonol	-7.7	
	Silymarin	-7.6	
	4',7-dihydroxy-3-methoxy-5,6-dimethylflavone	-7.6	

Protein	Ligand Chemical Name	Binding affinity (-kcal/mol)	Grid box parameters
	Glaucarubolone	-7.5	
	Emetine	-7.5	
	Chelerythrine	-7.4	
5MV1	Dihydrofisetin	-7.5	Centre: X:17.81 Y:-5.11 Z:22.18 Dimensions (A°): X:112.43 Y:39.65 Z:81.66
	Perivine	-7.5	
	Hainanolide	-7.4	
	Pelargonidin	-7.3	
	Naringenin	-7.2	
	Pelargonidin	-7.2	
	Glaucarubolone	-7.2	
	Chrysin	-7.2	
	Scutellarein	-6.9	
	Glycycoumarin	-6.9	
4HDH	Withaferin-a	-10.5	Centre: X:37.54 Y:9.65 Z:28.21 Dimensions (A°): X:76.50 Y:94.75 Z:93.61
	Sanguinarine	-9.9	
	Kaempferol	-9.5	
	Silymarin	-9.4	
	Pelargonidin	-9.3	
	Fisetin	-9.1	
	Morin	-9.1	
	Cryptopleurine	-9.1	
	Deoxypodophyllotoxin	-9.0	
	Camptothecin	-9.0	
5YWO	Glaucarubolone	-7.1	Centre: X:517.76 Y:321.92 Z:604.38 Dimensions (A°): X: 181.60 Y:84.40 Z:72.61
	Hainanolide	-6.7	
	Ternatin	-6.3	
	Quercetin	-6.2	
	5,4'-dihydroxy-3,7,3'-Trimethoxyflavone	-6.2	

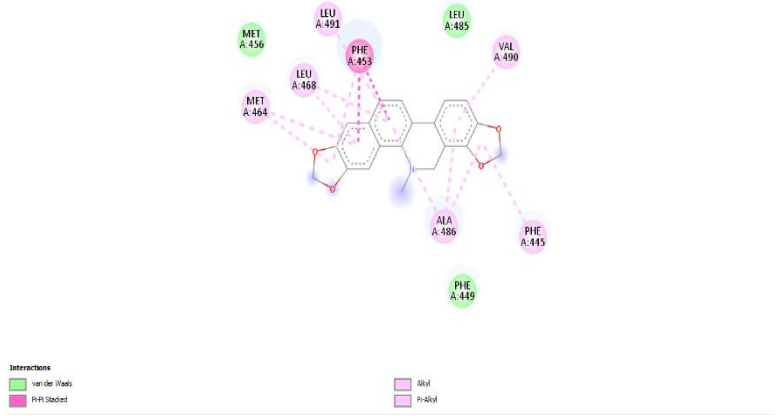
Protein	Ligand Chemical Name	Binding affinity (-kcal/mol)	Grid box parameters
	Quercetin-3,3'-Dimethylether	-6.2	
	Codeine	-6.1	
	Glycycoumarin	-6.1	
	Penduletin	-6.1	
	Arctigenin	-6.0	

Table 3. Classes of toxicity

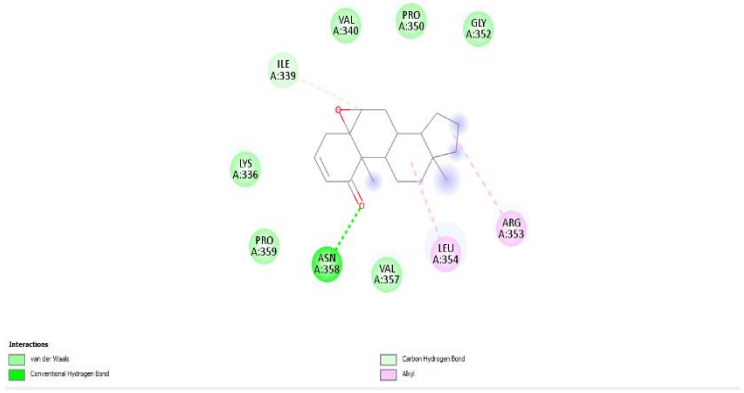
Ligand	Pubchem Id	Predicted ld50	Predicted toxicity class	Hepatotoxicity	Carcinogenicity	Immunotoxicity	Mutagenicity	Cytotoxicity
Atalaphillinine	122681	522mg/kg	4	Inactive	Inactive	Active	Inactive	Inactive
Hainanolide	188356	600mg/kg	4	Inactive	Active	Active	Inactive	Inactive
Glabranin	124049	2000mg/kg	4	Inactive	Inactive	Active	Inactive	Inactive
Perivine	647376	760mg/kg	4	Inactive	Inactive	Inactive	Inactive	Inactive
Arctigenin	64981	1500mg/kg	4	Inactive	Inactive	Active	Inactive	Inactive
Deoxypodophyllotoxin	345501	899mg/kg	4	Inactive	Active	Active	Inactive	Inactive
Withaferin-a	265237	300mg/kg	3	Inactive	Inactive	Active	Inactive	Active
Camptothecin	24360	50mg/kg	3	Inactive	Inactive	Active	Inactive	Active
Codeine	528431	85mg/kg	3	Inactive	Inactive	Inactive	Inactive	Inactive
Cryptopleurine	92765	100mg/kg	3	Inactive	Inactive	Active	Inactive	Active
Castelanone	44170	10mg/kg	2	Inactive	Inactive	Active	Inactive	Inactive
Chaparrinone	73154	10mg/kg	2	Inactive	Inactive	Active	Inactive	Active
Glaucaruboloe	441797	10mg/kg	2	Inactive	Inactive	Active	Inactive	Active



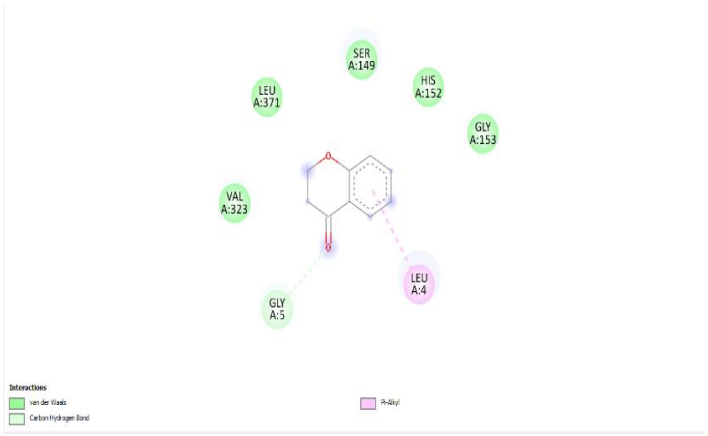
Pic. 1. 4K6M- Withaferin-A



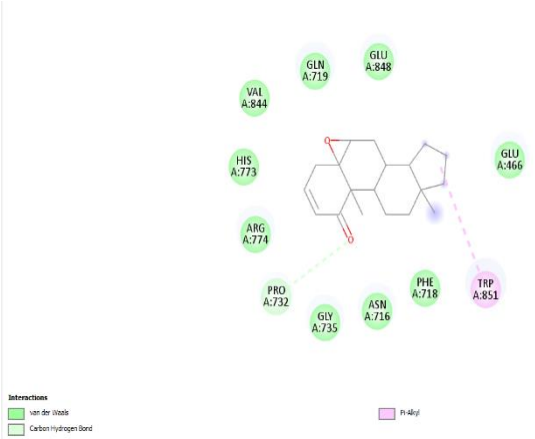
Pic. 2. 5WSN- Sanguinarine



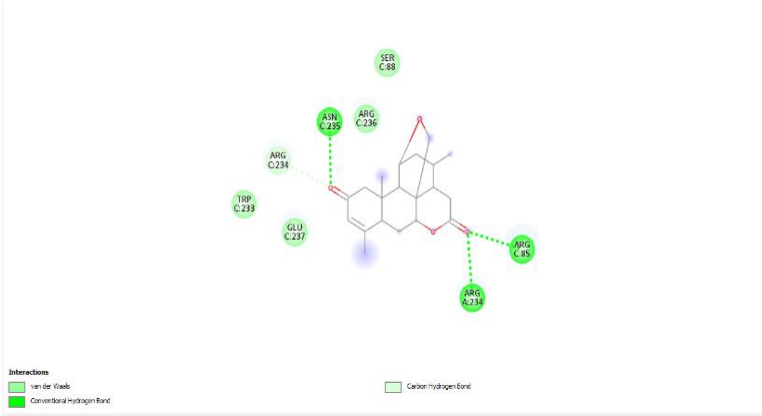
Pic. 3. 3P54-Withaferin-A



Pic. 4. 5MV1- DIHYDROFISETIN



Pic. 5. 4HDH- Withaferin-A



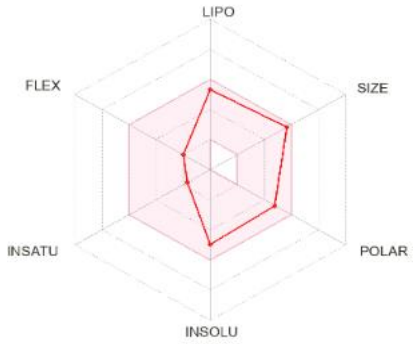
Pic. 6. 5YWO- Glaucarubolone

3.3 Bioavailability Radar and Toxicity Prediction

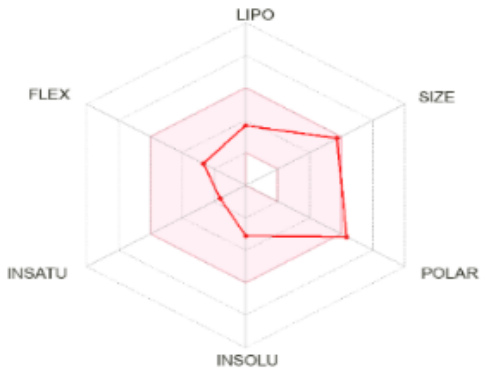
A drug's water solubility is vital for oral bioavailability and absorption. Swiss ADME web tool is a software used to estimate the physicochemical qualities, absorption, distribution, metabolism, elimination, and pharmacokinetic features of molecules, which are important requirements to go forward with clinical experiments. It includes six essential physicochemical properties: flexibility, lipophilicity, saturation, size, polarity, and solubility (Shweta et al. 2011).The molecular weight should be in the range of 150 to 500 g/mol, and the saturation, or the fraction of carbons in the sp3 hybridization, should not be less than 0.25. The parameters for solubility, or LogS should not be greater than 6, flexibility, or XLOGP3, lipophilicity, or TPSA, should be between -0.7 and +5.0, and polarity, or TPSA, should be between 20 and 130 Å6. Compared to alternative forms of medication, oral drug formulations are more widely preferred, simpler to administer, and more cost-effective. They also impose fewer restrictions in terms of sterility. One major challenge for drug developers lies in the fact that a majority of new compounds exhibit low solubility in water, which diminishes their suitability for oral administration. Furthermore, the therapeutic efficacy of these compounds is influenced by their high lipophilicity. Resolving these issues becomes essential to enhance the bioavailability of the medication. The substances evaluated for their effectiveness against JEV have been found to be orally bioavailable. During the development process, computational techniques can be employed to assess the safety of a drug, surpassing the advantages offered by in vitro and in vivo experiments. The PROTOX-II webtool was used to conduct a toxicity analysis on the shortlisted chemicals. In order to determine their toxicity, a number of parameters were used, including predicted LD50, cytotoxicity, carcinogenicity, hepatotoxicity, immunotoxicity, mutagenicity, and oral toxicity (Uzo et al. 2020). Out of the 287 phytochemicals with the highest binding affinity scores for their respective receptors, only 13 ligands were identified to fall within the optimal range indicated by the pink colour on the bioavailability radar (Fig. 2). These 13 ligands were determined to be free from hepatotoxicity and mutagenicity, and they exhibited either active or inactive characteristics for the other assessed toxicity parameters. Out of all the ligands shown in (Fig. 2), Castelanone, Chaparinone, and Glaucarubolone belonged to toxicity class II and had the same LD50 value of 10mg/kg, showing their effect if consumed. The four ligands—Withaferin-A, Camptothecin, Codeine, and Cryptopleurine were classified as having Class III toxicity (50< LD50≤ 300), indicating that they would be slightly toxic and irritating if ingested. The six phytochemical medications Atalaphillinine, Hainanolide, Glabranin, Perivine, Arctigenin, and Deoxypodophyllotoxin, on the other hand, were less hazardous than class II and III pharmaceuticals and fell into toxicity class IV (522< LD50≤ 2000) (Alesawy, et al. 2021).

3.4 Molecular Dynamic Simulation Analysis

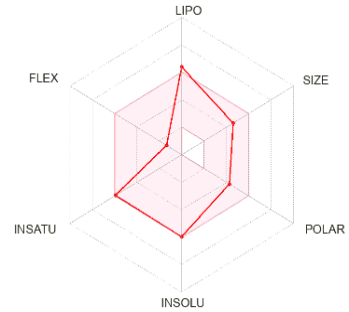
The analysis for RMSDs of the MD Simulation trajectories are shown above (Fig. 3). Among all the complexes, 4HDH and 4K6M complexed with withaferin A, a bioactive compound from Ashwagandha (*Withania somnifera*), exhibited minimal deviations throughout the simulation time. These was followed by 5MV1 complexed with dihydrofesitin in our likeability as it showed some deviation after 70ns. Considering, the RMSDs of the ligand in the complexes as shown (Fig. 4), we find that the ligands in all the complexes show comparatively sdtable deviation except the withaferin A molecule docked with 4K6M . This rules out this complexes as it shows a variable binding and may not have a strong reaction/response as a result. Amongst these Dihydrofesitin bounded with 5MV1 protein showed least deviation followed by withaferin A bounded with 3P54 at start of simulation, however they show larger deviations as the simulation progresses. This brings us to the withaferin A complexes with 4HDH, which showed a stable RMSD, which suggest a strong binding. Therefore, it is possible for it to possess a good physiochemical response on the protein. By following on the Radius of gyration(Rg) (Fig. 5) we find that theprotein 4HDH shows the least Rg. Thus, we can conclude that the protein remains complex and holds on to the ligand. This may allow a strong physiochemical response. Along with this, by comparing the root mean square fluctuation (RMSF) of the protein targets, we find that 4HDH shows a very low fluctuation along its residues and thus we can explain the low Rg.



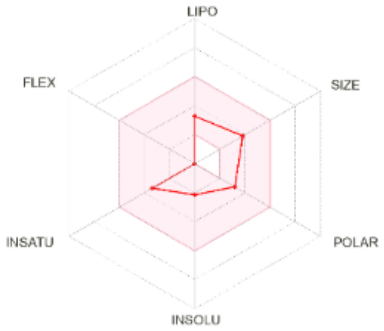
Withaferin-A
Pubchem ID: 265237



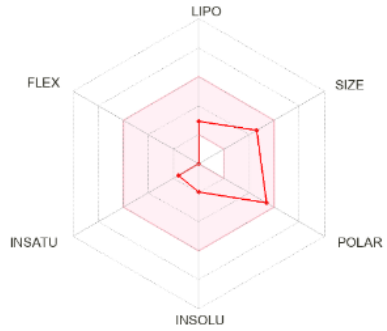
Castelanone
Pubchem ID: 441790



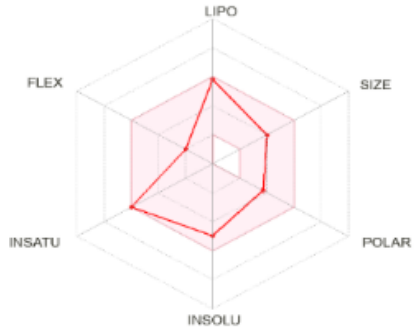
Atalaphillinine
Pubchem ID: 122681



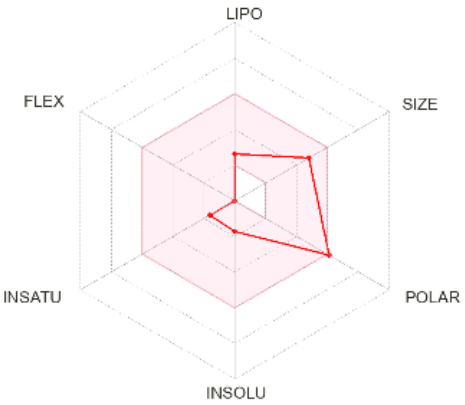
Hainanolide
Pubchem ID: 188356



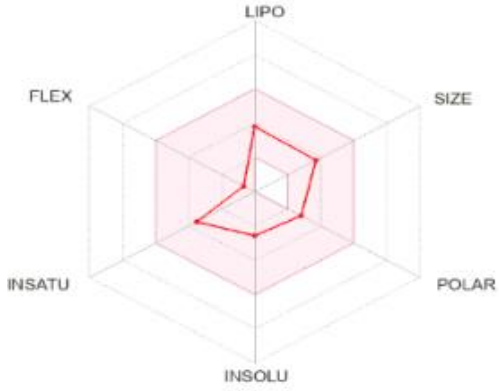
Chaparrinone
Pubchem ID: 73154



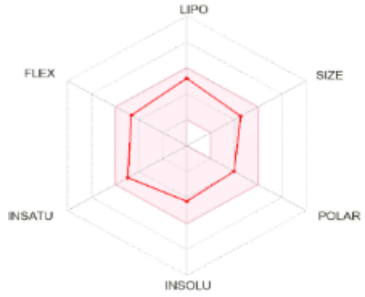
Glabranin
Pubchem ID: 124049



Glaucarubolone
Pubchem ID: 441797



Codeine
Pubchem ID: 5284371



Arctigenin
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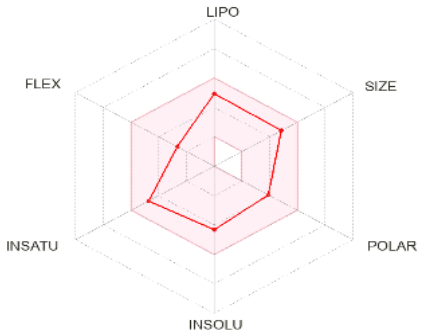

Deoxypodophyllotoxin
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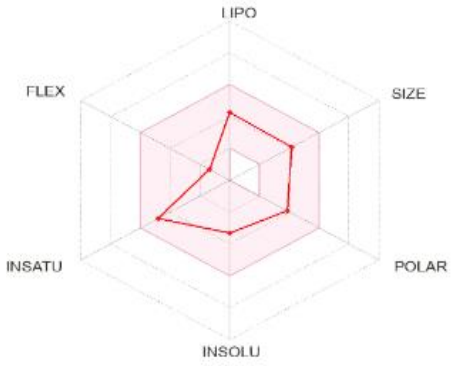
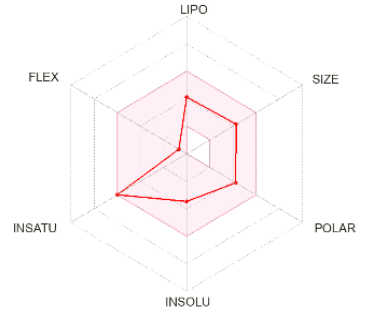
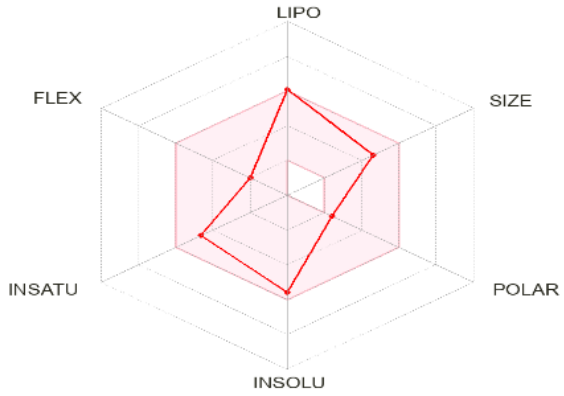


Perivine
Pubchem ID: 6473766



Camptothecin
Pubchem Id: 24360



Cryptopleurine Pubchem ID: 92765

Fig. 2. Bioavailability Radar plot of each shortlisted ligands

However, the complex of 5MV1 with Dihydrofesitin shows a comparatively higher RMSF (Fig. 6), thus it may not be as useful as a potential target for inhibiting proliferation of the virus. Lastly, by examining the SASA of the complexes, we find that 4HDH Complexed with withaferin A shows a decent area exposed to the solvent (water/cytosol), but is not as high as 4K6M as shown in (Fig. 7), which might have served an advantage in holding the ligand bound for a longer period of time.

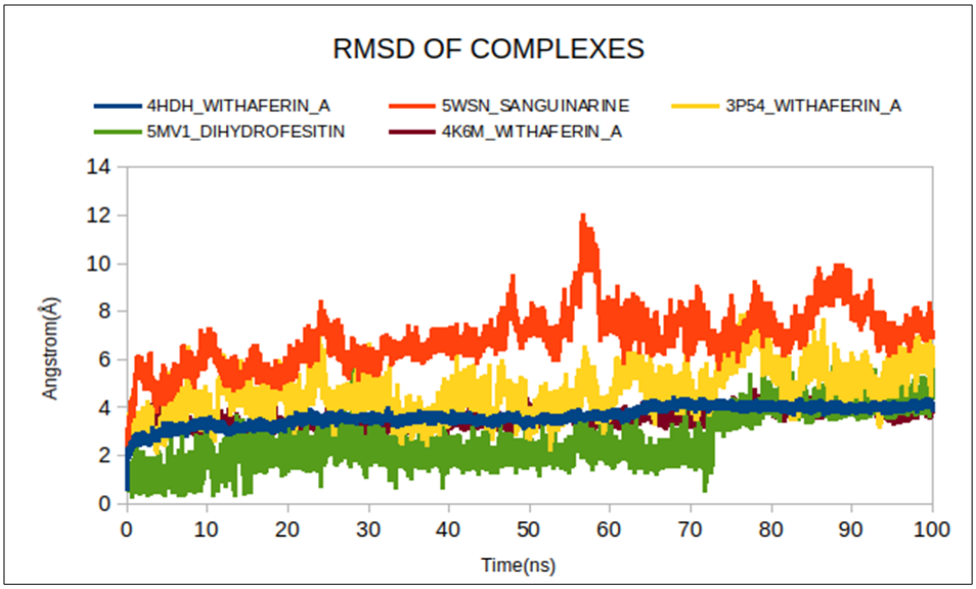


Fig. 3. Root mean square deviations of complexes

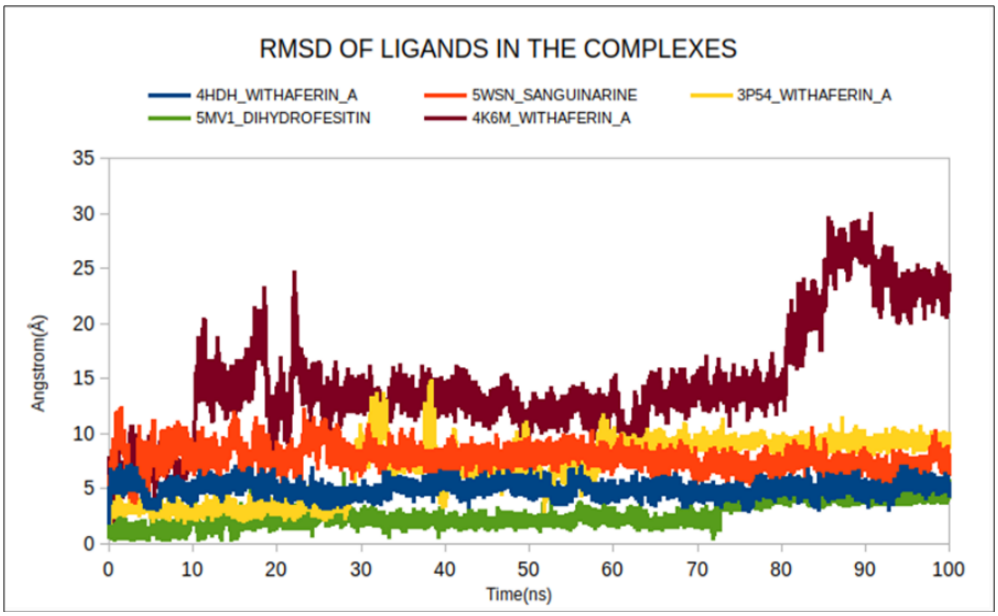


Fig. 4. RMSD of ligands in the complex

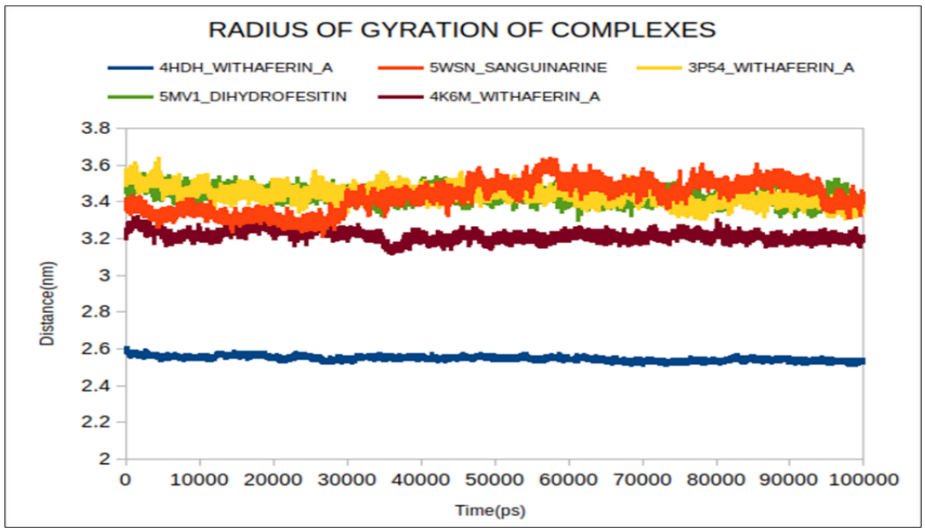


Fig. 5. Radius of gyration

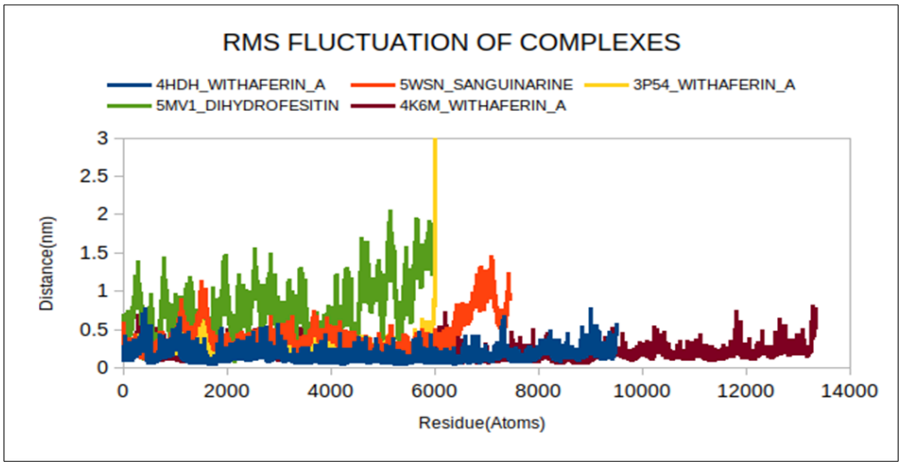


Fig. 6. Root mean square fluctuation

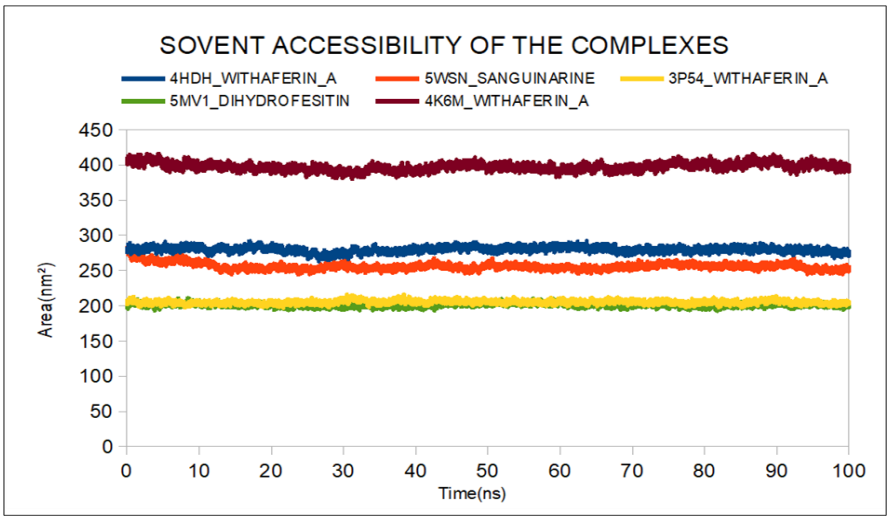


Fig. 7. Solvent accessible surface analysis

3.5 MM-GBSA End State Free Energy Calculations

By performing energy analysis we find the theoretical binding energy released at std. conditions. Out of which, the complex of 4HDH bound to withaferin A showed a good binding energy of -35.42 kJ/mol (Table 4). This gives us an indication that this good binding energy may be due to the spontaneity of the reaction leading to thew formation their complex. Above plots (Figs. 8 & 9) relate to the factors/components that relates to the binding energy of the complex 4HDH_Withaferin A. The Fig. 8 deals with the energy associated with the electrostatic interactions and the forces involved in the bound formation/interaction as well as the energy associated with solvation of the entire complex in water. The plot has significant negative energy which suggest that it would readily dissolve in water and find stability relatively quickly.

The Fig. 9 deals with the change in energies after formation of the complex, which states that the Delta (ΔG) is negative on totalling all the energies associated with complexation. These negative values denote that the structures are favourable and are most likely to lead to physiochemical changes after complex formation. Thus, may provide a therapeutic use to treat the viral infection. Through decomposition binding analysis, we identified specific residues involved in energy contributions (Fig. 10). Among these residues, the ligand (Withaferin A) exhibits regions of high positive enthalpy (red) during the simulation. This positive enthalpy can be associated with energy release, which is compensated by residues exhibiting negative enthalpy contributions (blue). This indicates that the energy required for the ligand to remain bound to the protein 4HDH is predominantly provided by residues with negative enthalpy contributions. Consequently, this binding interaction may potentially disrupt other processes mediated by these residues. Finally, by observing the Delta(ΔG) in the Decomposition Analysis (Fig. 11), we find that the major contribution to change in the decomposition comes the ligand, Withaferin A, which validates the association with the protein 4HDH inhibits the functions of the protein. Visualization is essential for making sense of complex data from MD simulations. It helps understand molecular movement, structure changes, and interactions. Trajectory visualization creates animations to show molecular evolution. 3D structural visualization reveals atomic arrangements. Energy plots track system stability. Radial Distribution Functions analyze particle interactions. Free energy surfaces depict favorable regions. Software like VMD, BIOVIA Discovery Studio, PyMOL, Chimera aided in visualization for the trajectory (Figs. 12 & 13). Meanwhile, the structures were extrapolate using GROMACS.

The analysis of Withaferin A within the complexes 4HDH and 4K6M, derived from *Withania somnifera*, provides essential insights into its interactions and potential therapeutic applications. Both complexes exhibit unique structures with Withaferin A, a bioactive compound known for its diverse pharmacological benefits. These complexes unveil how Withaferin A interacts with specific molecular targets, shedding light on its role in modulating biological pathways. Investigating binding sites and interactions within these structures offers a glimpse into possible mechanisms of action and their effects on cellular processes. Docking simulations show how ligands fit and interact with protein binding sites, providing a clear picture of their orientations. Tools like SwissADME and Lipinski's Rule of Five are used to check the compound's drug-like properties and bioavailability, confirming its potential as a therapeutic option. Molecular dynamics simulations add depth by analysing the behaviour of the protein-ligand complex over time, highlighting its stability and interactions. By studying these movements, researchers can understand the finer details of the binding process. Additionally, safety checks using tools like PROTOX-II offer insights into the potential toxicity of Withaferin A, supporting its role as a safe and effective drug candidate.

Table 4. MM-GBSA end state free energy calculations

	Protein Ligand	Average (kJ/mol)	SD(Prop.)	SD	SEM(Prop.)	SEM
A	4HDH_withaferin A	-35.42	0.53	4.2	0.01	0.04
B	5WSN_sanguinarine	-32.88	0.51	4.59	0.01	0.05
C	3P54_withaferin A	-24.54	6.15	5.96	0.06	0.06
D	5MV1_dihydrofesitin	-23.73	3.83	4.77	0.04	0.05
E	4K6M_withaferin A	-21.36	3.36	8.96	0.03	0.09

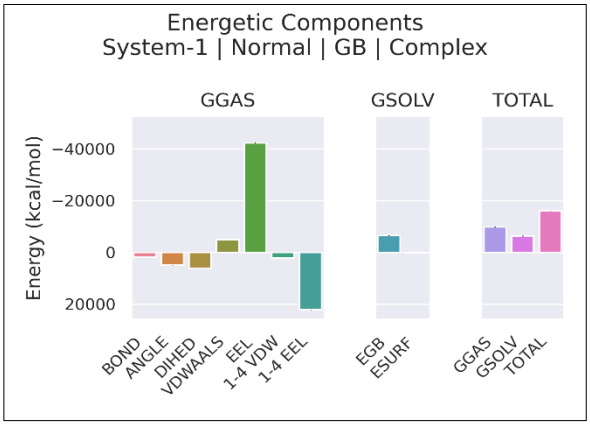


Fig. 8. GB Energies for the Complex [4HDH_withaferin A]

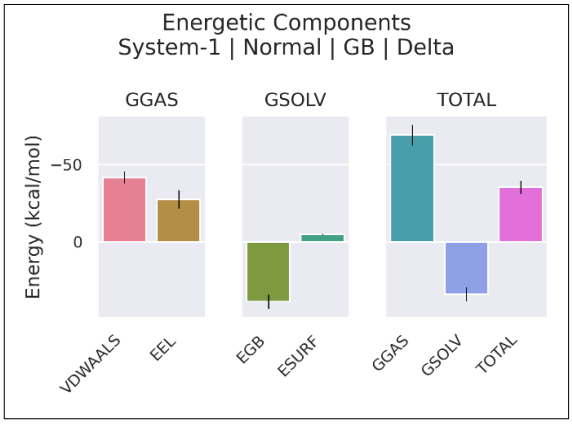


Fig. 9. GB energies for the change in free energy

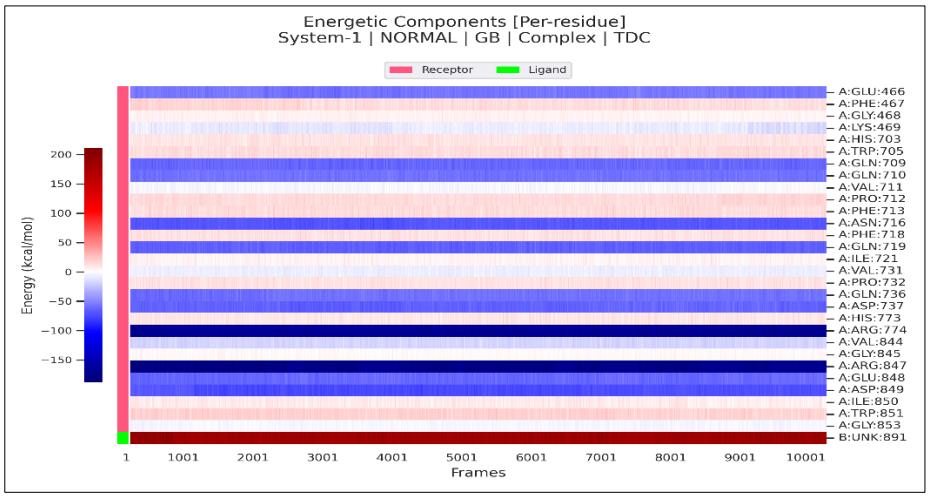


Fig. 10. GB Decomposition Energies for the complex

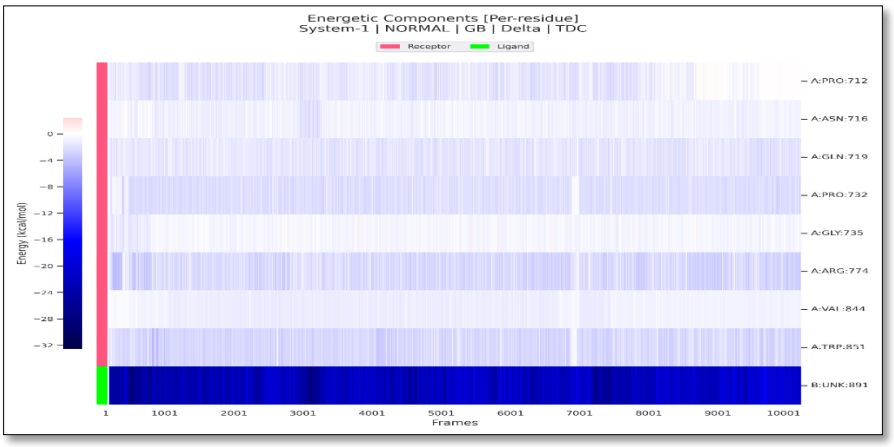


Fig. 11. GB Delta decomposition for the complex

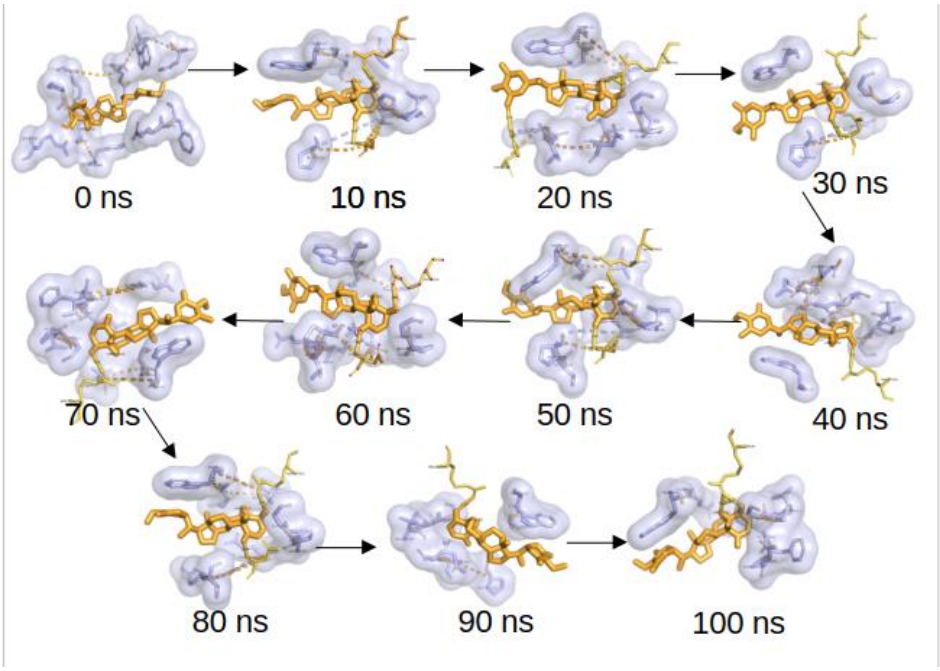


Fig. 12. Visualization of Trajectory of 4HDH_WithaferinA

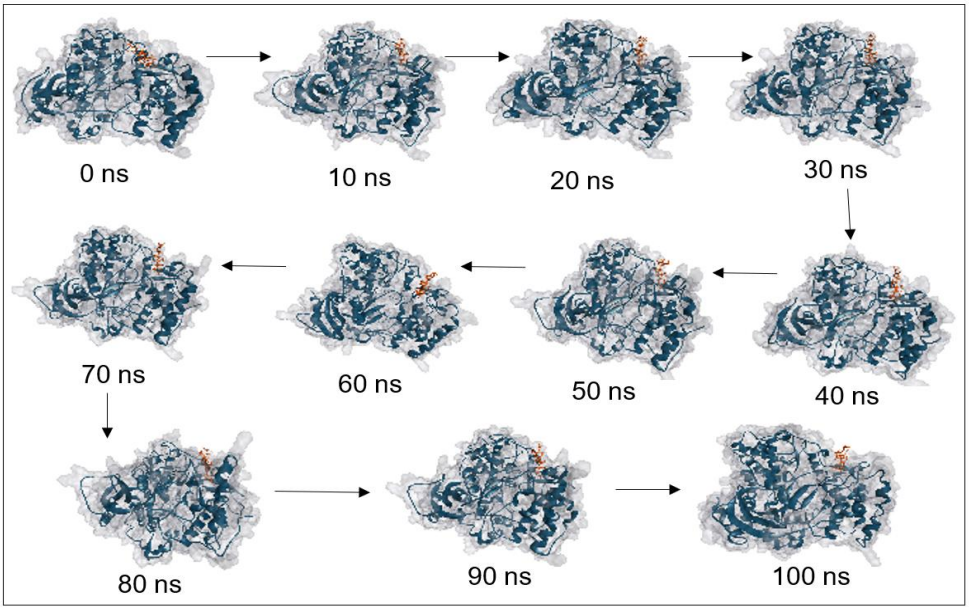


Fig. 13 Visualization of Trajectory in 2-dimensional plot

4. CONCLUSION

The analysis of Withaferin A within the complexes 4HDH and 4K6M, derived from Ashwagandha, provides essential insights into its interactions and potential therapeutic applications. Both complexes exhibit unique structures with Withaferin A, a bioactive compound known for its diverse pharmacological benefits. These complexes unveil how Withaferin A interacts with specific molecular targets, shedding light on its role in modulating biological pathways. Investigating binding sites and interactions within these structures offers a glimpse into possible mechanisms of action and their effects on cellular processes. Docking simulations visually demonstrate interaction orientations, while computational tools like Swiss

ADME and Lipinski's rule evaluate the compound's drug-like properties and bioavailability. Molecular dynamics simulations enrich understanding by exploring dynamic behaviours over time, revealing stability and interactionsVisualizing these trajectories helps unravel intricate motions that drive the binding process (Abate & Garabadu 2024). Safety assessment using tools like PROTOX-II provides insights into potential toxicity associated with Withaferin A. Withaferin A shows strong potential as an effective drug against Japanese encephalitis virus (JEV), according to a detailed analysis (Tiwari et al. 2023). It demonstrates a robust ability to bind with the 4HDH and 4K6M proteins, indicating its capacity to disrupt viral processes. Its adherence to Lipinski's Rule of Five enhances its suitability as a potential drug. Its classification within a safe toxicity range (class 3) and its lack of activity in hypo-toxicity, carcinogenicity, and mutagenicity tests suggest a low risk profile. Notably, its physical characteristics, such as flexibility, lipophilicity, and polarity, align well with the requirements for stable drugs. Its active response in immunotoxicity and cytotoxicity tests suggests it could both modulate the immune system and eliminate infected cells. Molecular dynamics simulations show promising binding to five protein targets, with the 4HDH complex demonstrating particularly strong binding energy, indicating stability. Structural analyses further support the suitability of the 4HDH_withaferin A complex. In conclusion, these findings highlight Withaferin A as a promising candidate for the treatment of Japanese encephalitis virus (JEV). Its multifaceted interactions and potential therapeutic properties warrant further investigation to confirm its effectiveness and safety in clinical settings. JEV, a mosquito-borne pathogen primarily affecting the central nervous system, causes brain inflammation and poses a significant risk of long-term neurological damage or fatality. While vaccination remains the most effective preventive measure, Withaferin A offers a prospective therapeutic option that merits deeper exploration (Tiwari et al., 2024). Numerous investigations encompass epidemiological analysis for transmission patterns, understanding the virus's pathogenesis, and the creation of vaccines and treatments. JEV remains a pressing public health issue across Asia, necessitating continuous research to refine comprehension and bolster preventive and curative strategies. Analysing Withaferin A's interactions in Ashwagandha-derived 4HDH and 4K6M complexes provides pivotal insights into its therapeutic potential. These interactions illuminate the compound's role in impacting biological pathways, revealing its effects on cellular processes through binding site explorations and docking simulations. Computational assessments such as Swiss ADME and Lipinski's rule confirm its drug-like attributes, while molecular dynamics simulations unveil stability and interactions. Safety evaluation with PROTOX-II highlights potential toxicity linked to Withaferin A. Overall, Withaferin A showcases promise as a potential JEV treatment, demonstrating robust binding to 4HDH and 4K6M proteins, adherence to Lipinski's Rule of Five, and a favourable toxicity profile. Its active modulation of immune and cytotoxic responses underscores its therapeutic potential. It also agrees on the approach of targetting RdRps (RNA-Dependent RNA Polymerase) as evident from the results of Withaferin A against 4HDH within the viral body from the paper by Alshammari SO (Alshammari 2024), wherein they tested phytochemicals from Brown algae for anti-viral activity and study conducted by Aftab Alam et al. with phytochemicals derived from Zingiberaceae plants (Aftab et al. 2024). The study also finds that a targeted approach against NS5 is highly effective, as demonstrated by the MD simulation results of Withaferin A binding to 4K6M, corroborating the findings reported by Tiwari R.K. et al (Tiwari et al. 2023). We also observed that even Protein E from the virus can have strategic importance as observed in MD simulations of Dihydrofestin with 5MV1. A study on SARS-CoV-2 explores a natural compound Wi-A as potential inhibitors of TMPRSS2, crucial for viral entry (Ullah et al. 2024). Wi-A binds to TMPRSS2's catalytic site, with stronger interactions. Wi-A's diverse content in *Withania somnifera* (Ashwagandha) suggests potential management of SARS-CoV-2 outbreak and drug development direction. Further research is needed for confirmation (Kumar et al. 2022, Yuda et al. 2024).

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

ETHICS APPROVALS

This study does not involve any experiments on either animals or human subjects, and therefore no ethics approval was required.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

Abate, S. K., & Garabadu, D. (2024). Virtual screening, molecular dynamics simulations, and antiviral evaluation of *Ocimum basilicum* phytoconstituents against Japanese encephalitis virus. *Preprint*. https://doi.org/10.21203/rs.3.rs-4888640/v1

Alam, A., Asma, A., Anjum, E. H. M. T. J. A. I. F. F. A. F. F. A. A. M. A. K. M. K. W., & Balaha, M. F. (2024). Zingiberaceae-derived phytomolecules inhibit Japanese encephalitis virus RNA-dependent RNA polymerase: A molecular dynamics study. *Journal of Biomolecular Structure and Dynamics*. https://doi.org/10.1080/07391102.2024.2102167

Alesawy, M. S., Elkaeed, E. B., Alsfouk, A. A., Metwaly, A. M., & Eissa, I. H. (2021). In silico screening of semi-synthesized compounds as potential inhibitors for SARS-CoV-2 papain-like protease: Pharmacophoric features, molecular docking, ADMET, toxicity, and DFT studies. *Molecules, 26*(24). https://doi.org/10.3390/molecules26247874

Alshammari, S. O. (2024). Marine brown algae-derived compounds as potential inhibitors of Japanese encephalitis virus RNA-dependent RNA polymerase. *Marine Drugs, 22*(1), 12. https://doi.org/10.3390/md22010012

Banerjee, P., Eckert, A. O., Schrey, A. K., & Preissner, R. (2018). ProTox-II: A webserver for the prediction of toxicity of chemicals. *Nucleic Acids Research, 46*, W257–W263.

Barrett, A. D. T. (2008). Japanese encephalitis virus. *Encyclopedia of Virology* (pp. 182–188). https://doi.org/10.1016/B978-012374410-4.00434-9

Bondi, A. (1964). Van der Waals volumes and radii. *The Journal of Physical Chemistry, 68*(3), 441–451. https://doi.org/10.1021/j100785a001

Chen, K. C., et al. (2019). Molecular interaction of the antiviral compound CW 33 and its analogues with the NS2B NS3 protease of the Japanese encephalitis virus. *International Journal of Molecular Medicine, 43*, 2024–2032.

Consortium, wwPDB. (2019). Protein Data Bank: The single global archive for 3D macromolecular structure data. *Nucleic Acids Research, 47*, D520–D528.

Dallakyan, S., & Olson, A. J. (2015). Small-molecule library screening by docking with PyRx. In *Methods in Molecular Biology* (Vol. 1263, pp. 243–250). Springer.

Eisenberg, D., & McLachlan, A. D. (1986). Solvation energy in protein folding and binding. *Nature, 319*, 199–203.

Eisenhaber, F., Lijnzaad, P., Argos, P., Sander, C., & Scharf, M. (1995). The double cubic lattice method: Efficient approaches to numerical integration of surface area and volume and to dot surface contouring of molecular assemblies. *Journal of Computational Chemistry, 16*(3), 273–284. https://doi.org/10.1002/jcc.540160308

GROMACS Development Team. (2023). *GROMACS Documentation Release 2023.1*.

Gupta, S. K., Singh, S., Nischal, A., Pant, K. K., & Seth, P. K. (2013). Molecular docking and simulation studies towards exploring antiviral compounds against envelope protein of Japanese encephalitis virus. *Network Modeling and Analysis in Health Informatics and Bioinformatics, 2*, 231–243.

Hubbard, R. E., & Kamran Haider, M. (2010). Hydrogen bonds in proteins: Role and strength. In *eLS*. Wiley. https://doi.org/10.1002/9780470015902.a0003011.pub2

Jha, V., et al. (2023). Multitargeted molecular docking study of phytochemicals on hepatocellular carcinoma. *Journal of Applied Biology & Biotechnology, 11*, 116–130.

Kumar, V., et al. (2022). Withanone and withaferin-A are predicted to interact with transmembrane protease serine 2 (TMPRSS2) and block entry of SARS-CoV-2 into cells. *Journal of Biomolecular Structure and Dynamics, 40*(3), 1–13. https://doi.org/10.1080/07391102.2022.2032035

Mishra, H., Singh, N., Lahiri, T., & Misra, K. (2009). A comparative study on the molecular descriptors for predicting drug-likeness of small molecules.

Modrow, S., Falke, D., Truyen, U., & Schätzl, H. (2013). Viruses with single-stranded, positive-sense RNA genomes. In *Molecular Virology* (pp. 185–349). Springer Berlin Heidelberg.

https://doi.org/10.1007/978-3-642-20718-1_14

Neidle, S. (2012). Design principles for quadruplex-binding small molecules. In *Therapeutic Applications of Quadruplex Nucleic Acids* (pp. 151–174). https://doi.org/10.1016/B978-0-12-375138-6.00009-1

Pettersen, E. F., et al. (2004). UCSF Chimera—a visualization system for exploratory research and analysis. *Journal of Computational Chemistry, 25*, 1605–1612.

Serhan, M., et al. (2019). Total iron measurement in human serum with a smartphone. In *AIChE Annual Meeting, Conference Proceedings* (Vol. 2019-November). American Institute of Chemical Engineers.

Shweta, M., Rashmi, D., & Mishra, S. (2011). In-vitro ADME studies of TUG-891, a GPR-120 inhibitor using Swiss ADME predictor. *Journal of Drug Delivery and Therapeutics, 9*(2-S), 2710. https://doi.org/10.22270/jddt.v9i2-s.2710

Silva, D., & Vranken, B. F. (2012). ACPYPE—AnteChamber PYthon Parser InterfacE. *Research Notes, 5*. Retrieved from http://www.biomedcentral.com/1756-0500/5/367

Singh, A., Kumar, A., & Srivastava, V. (n.d.). Molecular docking studies of antiviral drugs against NS3 helicase of Japanese encephalitis virus. Retrieved from http://autodock.scripps.edu/resources/adt

Tiwari, R. K., Pandey, V., Ojha, R. P., Pandey, V., & Pandey, M. (2024). Adenosyl derivatives as potential inhibitors of NS3 protease of Japanese encephalitis virus (JEV): In silico molecular insight into therapeutic discovery. *Computational and Theoretical Chemistry, 1241*, 114848. https://doi.org/10.1016/j.comptc.2024.114848

Tiwari, R. K., Pandey, V., Srivastava, H., Srivastava, A. K., & Pandey, V. (2023). Docking and MM study of non-structural protein (NS5) of Japanese encephalitis virus (JEV) with some derivatives of adenosyl. *Frontiers in Chemistry, 11*, 1145. https://doi.org/10.3389/fchem.2023.1145

Tiwari, R. K., Pandey, V., Srivastava, H., Srivastava, A. K., & Pandey, V. (2023). Docking and MM study of non-structural protein (NS5) of Japanese encephalitis virus (JEV) with some derivatives of adenosyl. *Frontiers in Chemistry, 11*, 1145. https://doi.org/10.3389/fchem.2023.1145

Udugade, S. B., Doijad, R. C., & Udugade, B. V. (2019). In silico evaluation of pharmacokinetics, drug-likeness, and medicinal chemistry friendliness of momordicin1: An active chemical constituent of *Momordica charantia*. *Journal of Advanced Scientific Research, 10*. http://www.sciensage.info/jasr

Ullah, A., et al. (2024). Identification of new pharmacophore against SARS-CoV-2 spike protein by multi-fold computational and biochemical techniques. *Scientific Reports, 14*, 2315. https://doi.org/10.1038/s41598-024-35252-9

Uzo, A., Christopher, O., & Christan, I. (2020). Molecular docking, toxicity and antimicrobial studies of p-nitrobenzene sulphonamide bearing leucine-isoleucine dipeptide carboxamides. *Preprints*. https://doi.org/10.22541/au.159129068.82389963

Yuda, G. P. W. C., Hanif, N., & Hermawan, A. (2024). Computational screening using a combination of ligand-based machine learning and molecular docking methods for the repurposing of antivirals targeting the SARS-CoV-2 main protease. *DARU Journal of Pharmaceutical Sciences, 32*, 47–65. https://doi.org/10.1007/s40199-024-00395-3

Yun, S. I., & Lee, Y. M. (2014). Japanese encephalitis: The virus and vaccine. *Human Vaccines & Immunotherapeutics, 10*, 263–279.

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