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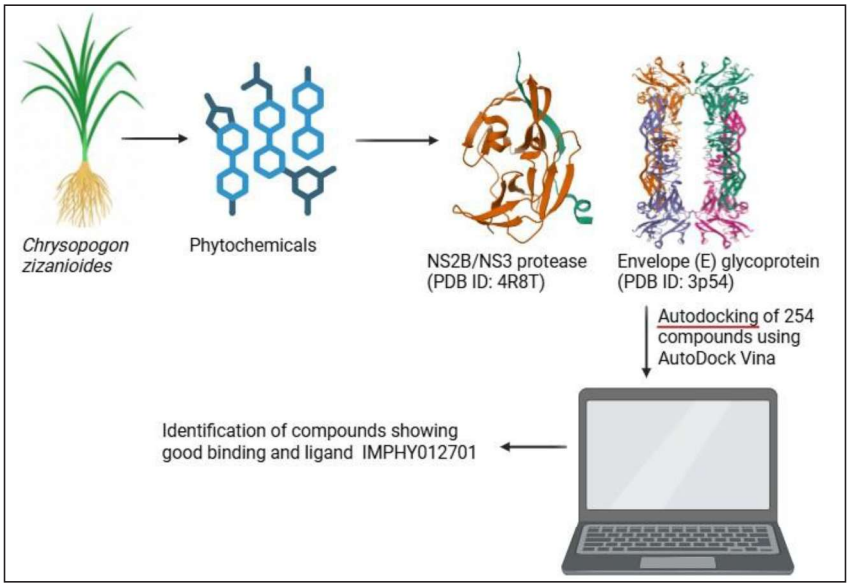
In Silico Screening of Phytochemicals from *Chrysopogonzizanioides* Targeting Key Proteins of Japanese Encephalitis Virus

Pardeep Yadav[1,2], Aakhya Chauhan[2] and Saurabh Kumar Jha[3*]
[1]Department of Biotechnology, Sharda School of Engineering and Technology, Sharda University, Greater Noida, Uttar Pradesh, India
Email: par.yadav2011@gmail.com
[2]Department of Zoology, School of Applied and Life Sciences, Uttaranchal University, Prem Nagar, Dehradun, Uttarakhand, India
Email: aakhya08@gmail.com
[3]Department of Zoology, Kalindi College, University of Delhi, Delhi, India
Email: jhasaurabh017@gmail.com
[*]Corresponding Author: jhasaurabh017@gmail.com

Abstract: Japanese Encephalitis Virus (JEV) is spread by mosquitoes and it is a major infection risk for people in Asia. Existing vaccines are good at preventing disease, but due to new viral forms, additional strategies are now required for treatment. For this study, molecule screening was performed virtually using *Chrysopogonzizanioides'* compounds against vital JEV proteins like NS2B/NS3 protease (PDB ID: 4r8t) and the envelope glycoprotein (PDB ID: 3p54). A total of 253 phytochemicals were picked, reduced for energy and put into PDBQT format using AutoDock Vina in PyRx for docking. Target proteins were built by improving the structure and performing flexible docking simulations latently. Bonds between molecules were examined to choose inviting interactions for drug development. Some of the compounds showed good binding and ligand IMPHY012701 showed the strongest connections against both targets which suggests it might work as a dual-action inhibitor. These results serve as a base for making plant-derived drugs for JEV and prove how structure-based virtual screening can speed up finding new drugs.



Keywords: *Chrysopogonzizanioides*, Japanese Encephalitis Virus (JEV), NS2B/NS3 protease inhibition, Plant-derived antivirals, Virtual screening.

I. Introduction

In Asia, JEV is a mosquito-carried flavivirus and a key reason for cases of viral encephalitis, making it a big public health problem across the region. Transmitted primarily by Culex

mosquitoes, especially *Culex tritaeniorhynchus*, JEV maintains a zoonotic cycle involving pigs and water birds as amplifying hosts. Humans are incidental, dead-end hosts, meaning they do not contribute to the virus's transmission cycle. The disease predominantly affects children under 15 years of age, with symptomatic cases being rare but severe. Approximately 1 in 250 infections leads to clinical illness, which can result in high fever, seizures, coma, and death. The case-fatality rate among those with encephalitis can be as high as 30%, and 30–50% of survivors suffer from permanent neurological or psychiatric sequelae (Japanese encephalitis n.d.) in 24 countries across the WHO South-East Asia and Western Pacific Regions, exposing more than 3 billion people to the risk of infection. Annual estimates suggest about 68,000 clinical cases and over 13,000 deaths globally. However, underreporting and limited surveillance capabilities in many endemic areas likely mean these figures are underestimates. A large-scale investigation from 2007 to 2021 in 14 countries where JE is found that vaccination stopped 193,676 cases, 43,446 deaths and 77,470 sequelae, saving people 6.6 million years when disability and health loss are taken into account and saving more than USD 19 million in treatment expenses (Japanese encephalitis n.d.; Moore 2021; Mulvey *et al.,* 2021).

Prevention of JE is most successful through vaccination. Several different vaccines have been developed and introduced, including mouse brain vaccines, weakened live viruses and vaccines with pieces from other animals. The live attenuated SA14-14-2 vaccine from China is being used in India, Nepal, Cambodia, South Korea, Thailand and Sri Lanka. It is known for being strong enough to trigger an immune response, inexpensive and safe to use. After just one dose of its chimeric live-attenuated vaccine, Sanofi Pasteur's vaccine for JE can induce serum antibody positivity in 99.1% of vaccinated populations (Li *et al.,* 2025).

These developments have not fully overcome all the issues. Because of the appearance of genotype V (GV), there are worries about how effective vaccines that use genotype III (GIII) still are. Evidence suggests that GIII-based vaccines may not protect individuals against GV viruses, making them more likely to suffer outbreak-related problems. As a result, we must regularly check the strains of JEV and make vaccines that protect against many types of the virus (Mulvey *et al.,* 2021).

Innovations related to vaccine technology are being examined. Specifically, researchers have made mRNA-LNP vaccines that protect mice from JEV and are poised to proceed to clinical trials in people. New approaches can enable fast design and rollout, particularly when dealing with new types of JEV strains (Zhu *et al.,* 2024).

Typical diagnostic strategies in virology, for example, PRNT, ELISA and RT-PCR, are regularly applied, but they have disadvantages such as high costs, lengthy procedures and the need for unique equipment and expertise. The latest developments aim to make sensitive and affordable diagnostics, using immunosensors and electrochemical sensors along with the benefits of nanotechnology (Mohsin *et al.*, 2022).

Non-structural proteins NS3 and NS5 are involved in replicating the virus and may therefore be targeted by medicines. NS3 has protease and helicase functions, NS5 acts as an RdRp and it blocks the immune response triggered by host interferon. Treatments that target these proteins might be used to develop drugs against JEV (Li *et al.,* 2025; Zhu *et al.,* 2023).

In addition, microRNAs (miRNAs) now play an important role in the development of JEV disease. It has been determined that miRNA-124 blocks JEV replication by targeting host genes involved in viral growth. Working with these miRNAs might provide new approaches for treating infections caused by JEV (Basu and Dutta, 2017).

Although excellent steps have been taken to address JEV, there are still challenges that require more efforts in identifying new variants, enhancing diagnostics and discovering potent antiviral medications. Reducing the number of cases in regions affected by this disease will depend on combining vaccination with enhanced watching, study of trends and actions by the public health system (Mulvey *et al.,* 2021).

II. Material and Methods

A. Ligand Preparation

A total of 253 chemicals from *Chrysopogonzizanioides* were identified and collected from the peer-reviewed literature and databases like IMPPAT 2.0 and PubChem, in 3D .sdf form. Using the MMFF94 force field on Open Babel, the geometries of these structures were adjusted to make them free of strain. Once minimization was accomplished, each ligand was changed to PDBQT format in AutoDock Tools which included torsion degrees of freedom, Gasteiger charges and polar hydrogens. As a result, each compound could be used correctly in molecular docking tests on the protein targets.

B. Target Protein Preparation

Two significant aggregates of the JEV were chosen as the targets for carrying out docking experiments on their three-dimensional crystal structures. The NS2B/NS3 protease (PDB ID: 4R8T) was included for its role in cleaving the viral polyprotein, as was the envelope (E) glycoprotein (PDB ID: 3p54), necessary for host cell identification and entry of the virus. Both structures were found in the .pdb format at the RCSB Protein Data Bank. PyMOL and UCSF Chimera were both used to refine and prepare the protein. Heteroatoms,

water molecules and any co-crystallized ligands or ions were first eliminated to avoid non-specific interactions docking results. Visual observation of the proteins was used to confirm their shape.

Following this, polar hydrogens were inserted and the Gasteiger charge was assigned through AutoDock Tools (ADT). The rotatable bonds and torsions were defined where necessary, and the refined protein structures were saved in PDBQT format, compatible with AutoDock-based docking simulations. This thorough preparation ensured that the binding sites were exposed and accessible for accurate ligand-protein interaction screening.

C. Structure Based Virtual Screening

Virtual screening of 253 phytochemicals retrieved from the IMPPAT 2.0 database, which catalogues phytochemicals from Indian medicinal plants, was carried out using PyRx 0.8, a virtual screening tool that integrates molecular docking via the AutoDock Vina engine. This screening was performed to assess the binding potential of the selected phytochemicals against two essential proteins of the, namely the NS2B/NS3 protease (PDB ID: 4r8t) and the envelope glycoprotein (PDB ID: 3p54). The ligands, previously prepared in PDBQT format, along with energy-minimized target proteins, were imported into the PyRx environment. To ensure unbiased blind docking, grid boxes were defined to encompass the entire ligand-binding or active regions of both target proteins. For the NS2B/NS3 protease (4r8t), the grid was centered at x = -18.119, y = -23.3628, z = 8.8772 with dimensions x = 75.3322 Å, y = 59.9887 Å, z = 53.9207 Å, while for the envelope glycoprotein (3p54), the grid center was set at x = -14.0553, y = -20.2039, z = -13.2723 with dimensions x = 53.6412 Å, y = 78.0345 Å, z = 163.8087 Å. The docking exhaustiveness parameter was maintained at the default value of 8 to balance accuracy and computational efficiency. Each ligand was docked into the binding site of both proteins, and binding affinities were recorded in kcal/mol for the top-ranked conformations. The docking results were subsequently exported, analyzed, and compounds exhibiting the most negative binding energies were shortlisted. These selected ligands were subjected to further interaction analysis and visual inspection using PyMOL and Discovery Studio Visualizer. Through this approach, potential lead compounds from *Chrysopogonzizanioides*—as listed in the IMPPAT 2.0 database—were identified that may interfere with key viral functions by targeting the JEV protease and envelope proteins.

D. Molecular Docking

Molecular docking studies were conducted using the latest version of AutoDock Vina, integrated as a plugin within the most recent release of UCSF Chimera. The crystal structures of the selected JEV target proteins—NS2B/NS3 protease (PDB ID: 4R8T) and envelope glycoprotein (PDB ID: 3P54)—were prepared by removing water molecules, adding polar hydrogens, and assigning Gasteiger charges. Top 10 Screened energy-minimized phytochemicals retrieved from the IMPPAT 2.0 database were used as ligands in molecular docking. The grid box parameters, including dimensions and center coordinates, were adopted from validated PyRx-based virtual screening protocols to ensure consistency and reproducibility in binding site definition. All docking simulations were carried out using the default exhaustiveness setting of AutoDock Vina, ensuring a balance between accuracy and computational efficiency. The docked complexes were evaluated based on their binding affinities (in kcal/mol), and further analyzed to identify hydrogen bonds, hydrophobic interactions, and the key amino acid residues involved in ligand binding. This protocol enabled the identification of potential inhibitors targeting critical viral proteins of JEV.

III. Result and Discussion

A. Screening of Compounds

A structure-based virtual screening of 253 chemicals from *Chrysopogonzizanioides* was performed using PyRx version 0.8, a free and widely used virtual screening platform that integrates AutoDock Vina for docking calculations. Two target proteins were selected: NS2B/NS3 protease (PDB ID: 4R8T) and envelope glycoprotein (PDB ID: 3P54). The protein structures were prepared in PDBQT format by removing water molecules and other non-standard residues, followed by the addition of polar hydrogens and assignment of Gasteiger charges using AutoDock Tools. Both the ligands and energy-minimized proteins which were readied in PDBQT format, were imported to use within PyRx. We marked out areas on the proteins, called grid boxes that include their binding or active regions for unbiased blind docking. For the NS2B/NS3 protease (4r8t), I centered the grid at x = -18.119, y = -23.3628, z = 8.8772, with dimensions x = 75.3322 Å, y = 59.9887 Å, z = 53.9207 Å. This study kept the default value of 8 for the docking exhaustiveness parameter to ensure accuracy at a reasonable processing rate. All ligands were fitted into the active sites of the two proteins and the binding affinities were noted for the top results in kcal/mol. To achieve both good accuracy and speed, the default value of 8 was used for the docking exhaustiveness parameter. All ligands were fitted into the binding site of the proteins and the binding affinities for the best conformations were reported in kcal/mol. Following docking, the results were exported, examined and the compounds with the most negative binding energies were chosen (Table S1 and S2).

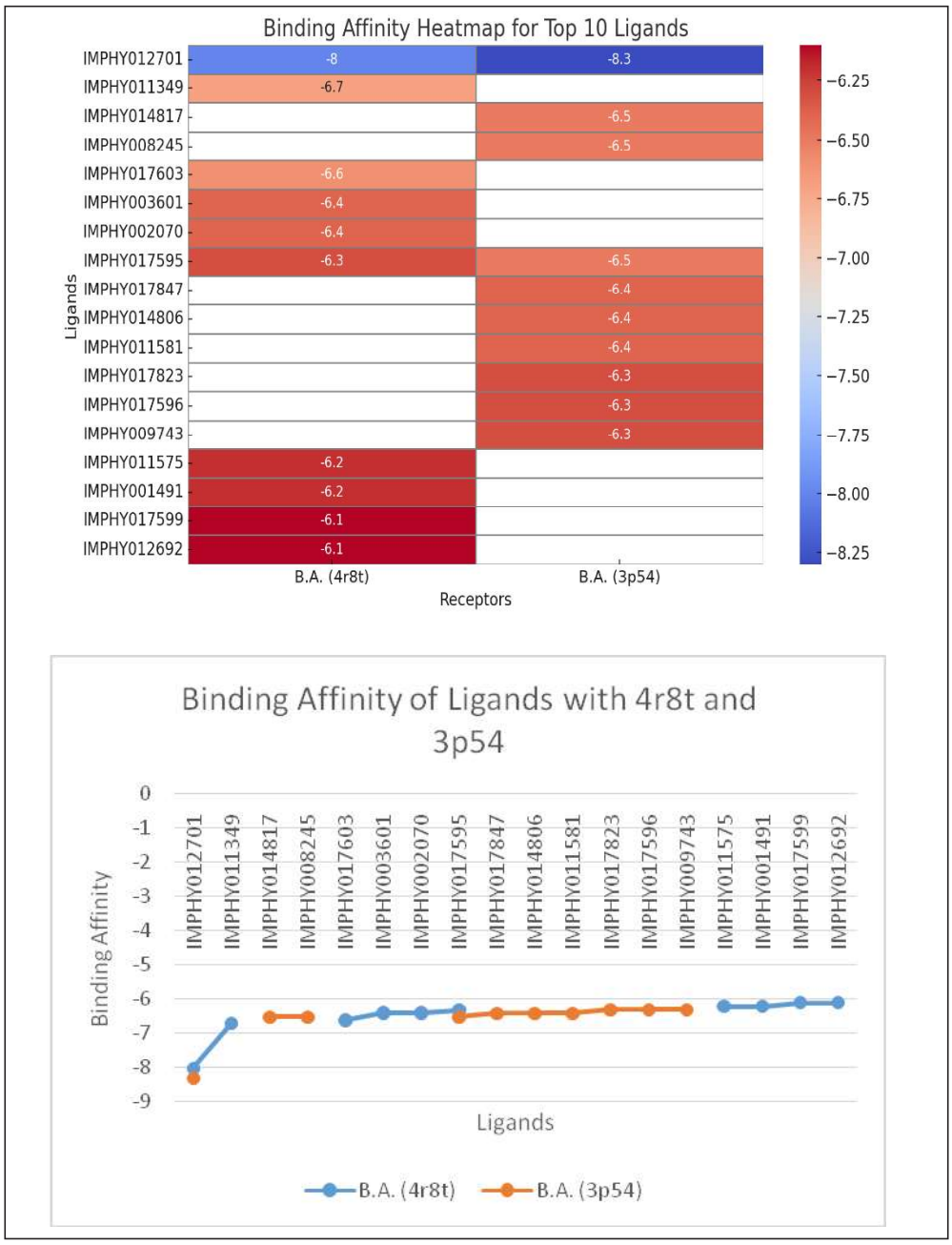


Fig. 1

Fig. 1 represents the heatmap illustrates the binding affinities (B.A.) of various ligands with two protein targets—4r8t and 3p54—highlighting the interaction strength where lower B.A. values indicate stronger binding. Ligands are listed on the X-axis, while the Y-axis denotes their corresponding affinity values. Color coding distinguishes between the proteins: blue shades represent affinities with 4r8t, and red shades indicate affinities with 3p54. Missing data points are excluded for clarity. Notably, ligand IMPHY012701 exhibits the strongest binding to both proteins, particularly to 3p54, as reflected by its highly negative affinity value. This visual representation aids in identifying the most promising ligands for further study.

B. Docking Results

A structure-based virtual screening was carried out using AutoDock Vina through the UCSF Chimera interface to assess the binding affinity of top 10 screened chemicals from *Chrysopogonzizanioides* against two proteins: NS2B/NS3 protease (PDB ID: 4R8T) and envelope glycoprotein (PDB ID:

3P54). All ligands were docked at predefined active sites, and the resulting binding affinities (kcal/mol) and root mean square deviation (RMSD) values were same as found in screening (Table S3 & S4). Among all the ligands, IMPHY012701 has the strongest interaction with both proteins in particular with 3p54, as its affinity value strongly suggests (Fig. 2).

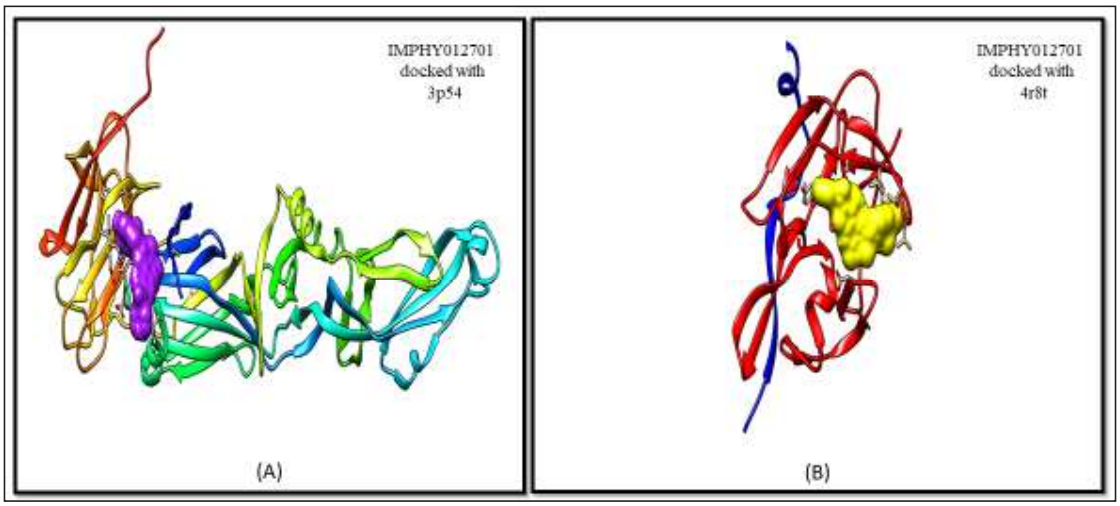


Fig. 2: Interaction of IMPHY012701 Ligand with (A) 3p54 and (B) 4r8t Protein Molecules

IV. Conclusion

The present study employed a structure-based virtual screening approach to identify potential antiviral compounds from *Chrysopogonzizanioides* against key JEV proteins—NS2B/NS3 protease and the envelope glycoprotein. A total of 253 phytochemicals were studied for their binding strengths with each target by using PyRx, AutoDock Vina and UCSF Chimera. According to the evaluation, a number of compounds stood out for their high affinity for the proteins, with IMPHY012701 showing the highest favourable interactions toward both viral proteins and especially the envelope glycoprotein (PDB ID: 3p54).

The NS2B/NS3 protease is crucial for dividing the polyprotein and the envelope glycoprotein is responsible for viral entry, so both proteins can be important targets for treatments against the virus. Since strong affinity is seen, these molecules may target JEV replication and affect the pathways involved in JEV infection. Specificity of these phytochemicals towards target proteins is possible due to hydrogen bonding and hydrophobic contacts among their molecules.

This simulation study offers a solid starting point for designing antivirals that come from natural materials. These results are encouraging, but they must be confirmed by tests carried out outside the body to check the drug's behaviour, safety and ability to fight the virus. Besides, changing the structure of lead compounds can make them easier for the body to use and target where they are needed.

The current research suggests that the phytochemicals from *Chrysopogonzizanioides* could be valuable for treating JEV. Linking computer-based drug research with traditional medicine gives a solid approach to finding new antiviral drugs against neglected diseases such as JEV.

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