IN SILICO INVESTIGATION OF PHYTOCHEMICALS DERIVED FROM *CATHARANTHUS ROSEUS* AS POTENTIAL THERAPEUTIC AGENTS TARGETING THE A42R PROTEIN OF THE HUMAN MONKEYPOX VIRUS

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BIOTECHNOLOGY

by

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CANDIDATE'S DECLARATION

I ESHITA, Roll Number: 23/MSCBIO/83, hereby certify that the work which is being presented in the thesis entitled-"In silico investigation of phytochemicals derived from *Catharanthus roseus* as potential therapeutic agents targeting the A42R protein of the human monkeypox virus" in partial fulfilment of the requirements for the award of Master of Science, submitted in the Department of Biotechnology, Delhi Technological University is an authentic record of my work carried out during the period from January 2024 to June 2025 under the supervision of Dr. Navneeta Bharadvaja. The matter presented in the thesis has not been submitted by me for the award of any other degree of this or any other Institute.

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Eshita

Candidate's Signature

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CERTIFICATE

Certified that Eshita (23/MSCBIO/83) has successfully carried out the research work presented in this thesis under my supervision entitled as "In silico investigation of phytochemicals derived from *Catharanthus roseus* as potential therapeutic agents targeting the A42R protein of the human monkeypox virus", for the award of the degree of Master of Science in Biotechnology.

The thesis is an original piece of research carried out independently by the student. The findings presented in this study are original and have not been previously submitted, whether wholly or partially, for the conferral of any academic degree or diploma at this or any other university or institution.

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In Silico Investigation of Phytochemicals Derived from *Catharanthus roseus* as Potential Therapeutic Agents Targeting the A42R Protein of the Human Monkeypox Virus.

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ABSTRACT

The Human Monkeypox virus (MPXV) causes an animal-borne disease and belongs to the Orthopoxvirus genus. In 2022, the largest outbreak of this disease led to an epidemic in various countries. As stated by the World Health Organisation (WHO), physical contact with affected animals, persons, or contaminated surfaces may serve as possible transmission routes for the virus infection. Symptoms may appear after an incubation period of about 7-14 days, including myalgia, fever, fatigue, body aches, headaches, skin lesions, and lymphadenopathy.

JYNNEOS and ACAM2000 are both FDA-approved vaccines used to prevent Monkeypox disease however, the latter vaccine can cause severe conditions like pericarditis and myocarditis in immunocompromised vaccinated individuals. Thus, drugs that can target the virus are in high demand, according to current circumstances. Therefore, *Catharanthus roseus,* a medical plant with active phytochemical compounds, can serve as a potential source for synthesizing herbal drugs. In this research, using molecular docking, the leading active compounds observed in the plant showed remarkable interactions with suitable binding affinities. Barassinolide surpasses other phytochemicals regarding binding energy, i.e., -8.7 kcal/mol, making it the most promising phytochemical that can be used as a therapeutic target.

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CHAPTER 1

INTRODUCTION

The Monkeypox virus was first reported in Africa and belongs to the *orthopoxvirus* genus of the *Poxviridae* family. The 'monkeypox' name came after it was found in macaque monkeys. However, the origin of the virus is unknown to date, but it is estimated that it is transmitted via various small mammals and rodents. Humanity's history has seen various viral epidemics lead to major effects on the health of the world population, as well as on society. HIV-1 (Human Immunodeficiency Virus-1), smallpox, Ebola, influenza, SARS-CoV-2 (severe acute respiratory syndrome coronavirus-2), and SARS (severe acute respiratory syndrome) have all demonstrated the devastating consequences of viral outbreaks, highlighting the urgent need for effective surveillance, treatment, and prevention strategies [1]. Human Monkeypox Virus was initially recorded in 1958, when a vesicular disease occurred among monkeys mobilized from Africa to Denmark and Copenhagen for study. in 1970, on August first human case of hMXPV was recorded in a 9-year-old child having skin lesion symptoms similar to smallpox, in Bukenda village is located in the equatorial region of the Democratic Republic of Congo (DRC) [2].

The World Health Organization (WHO) Director-General, on 23rd July, amid the 2022 outbreak of the Human Monkeypox Virus, with the majority of cases in Europe, declared it a Public Health Emergency of International Concern (PHEIC) [3]. The 2022 outbreak affected 118 countries, having confirmed cases or suspected cases. Overall, 64851 verified and 3673 presumed cases were documented as of 2022-09-23. Out of 64851 confirmed cases, 248 cases had a travel history [4]. Under the present circumstances, there are 3 FDA-authorized vaccines to treat smallpox and

monkeypox diseases. As of now, we do not have any antiviral medications specifically for the monkeypox infection treatment. Although some medications for smallpox treatment, such as cidofovir, tecovirimat, and Brincidofovir, can be used for the monkeypox infection treatment [1]. Therefore, it is crucial to explore potential drug targets. Historically, herbal medicines were widely used to treat various infections and diseases because of their abundant therapeutic bioactive substances, which laid the foundation for the development of plant-based drug formulations. Plant-based medications have shown great results against several viruses, such as influenza viruses, coronaviruses, and poxviruses. This opens the door for exploring antiviral plant-based medications for the Human Monkeypox Virus. Researchers have worked on several alkaloids, triterpenes, flavonoids, and phenolic compounds metabolites known for their antiviral properties. Recent studies showed that curcumin derivatives are powerful antiviral agents effective against smallpox and monkeypox viruses [5]. Utilizing computational methods like molecular docking, researchers can efficiently screen large libraries of chemical compounds to identify phytochemicals with potential therapeutic effects.

This dissertation explores the potential phytochemicals for the cure of the Monkeypox virus by utilizing in silico methods. We will focus our study on finding the phytochemicals that can inhibit the A24R protein of the Human Monkeypox Virus. Additionally, we investigate the ability of various compounds extracted from *Catharanthus roseus* to inhibit the same protein. Using molecular docking simulations, we examine the binding affinity and potential effects of these compounds, thereby enhancing our understanding in the development of novel therapeutic strategies.

1.2 Importance of Targeting the A42R Protein

The A42R protein (PDB-4QOW) encoded by the gp153 gene in hMXPV is significantly similar in terms of amino acid sequence to the profilin protein of eukaryotic cells [6]. The structure A42R protein is reported to be the 1st and only hMPXV protein documented in the Protein Data Bank (PDB). In 1990, studies were conducted to determine its biochemical significance. Further studies showed the significance of the protein in viral replication in the host's cells [7].

The A42R protein, found in orthopoxviruses, is known to share structural similarity with host actin-binding proteins, thus suggesting its role in manipulating the cellular machinery of the hosts to benefit viral replication and spread [8]. However, A42R interacts weakly with actin where whereas other cellular profilin proteins interact strongly [9]. Actin is mainly related to the disease-causing ability of different poxviruses by influencing the viral transmission to the surrounding host cells.

In the studies of other homologous profilin proteins present in various poxviruses, it is reported that alpha-tropomyosin (a cellular 38kDa actin-binding protein) interacts directly with the profilin-like protein of the virus and colocalizes with surface tubules, suggesting the involvement of tropomyosin in virus mobility [10]. A42R, interaction with phosphatidylinositol lipids, this interaction suggests that it uses the mechanism that manipulates the host cell and wraps the virus, required to fuse it with the plasma membrane, and also protects it in the cytoplasm [9] [11]. Given its critical role in cytoskeletal modulation and its uniqueness to the viral proteome, A42R presents a promising and selective target for antiviral therapy. Despite its importance, A42R remains largely unexplored in terms of drug targeting, presenting an opportunity for the discovery of first-in-class inhibitors. This highlights the A42R protein as a valuable target for developing new antiviral treatments against the monkeypox virus. By focusing on this protein, researchers may be

able to interfere with important stages of the virus's life cycle, potentially stopping it from spreading inside the body. Targeting A42R could also help create therapies that are more specific to the virus, reducing the chances of harming healthy human cells and improving treatment safety.

1.3 Phytochemicals as a Therapeutic Approach

Phytochemicals are bioactive compounds derived from plants that have become a cornerstone in modern drug discovery, contributing significantly to the pharmaceutical market. Compared to randomly synthesized molecules, drugs obtained from natural sources often demonstrate better biocompatibility, improved safety profiles, and fewer side effects. The treatment via plant-derived compounds is known as phytotherapy, provides a promising and holistic approach. The phytochemicals perform diverse biological roles, which include disrupting cell membranes, inhibiting key proteins, or interacting directly with nucleic acids such as RNA or DNA. Unlike synthetic drugs, the effectiveness of phytochemicals often results from synergistic interactions, where multiple compounds enhance each other's effects or act on different points of the same biological pathway.

Mechanisms underlying these interactions may involve improved bioavailability, altered transport within cells, transformation of prodrugs into active forms, or neutralization of harmful metabolites. In addition, many phytochemicals can modulate entire signalling cascades through multi-target activity, offering broader therapeutic coverage [12].

As the viral disease epidemics are threatening the entire world, the 2022 monkeypox outbreak and the 2020 coronavirus outbreak are recent examples that highlight the urgent need to develop effective antiviral drugs against different kinds of viruses. Although some antiviral drugs are on the market at present for the treatment of some viral diseases, for some, there are no antiviral medicines or vaccines

present in the market. Synthetically developed drugs are directly or indirectly related to harmful side effects.

Due to this, the demand for antiviral drugs formulated via plant compounds is on the rise, as their toxicity is low and they have a low possibility of developing resistance. Phytochemicals have been in use for a long period, used to cure several illnesses, and studies show that they are a great candidate for a drug as they exhibit anti-viral, antibacterial, anti-cancerous, and antioxidant properties. Most of these compounds act by interfering with viral processes, either by blocking the virus's entry into the host cell or by disrupting its replication cycle [13]. Around 2,500 plant species with medicinal properties have been identified worldwide and are traditionally used to manage a vast range of medical conditions in humans and other animals. The bioactive phytochemicals found in this plant have shown promising antiviral properties, making them valuable candidates in the fight against various viral infections, such as alkaloids, quinone, flavonoids etc [14]. Despite the vast therapeutic promise of phytochemicals, their application still faces several challenges, such as standardization, bioavailability, efficacy, and safety in clinical use. However, recent progress in computational methods like molecular docking has greatly improved the ability to screen and identify effective phytochemicals, thereby speeding up the drug discovery in this area.

1.4 In Silico Approach in Drug Design

In silico techniques are becoming gradually vital in modern drug discovery, offering a cost-effective approach to identifying potential therapeutic candidates. These computational strategies help reduce reliance on animal testing, support the rational development of new and safer drugs, and enable the repurposing of existing pharmaceuticals. By streamlining various stages of the drug discovery process, CADD tools provide valuable insights for medicinal chemists and pharmacologists, enhancing efficiency and

precision in the search for effective treatments [15]. Ligand-protein docking is a potent software tools for estimating the binding affinities of selected ligands and with chosen proteins, with exceptionally precise and effective. This approach is essential in the drug discovery and design process, as it helps identify promising lead compounds that can bind selectively to targeted proteins, thereby influencing their biological function [16].

At its foundation, ligand-receptor binding simulation is a computer based technique that is used study how different ligands interact with proteins binding site in three-dimensional space. Using specialized algorithms that consider factors like the ligand's flexibility, possible structural changes in the protein, and electrostatic compatibility, docking tools can estimate the most stable and energetically favourable way a ligand fits into the protein's binding pocket [17].

The precision and dependability of molecular docking results are influenced by multiple factors, such as the resolution and quality of the target protein structure, the selection of an appropriate scoring function, and the consideration of solvent interactions and protein flexibility [18]. Furthermore, comparing docking outcomes with experimental evidence like crystal structures or measured binding affinities is crucial for evaluating the accuracy and effectiveness of docking algorithms. Due to its computational nature, molecular docking has become a potent tool in virtual screening efforts, enabling the efficient identification of promising and effective drug candidates from extensive chemical libraries. Researchers can select and prioritize potential leads by screening thousands of compounds in silico.

1.5 Study Objective and Scope

This research primarily seeks to employ molecular docking strategies to identify potential bioactive phytochemicals present in *Catharanthus roseus*, targeting the desired protein found in Human

Monkeypox Virus leads to inhibition of the virus. Specifically, the research aims to:

1. Select a protein which is widely known for its essential role in infection mechanism, based on a comprehensive review of the literature and expert consultation.

2. Build a database of phytochemicals with potential ligand activity by sourcing compounds from various chemical libraries and bioactivity databases.

3. Perform molecular docking simulations to evaluate interactions between the target protein and selected plant compounds acting as ligands, taking into account ligand flexibility and potential structural alterations in the receptor.

4. Assess the precision and predictive strength of the docking results by comparing them with available experimental data, where applicable, to ensure the reliability of the computational method.

5. Examine the highest-ranking docking poses and scoring parameters to select the most promising leading compounds for further research, taking into account factors such as binding affinity, target specificity, and drug-like properties.

6. Evaluate the contribution of the findings for designing novel treatments by targeting the A42R protein found in the human monkeypox virus, emphasizing their potential to contribute to effective antiviral drug development using plant-derived compounds.

The scope of this research involves computational and theoretical investigations to explore the molecular mechanisms through which plant-derived phytochemicals may inhibit the A42R protein found in monkeypox virus. While the study is centred around molecular docking as a predictive approach, it also incorporates concepts from structural biology, pharmacology, and bioinformatics to develop an in-depth understanding of the interactions between chosen phytochemicals and the targeted viral protein. This integrative strategy aims to support the identification of novel antiviral candidates capable of disrupting viral function at the molecular level.

CHAPTER 2

Literature Review

The Human Monkeypox virus, a concerning viral animal borne disease in humans, this virus is related to Orthopoxvirus genus in the Poxviridae family [19]. It is a dsDNA virus, has a 200kb long DNA sequence containing highly conserved genes encoding for replication machinery and viral assembly complex in the middle of the genome, and various genes related to its virulence and host determination in the terminal ends. Based on the morphology of MPXV, which resembles that of other orthopoxviruses (OPXVs), the virions appear as ovoid or brick-shaped structures surrounded by a lipid-protein which is the outer membrane. The MPXV genome encodes all the proteins necessary for viral DNA replication, transcription, and the assembly of new virions, and their release from the cell [20]. The MPXV may be classified mainly into 2 genetic groups, i.e., the Clade I-Congo basin and Clade II-west African group. Former exhibits higher death rates in comparison to the latter, due to some deletions and fragments in open reading frames (ORF), which can be the reason for the low virulence of the West African group [2][21]. Research shows that the Central African monkeypox virus interferes with triggering T-cells and also suppresses cytokine release, which causes inflammation in infected host cells. Moreover, this strain appears to specifically impair the host's immune response by suppressing apoptosis and blocking the transcription of certain immune-related genes [22].

Human monkeypox typically presents with symptoms observed mainly in children and adults from Central and West Africa. The illness starts with a viral prodrome lasting 1–3 days, marked by fever, chills, headache, body aches, muscle aches, and back pain. Subsequently, a maculopapular rash develops, which is usually

monomorphic and spreads in a centrifugal pattern. The rash evolves through vesicular and pustular stages before forming crusts throughout 2 to 3 weeks. Marked lymphadenopathy is a distinguishing feature of monkeypox [23].

The transmission routes of the Human Monkeypox Virus can be divided into zoonotic transmission (1˚transmission) and interhuman transmission (2˚transmission). This involves injuries from infected animals, eating poorly cooked infected meat, exposure to infected blood or bodily fluids, and direct contact with cutaneous/ mucosal lesions, sexual contact with infected humans, respiratory droplets, and contact with contaminated surfaces/objects. A few studies show that pregnancy during the infection can lead to vertical transmission and perinatal loss [24][25].

Monkeypox is a reappearing viral threat, a global health issue because of its interhuman transmission and the absence of defined antiviral treatments. Its symptoms can range from fever and rash to severe systemic complications, particularly in immunocompromised individuals. This study aims to contribute to the search for effective therapeutic strategies by targeting the A42R protein found in the monkeypox virus using plant-derived phytochemicals. The docking simulation serves as a key computational approach to evaluate the binding potential of these compounds. By simulating protein-ligand interactions, this method helps identify promising lead molecules with inhibitory activity against A42R, supporting the development of novel plant-based antivirals.

2.2 Exploring Molecular Targets for Antiviral Drug Development in Orthopoxviruses

The World Health Organization (WHO) have currently classified human monkeypox virus (hMPXV), an animal-borne infection, as a global Health Emergency and states that at present, we lack specific treatments potential for Mpox infection. Medicinal herbs play a crucial part in both the medical field and economical development,

especially in developing countries.

The main structural proteins, like A29,L1R, and,H3L, exhibit critical roles in the virulence of the monkeypox virus and play a vital role in both viral invasion and host immune activation. Proteins like A35R, B6R, and A42R responsible for viral growth in host cells, can be identified as potential vaccine targets. MPXV employs several immune evasion strategies, including hiding its DNA, downregulating interferon production, inhibiting T and natural killer cells stimulation, also blocking regulated cell death. These mechanisms present significant challenges in the development of effective vaccines and treatments [26].

Orthopoxvirus replication involves a complex series of steps, each presenting potential molecular targets for antiviral intervention. Key targets include structural and entry proteins such as A27L, H3L, and B5R that facilitate viral attachment and fusion, as well as transcriptional regulators like Rap94 and A8L, which initiate early gene expression. DNA replication is primarily driven by the E9L DNA polymerase and associated proteins, making them effective targets for nucleoside analogs such as cidofovir and CMX001. Additional enzymes like viral thymidine kinase (J2R), ribonucleotide reductase, and protein kinases (B1R, F10L) also regulate viral replication and interfere with host immunity. Later stages involving genome packaging, virion assembly, and egress rely on proteins like I7L, D13L, F13L, and A36R, several of which are inhibited by drugs like ST-246 (Tecovirimat) and mitoxantrone. Together, these components of the viral life cycle offer multiple strategic points for therapeutic targeting [27].

Unlike bacterial and fungal infections, viruses rely on living host cells for replication, which makes their control particularly challenging. They integrate into host cells at both functional and structural levels, making it difficult to distinguish and eliminate them. Additionally, certain viruses can remain dormant as latent infections, posing long-term health concerns. The limited availability of effective antiviral treatments, coupled with the risks

of drug toxicity and the emergence of cross-resistant viral strains, further complicates management. Consequently, increasing attention is being directed toward plant extracts and phytochemicals as promising alternative strategies for combating infectious diseases in livestock, with ongoing research exploring their antiviral potential [28].

2.3 Phytochemicals of *Catharanthus roseus* and Their Antiviral Potential

Originally indigenous to Madagascar, *Catharanthus roseus,* typically referred to as the Madagascar periwinkle, is a well-known therapeutic plant recognized for its extensive pharmacological properties, related to Apocynaceae family. Studies suggest that the bioactive compounds found in this plant demonstrate higher antibacterial, antiviral, antifungal, and anticancer properties. Several phytochemicals, such as vindolinine, periformyline, leurocristine, perivine, perividine, vincaleukoblastine, pericalline, carosine, and leurosivine have demonstrated antiviral activity against both vaccinia virus, poliovirus type III, and dengue virus type 2. [29][30]. Further, *C. roseus* extract demonstrated wide-ranging antibacterial activity against *Salmonella typhi, Pseudomonas aeruginosa, Staphylococus aureus,* and *shigella boydii.* It also exhibits antifungal activity against *Ganoderma philippii, Rigidoporus microporus, and Phellinus noxius* [31]. Vincristine and vinblastine, originally extracted from *Catharanthus roseus*, were notably the first plant-based anticancer compounds introduced into clinical practice. More recently, several newly extracted indole alkaloids present in this plant, such as 14′,15′-didehydrocyclovinblastine, 17-deacetoxyvinamidine, catharoseumine, and 17-deacetoxycyclovinblastine have shown potent inhibitory effects on cultured human cells. In addition, phytochemicals like vindolicine, vindolinine, vindolidine, and vindoline, extracted from the *C.*

roseus, have demonstrated promising antidiabetic properties. Mentioned findings demonstrate that this flora remains a valuable reservoir of bioactive compounds and warrants further scientific exploration [32]. *Catharanthus roseus* is also useful in the treatment of hypertension and diabetes mellitus. This plant also exhibits anti-inflammatory, antimicrobial, antithrombotic, antioxidant, anti-allergic, and vasodilatory effects and cardio-protective properties [33].

The broad spectrum of bioactive compounds found in *Catharanthus roseus*, including several indole alkaloids with known antiviral, anticancer, and antidiabetic properties, reinforces its importance as a valuable resource in modern drug development. Given the urgent need for effective antiviral therapies against emerging infections such as monkeypox, the exploration of *C. roseus*-derived phytochemicals offers a promising strategy. In this study, these compounds were analysed via in molecular interaction modelling to assess their binding potential against the A42R protein found in monkeypox virus, a structurally unique and functionally important viral target. The integration of traditional medicinal knowledge with computational tools not only supports the relevance of *C. roseus* in antiviral research but also lays the groundwork for the development of plant-based therapeutics against Orthopoxvirus infections.

2.4 Structural and Functional Insights into the A42R Protein and Related Orthopoxvirus Targets.

Structurally, A42R protein (PDB-4QOW) is similar to host actin-binding protein in terms of amino acid sequence it is composed of 157 amino acid residues, that is encoded by the gene gp153 in monkeypox virus. The protein structure was resolved using X-ray diffraction at a high resolution of 1.52 Å, revealing a compact globular folds, typical of profilin family proteins. The structure includes two chains (A and B) [34][6].

The aim to consider A42R protein as the target protein of

monkeypox virus is because this is the first and only monkeypox virus protein documented in the protein data bank, i.e., PDB [7]. Various studies have been carried out till now to study its purpose and roles in the spread. Some studies suggest that it plays a role in viral replication in host cells via manipulating the host cellular machinery, contributing to viral replication and viral spread [8].

Hence, by adopting a similar structure, A42R may interfere with host cytoskeletal dynamics and intracellular transport, aiding viral replication and spread. Its expression during infection and conservation across Orthopoxviruses suggests an essential role in viral pathogenesis, potentially contributing to immune evasion or host-cell manipulation.

Former studies on Orthopoxvirus proteins have mainly focused on well-defined targets, namely DNA polymerase (E9L), F13L, contributing to virion egress, or surface glycoproteins like H3L, and B5R. However, research on A42R remains limited; despite this, structural analyses confirm its similarity to eukaryotic profilins, and recent investigations propose its involvement in viral modulation of host cytoskeletal machinery. Having a viral origin and functional importance, A42R remained an unexplored target for antiviral drug discovery, mainly through in silico screening of natural compounds [27]. So, in this dissertation, we will target the A42R protein found in monkeypox virus with the phytochemicals of *Catharanthus roseus*.

2.5 Computational Drug Design Techniques.

Molecular docking is a frequently used computational technique for drug development by analyzing binding interactions between ligands, such as bioactive compounds, and targeted proteins. With applications spanning lead identification, optimization, virtual screening, and structure-based drug design [17]. This approach simulates the geometric fit and non-covalent interactions between a ligand and its target receptor within the binding site, allowing for the

analysis of the most stable binding conformation and its corresponding binding affinity.

Various molecular docking algorithms have been developed, having their features and strengths. AutoDock, AutoDock Vina or DOCK are widely used, employ various functions and algorithms to navigate the ligand conformational space and enhance their binding interactions with the target protein. These tools are incorporated with various criteria such as ligand-protein flexibility and solvent effects, increasing the dependability and predictive accuracy of docking simulations [17].

In this dissertation, molecular docking is employed as a core computational strategy to identify the potent phytochemical inhibitors of the A42R-targeted protein found in monkeypox virus. By simulating the interaction between selected plant-derived compounds from *Catharanthus roseus* and the viral protein's active site, the study aims to predict binding affinities and explore the structural compatibility of these ligands. This strategy enables the identification of potential bioactive compounds based on their binding strength, and orientation with the reactive site of selected proteins from the virus. By combining the principles of structural biology and pharmacoinformatic, this approach supports the early phase screening process, making it cost effective and time-efficient procedure to prioritize candidate molecules for further research. This in silico framework lays a foundational step toward the development of novel, plant-based antiviral therapies against monkeypox.

CHAPTER 3

METHEDOLOGY

The employed methodology in this research is the integration of computational techniques with molecular docking stimulation to find the potent bioactive compound present in *Catharanthus roesue*, inhibiting target protein A42R of monkeypox virus.

In this dissertation, for virtual screening of various ligands (bioactive compounds) interacting with the targeted protein, we employed PyRx.

Below, the given details explain the methods utilized for protein preparation, ligand selection, molecular docking, and validation procedures.

1. Downloading the protein

- Utilize Protein Database Bank, i.e., PDB to download the selected protein, in our case, it is protein A42R.
- Download the protein in the PDB format.
- Store the downloaded protein in a new separate folder

2. Download the ligands

- Use either PubChem or IMPPAT. The former is the database of chemical substances and their properties, while the latter is the database of Indian medicinal plants and their natural compounds.
- Search for the ‘Brassinolide’ as a compound. Download the corresponding 3D conformer in SDF format. Store it in a new separate folder.
- Download the Teasterone, Typhasterol, Quercetin, Kaempferol, 2,2,6-trimethyl cyclohexanone, and Myristic acid in the same format mentioned in the immediate above

step and save all ligands in the same folder.

3. Preparing the protein

- First of all, for protein docking, we should know about the binding residues and binding location. They may exist in 2 chains of targeted protein or may reside in just one of the chains of protein. Study the targeted protein structure through the research papers published related to the protein structure.
- Now, the catalytic binding site of the A42R protein is known.
- Protein preparation, it was loaded in Biovia Discovery Studio for the removal of non-essential components that may hinder the binding of the specific bioactive compounds with the selected protein, such components are water molecules, heteroatoms, and ligands. For better interaction results addition of polar hydrogens to the protein is suggested. This led to the stabilization of ligand-protein and electrostatic interactions, improving the efficiency and precision of the molecular docking results.
- We must remove the extra chains from the targeted protein structure to prevent any complexity
- In this study, we kept chain A and removed the other chain i.e. chain B, as protein A42R consists 2 chains only, having chain A and B.
- After the preparation of the protein store the file as
- Now we have the prepared targeted protein, we can proceed further for docking.

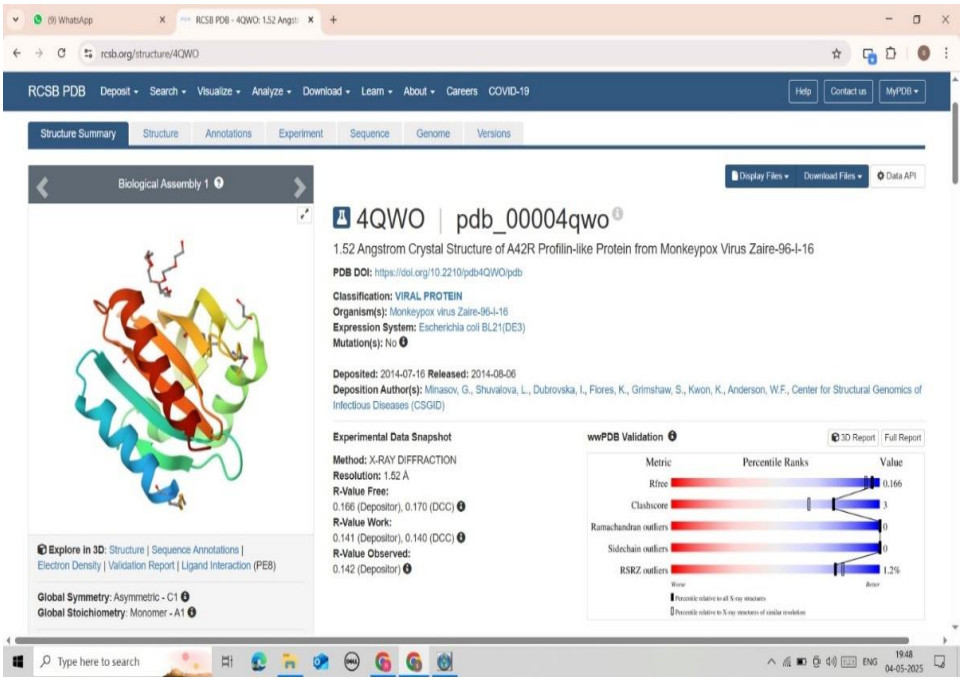


Figure 3.1- Structural details of A42R protein (PDB ID: 4QWO) from the RCSB PDB database.

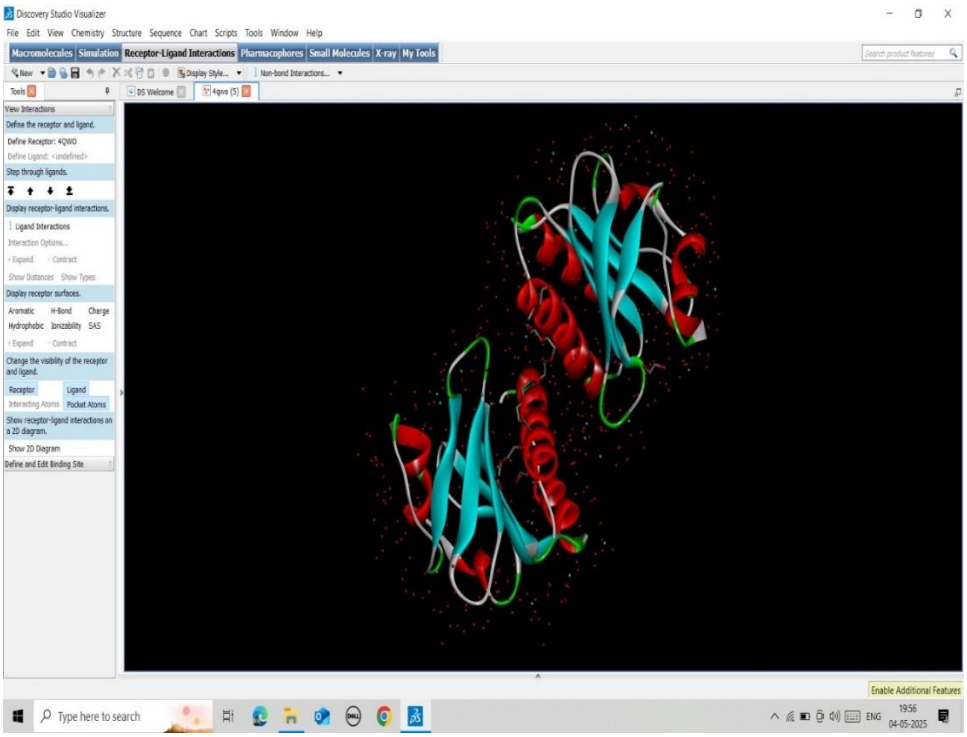


Figure 3.2- Loading of protein in Discovery Studio/protein before modification

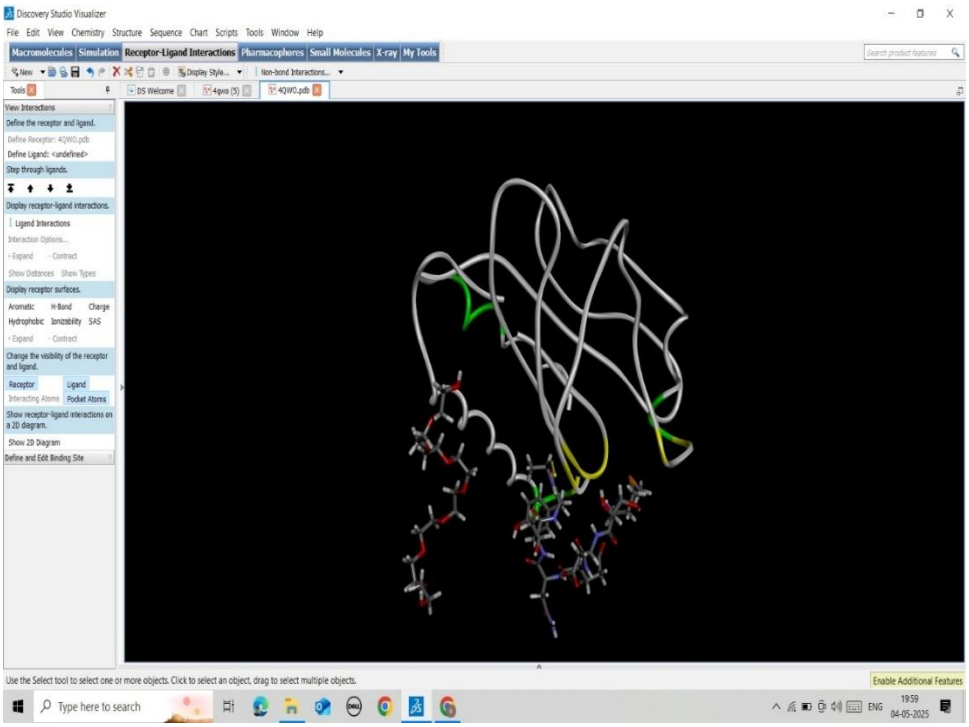


Figure 3.3- Protein after modification

4. Performing docking using PyRx.

- We will be using Autodock Vina as docking tool. Using Vina algorithm to dock it in PyRx. Open the PyRx graphical interface and proceed with the following steps:

4.1 Protein Loading

- Click on "File" and select "Load Molecule," or alternatively, click the first icon in the top-left corner. Then, upload the downloaded protein structure file, referred to here as "4QWO.pdb."
- To convert the protein file from PDB to PDBQT format, right-click on "4QWO," go to "Display," and select "Macromolecule."

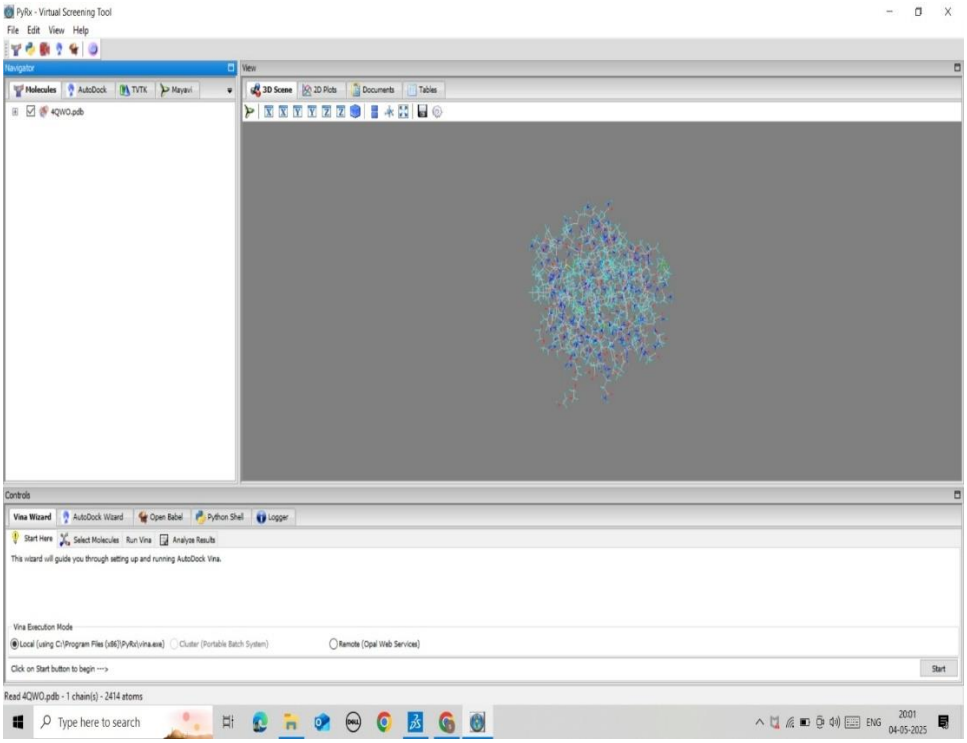


Figure 3.4- Loading protein in PyRx.

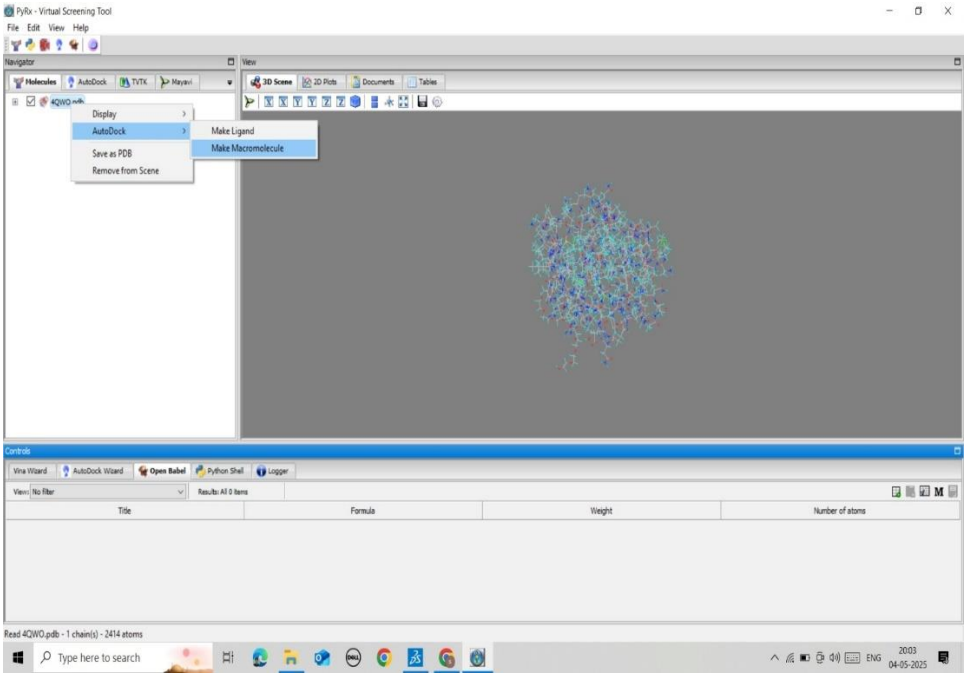


Figure 3.5- Conversion of protein file pdb to pdbqt

4.2 Ligand loading

- In PyRx, click on OpenBabel and choose "Insert New Item" located at the bottom right corner. Then, upload each ligand individually by selecting them one by one from the folder.

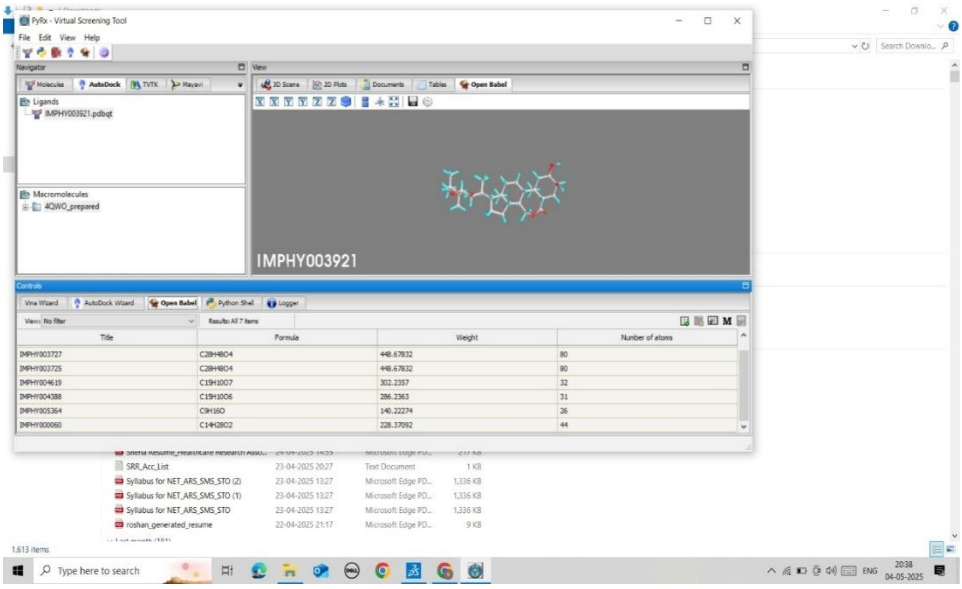


Figure 3.6- Loading of ligands

- Once all ligands are uploaded, right-click on a ligand and choose "Minimize All" to optimize their energy states.

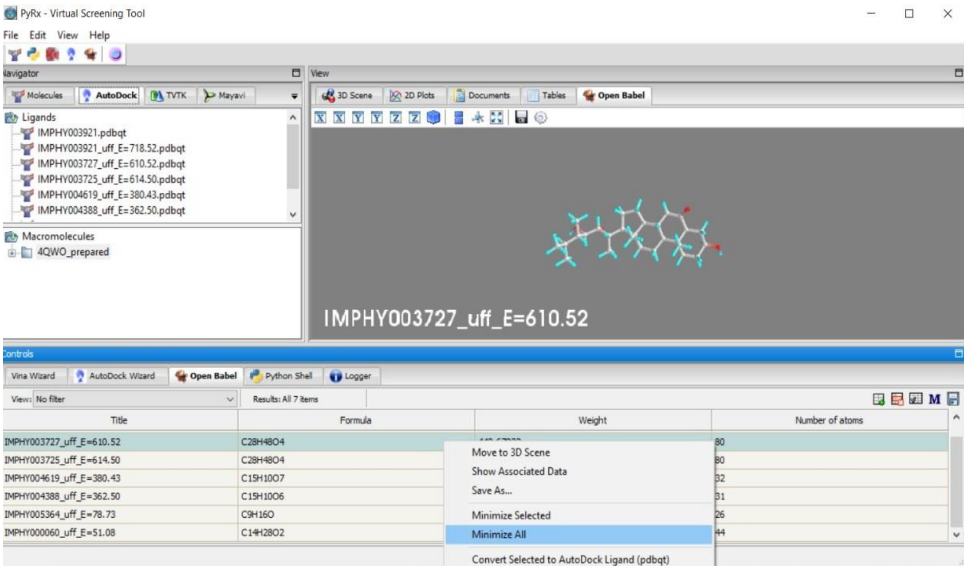


Figure 3.7- Minimization of the energy of the ligand

- Next, right-click again and select "Convert All to AutoDock Ligand (pdbqt)" to convert all ligands into the pdbqt format.

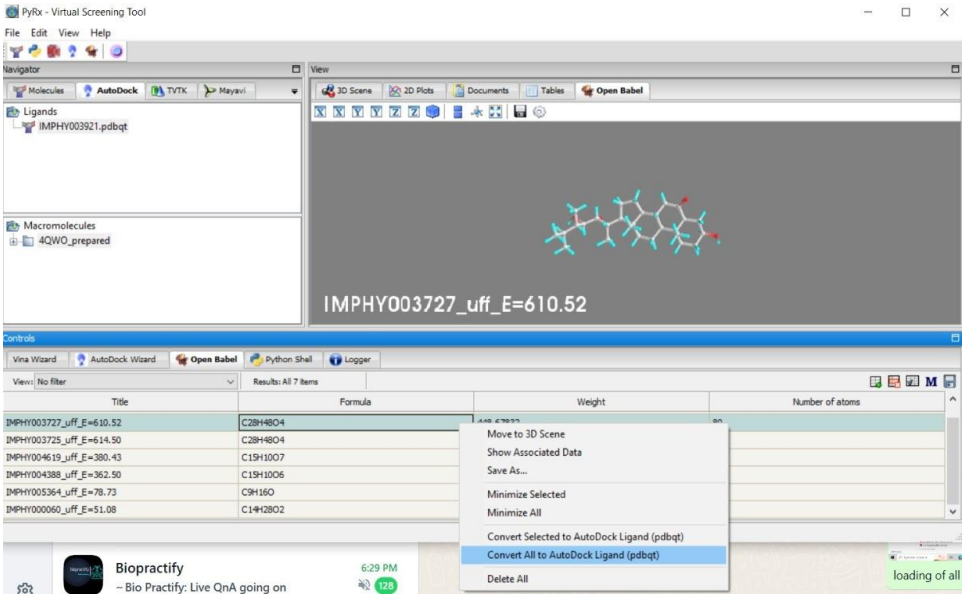


Figure 3.8- Conversion of all ligands to pdbqt format.

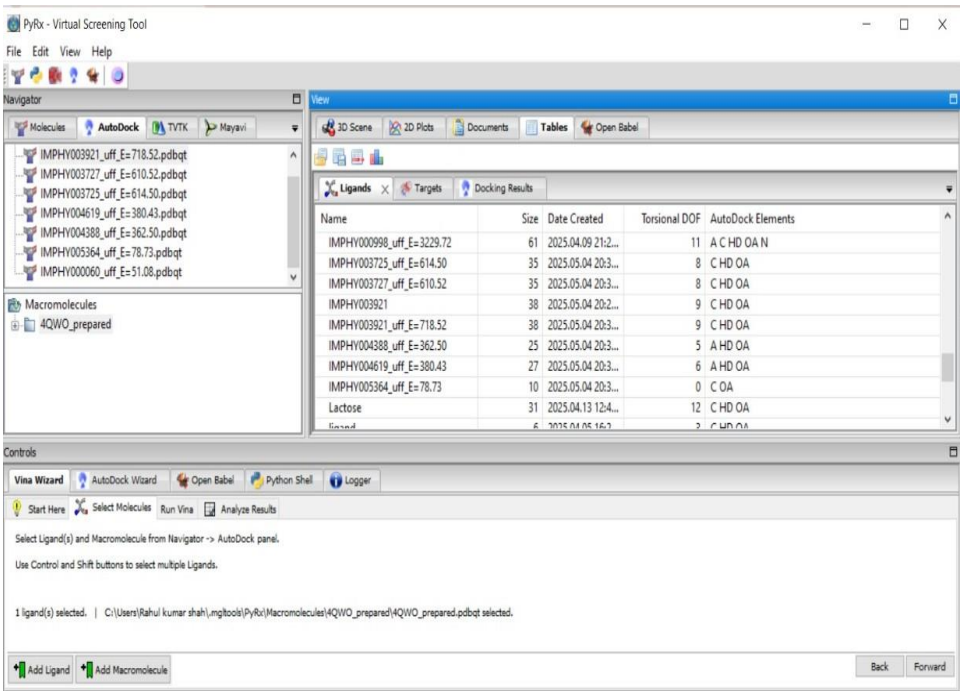


Figure 3.9- Display of all ligands

5. Defining ligands and protein.

- Under the "Molecules" tab, both the loaded protein and ligands are displayed. To distinguish them, right-click on the protein, go to "Autodock," and select "Make Macromolecule." Similarly, right-click on each ligand, choose "Autodock," and then "Make Ligand." This process will automatically generate their PDBQT files, which will appear under the "Autodock" tab.

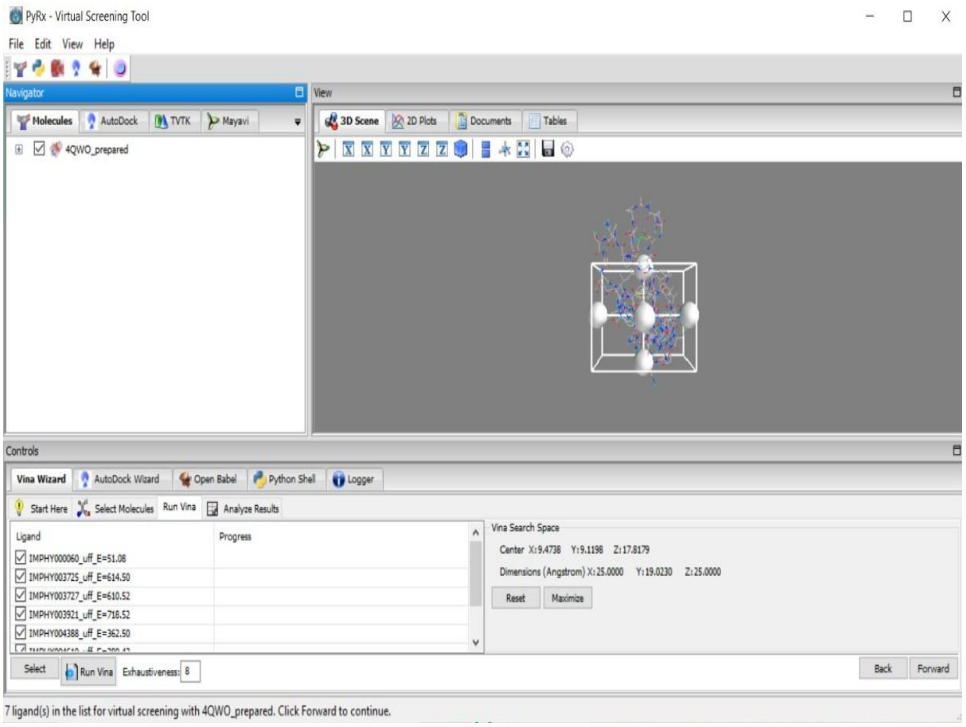


Figure 3.10- Undefine grid box

6. Defining grid box

- Now, click on "Vina Wizard" and select the "Start" option located at the bottom right corner. Using the Shift and Control keys, select the protein and ligand one by one.
- Click "Forward" to proceed, and a grid box will appear. Then, go to the "Molecules" tab on the right side and click the "+" icon next to the loaded protein.
- All residues within the chain will now be visible. To identify

the binding residues, right-click on a residue and select **Atoms → Display → Label → Atoms**. This will display the atoms on the protein structure. Next, adjust the grid box to ensure it encompasses all the selected binding residues. It is not necessary to include the ligand within the grid box

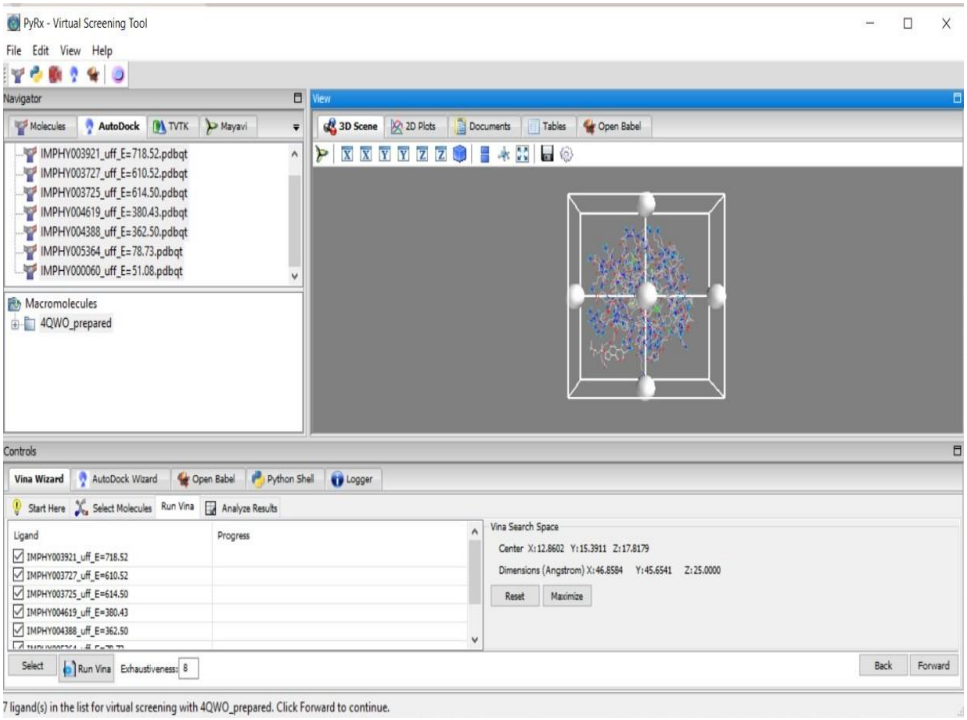


Figure 3.11- Defining of grid box

7. Running Vina for docking

- To set the exhaustiveness level, enter the desired value in the box at the bottom-left corner.
- Once all parameters are set, click the "Forward" button to begin the docking process. The progress will be displayed on-screen, and upon completion, the bottom panel will show all generated poses along with their binding affinities and RMSD values.
- Export the results to an Excel file for further analysis.
- Next, review the docking results and identify the ligand with the **most negative binding energy**, indicating the strongest binding.

- Then, go back to the PyRx tab, click on **AutoDock**, select **Macromolecule**, and choose the top-performing ligand. Right-click on it, select **Display**, and all models of the ligand will appear.
- Choose your preferred model and save it in **PDB format** for further analysis.

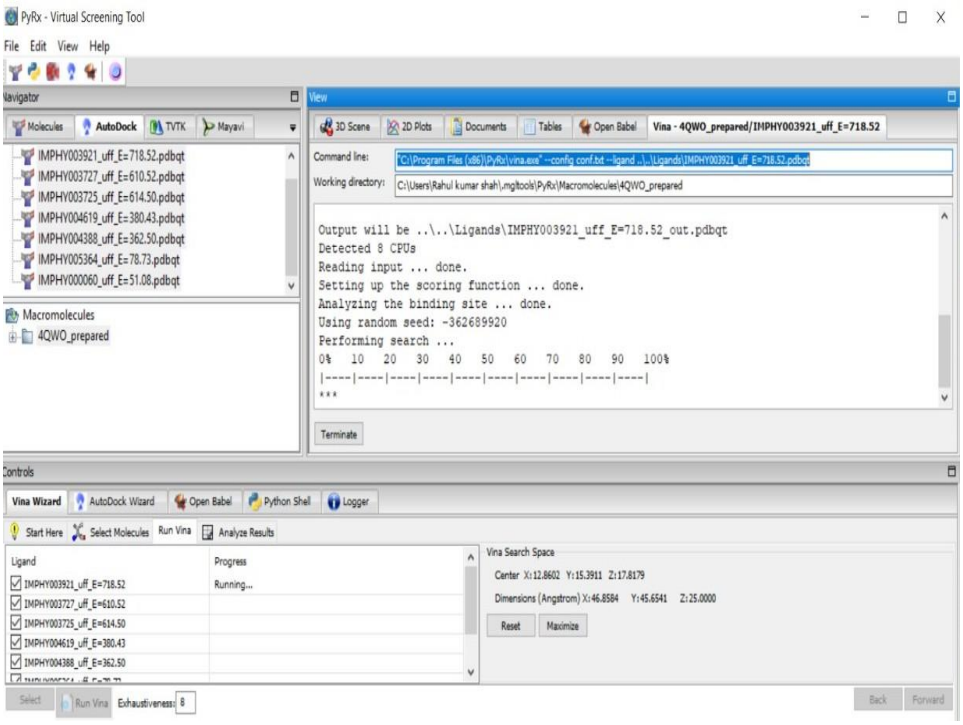


Figure 3.12- Running of the Vina wizard

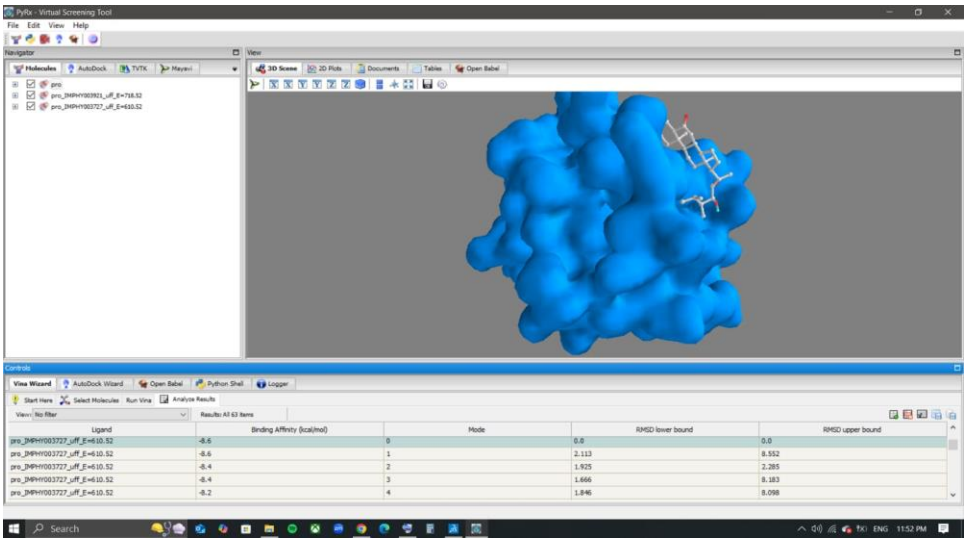


Figure 3.13– Macromolecular structure of protein with different ligands

8. Open Discovery Studio

- Now, start a fresh project in Biovia Discovery Studio

9. Protein Structure Import:

- Open Discovery Studio and import the structure of the target protein.
- Choose your protein structure file (such as *.pdb) by using File > Open.

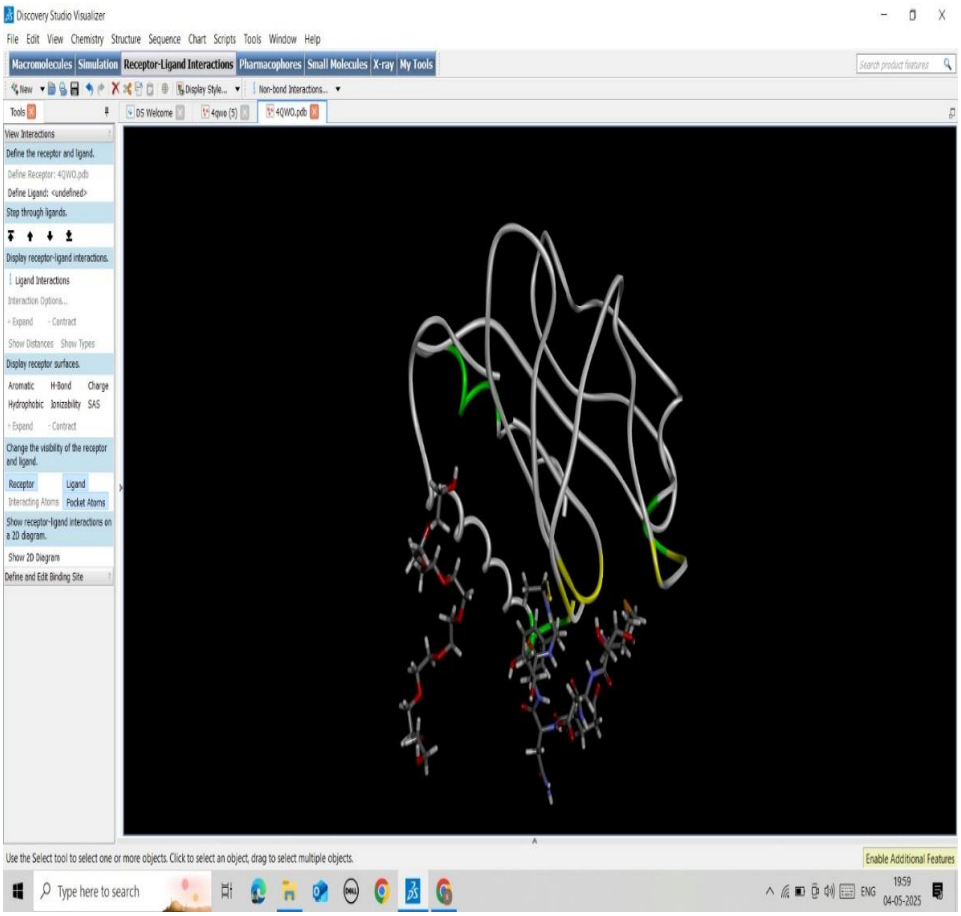


Figure 3.14- Visualization of protein structure in Discovery Studio

10. Import Docked Ligand Conformations

- Import the confirmation of docked ligand in Discovery Studio.
- Click file and select the converted ligand file, i.e., pdb.

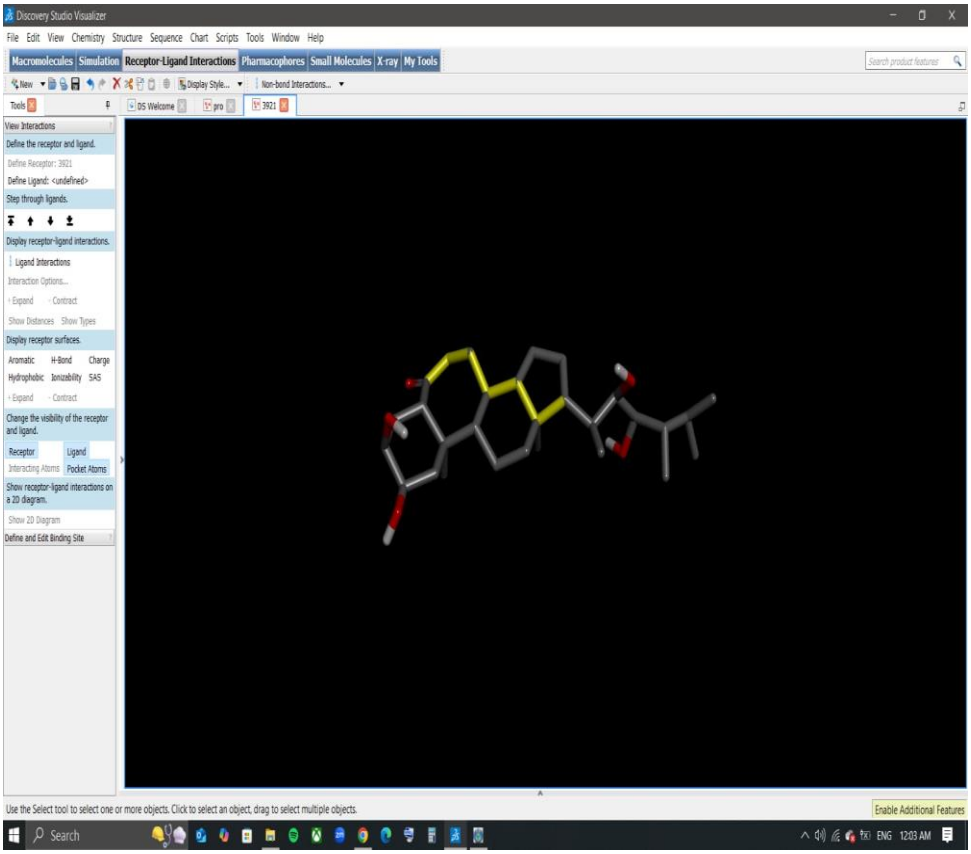


Figure 3.15- Structure of Brassinolide

11. Visualize Docked poses:

- Protein and ligand to be displayed in the 3D workspace.
- Click the View > sequence panel to make sure that the correct structures are loaded.
- Modify the display settings for better visualization of the interactions, using representations like stick or surface view to highlight the binding between the phytochemicals and the selected protein.

12. Analyse Binding Affinities:

- Review the binding affinity values for each docked pose.
- These values can be obtained from the PyRx log files or the docking output summary.
- Document the binding energies (typically expressed in kcal/mol) for reference and comparison.

13. Investigate Binding Interactions:

- Utilize the Analyze > Receptor-Ligand Interactions tool to explore key interactions between the ligand-protein.
- Analyse different bonds
- Cross-check the interacting residues for consistency with known active site features or functionally important regions of the binding pocket.

14. Assess Docked Poses:

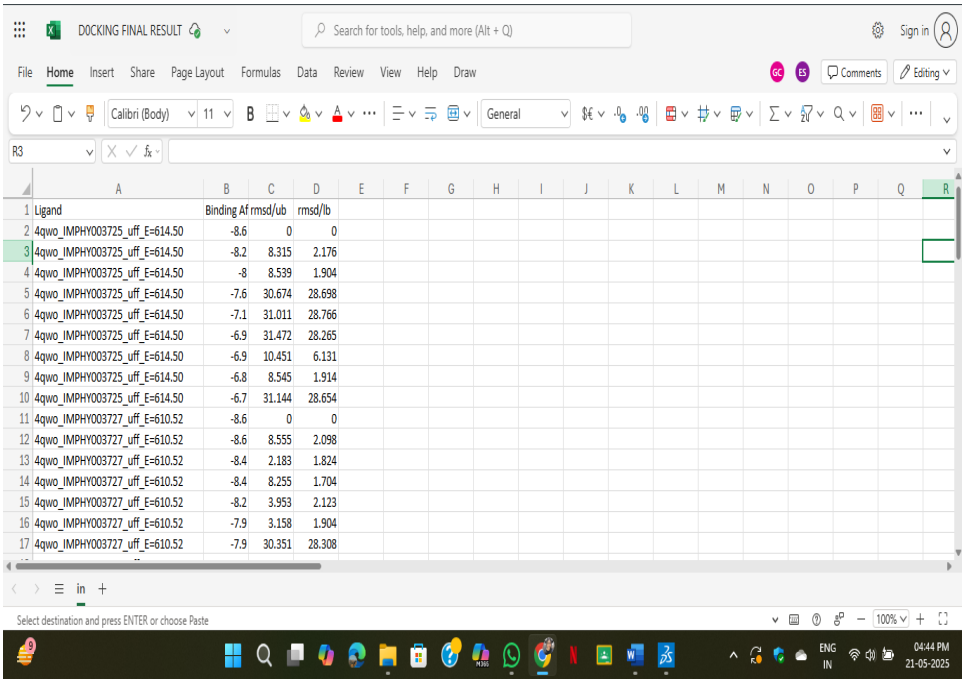
- Evaluate multiple docked conformations to determine if a consistent binding mode is present.
- Use the View > Compare feature to overlay poses and visually assess structural similarities.
- Consider the RMSD values to quantify the variation among complex conformation and support the selection of the most reliable conformation.

CHAPTER 4

RESULTS

The results of molecular docking simulations show the most potent drug candidates to the cure of the monkeypox virus. By virtual screening of various bioactive compounds from *Catharanthus roseus* against protein A42R, some of the compounds showed favourable binding affinities and potent therapeutic effects. The phytochemical exhibiting the highest binding affinity is selected for further analysis, and its interaction with the target protein is visualized using Discovery Studio. Additionally, the binding affinity of cidofovir a compound already used in the treatment of monkeypox infection is evaluated against the target protein A42R for comparative assessment.

The images below show the docking results of various bioactive compounds with the A42R protein.



DOCKING FINAL RESULT

	Ligand	Binding Af	rmsd/ub	rmsd/lb
2	4qwo_IMPHY003725_uff_E=614.50	-8.6	0	0
3	4qwo_IMPHY003725_uff_E=614.50	-8.2	8.315	2.176
4	4qwo_IMPHY003725_uff_E=614.50	-8	8.539	1.904
5	4qwo_IMPHY003725_uff_E=614.50	-7.6	30.674	28.698
6	4qwo_IMPHY003725_uff_E=614.50	-7.1	31.011	28.766
7	4qwo_IMPHY003725_uff_E=614.50	-6.9	31.472	28.265
8	4qwo_IMPHY003725_uff_E=614.50	-6.9	10.451	6.131
9	4qwo_IMPHY003725_uff_E=614.50	-6.8	8.545	1.914
10	4qwo_IMPHY003725_uff_E=614.50	-6.7	31.144	28.654
11	4qwo_IMPHY003727_uff_E=610.52	-8.6	0	0
12	4qwo_IMPHY003727_uff_E=610.52	-8.6	8.555	2.098
13	4qwo_IMPHY003727_uff_E=610.52	-8.4	2.183	1.824
14	4qwo_IMPHY003727_uff_E=610.52	-8.4	8.255	1.704
15	4qwo_IMPHY003727_uff_E=610.52	-8.2	3.953	2.123
16	4qwo_IMPHY003727_uff_E=610.52	-7.9	3.158	1.904
17	4qwo_IMPHY003727_uff_E=610.52	-7.9	30.351	28.308

Figure 4.1

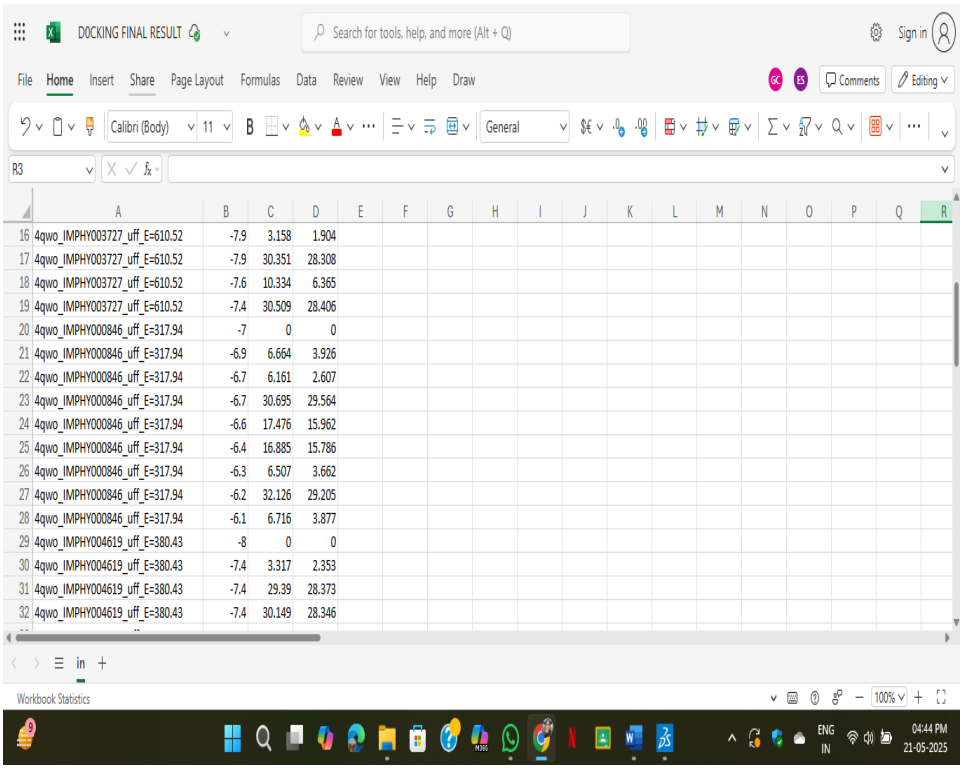


Figure 4.2

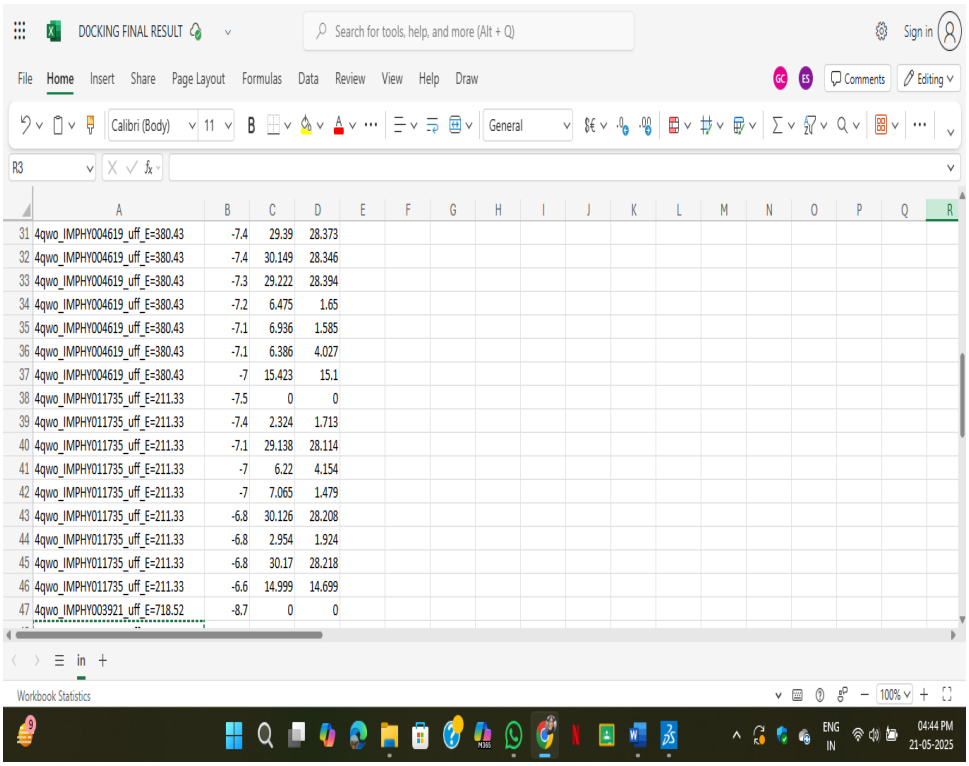


Figure 4.3

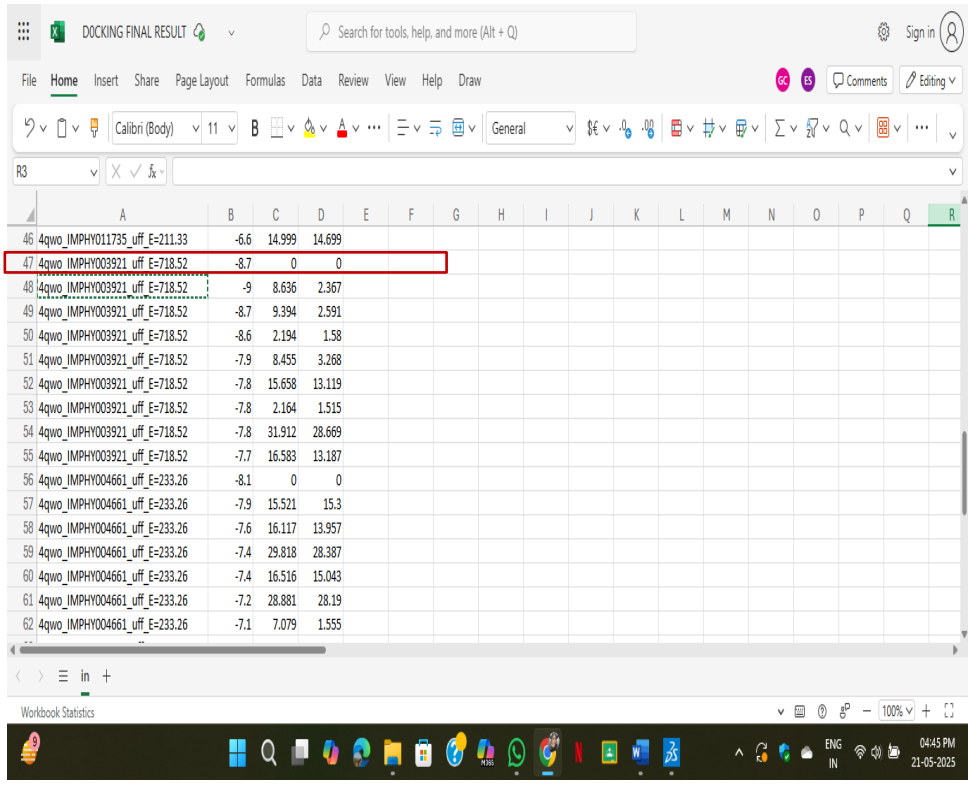


Figure 4.4

By analysing the molecular docking results, we can conclude that Brassinolide exhibits the highest binding score OF -8.7 Kcal/mol ith energy E=718.52.

- **Structure of the studied natural bioactive compounds present in *Catharanthus roseus*.**

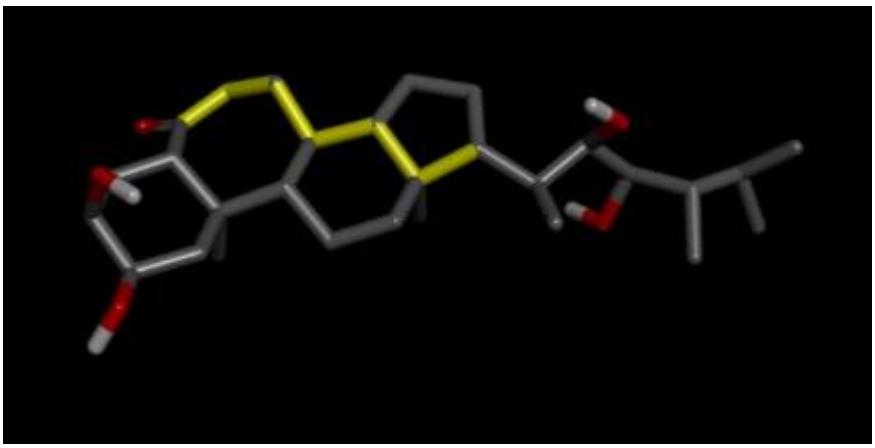


Figure 4.5- Brassinolide

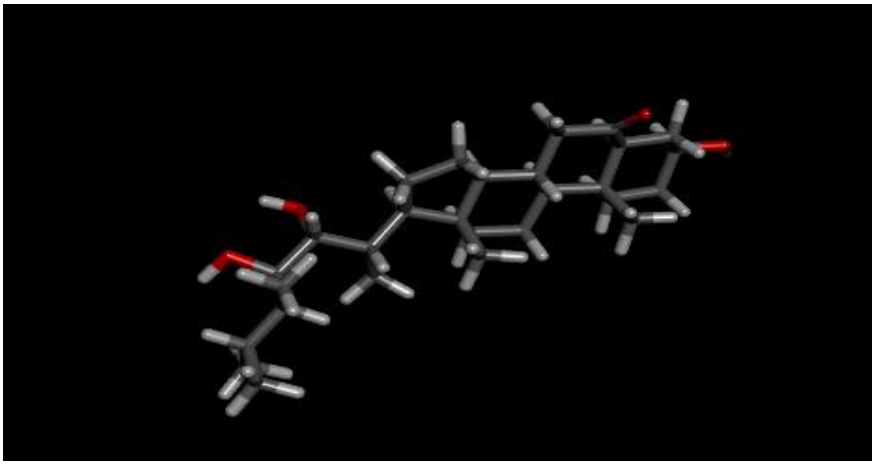


Figure 4.6- Teasterone

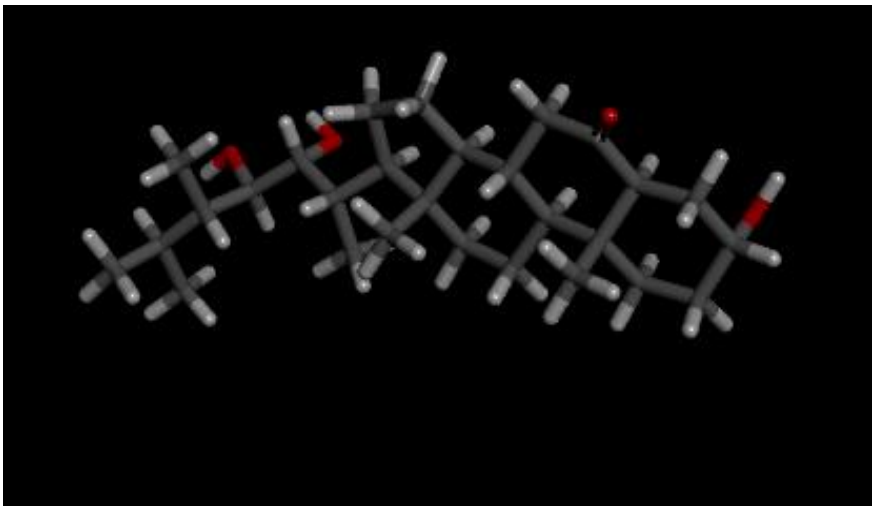


Figure 4.7- Typhasterol

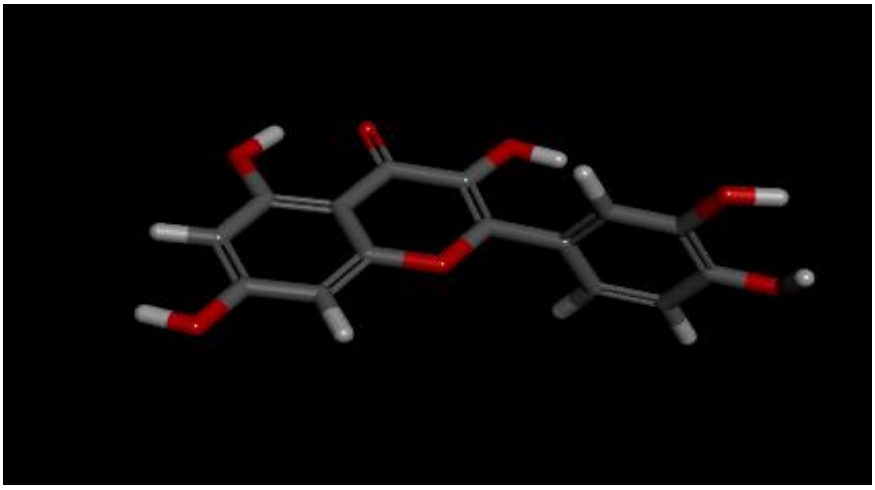


Figure 4.8- Quercetin

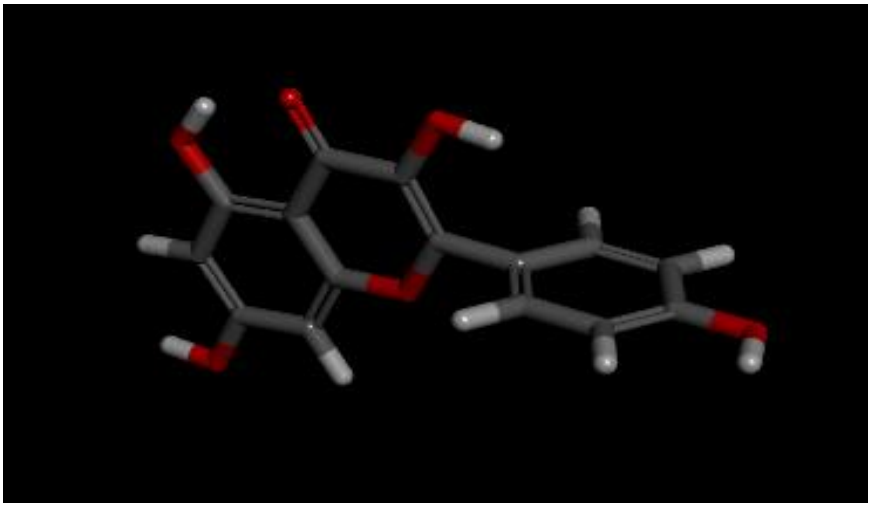


Figure 4.9- kaempferol

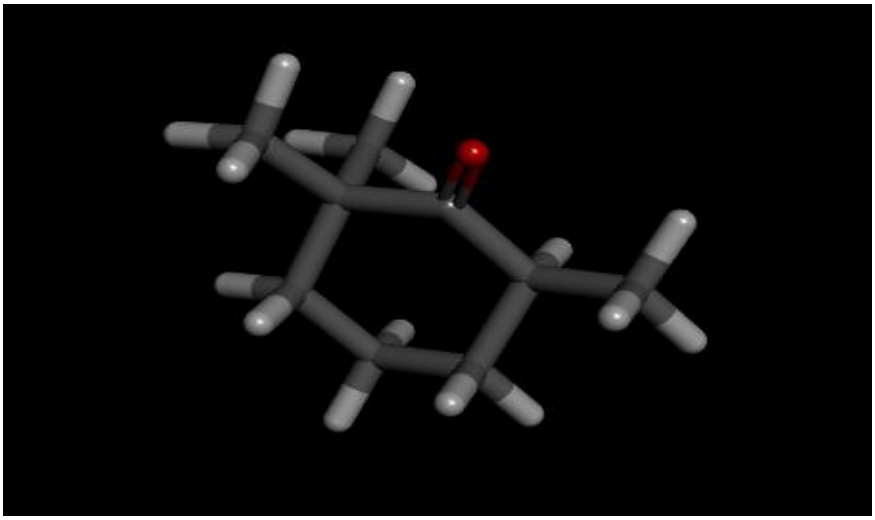


Figure 4.10 - 2,2,6-trimethyl cyclohexanone

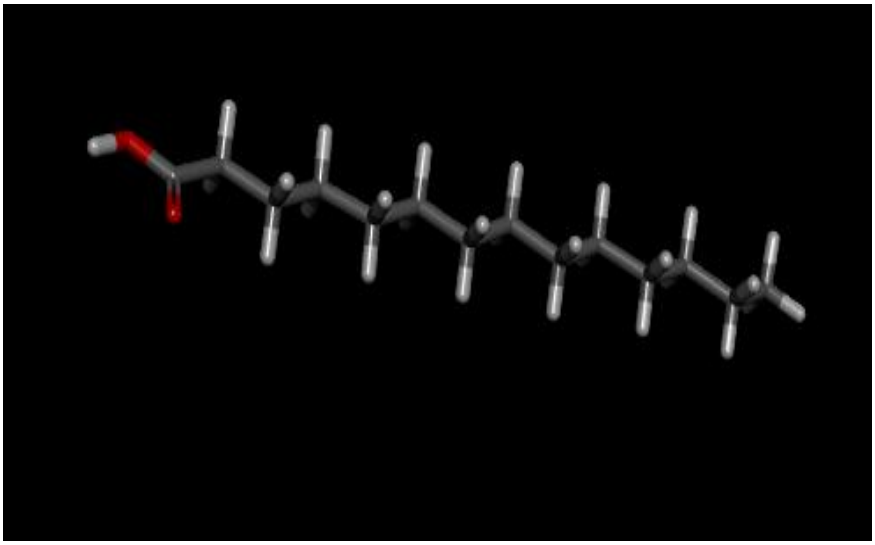


Figure 4.11- Myristic acid

- **The Following Images Show 2-D Structural Interactions Between Protein-Ligand (4QWO)**

➢ **Docking of Brassinolide with A42R protein**

- Interaction diagram showing the binding of Brassinolide with the target protein.
- E score =718.52, binding affinity -8.7 kcal/mol, and rmsd value=0

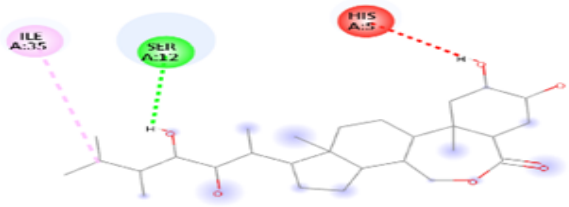


Figure 4.12

➢ **Docking of Teasterone with the A42R protein**

- Interaction diagram showing the binding of Teasterone with the target protein.
- E score =610.52, binding affinity -8.6kcal/mol, and rmsd value=0

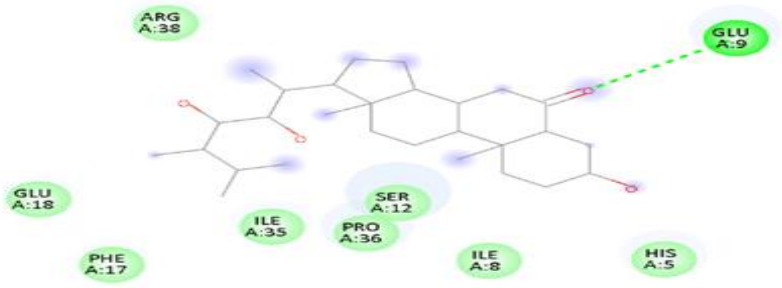


Figure 4.13

➢ **Docking of Typhasterol with the A42R protein**

- Interaction diagram showing the binding of **Typhasterol** with the target protein.
- E score =614.50, binding affinity -8.6 kcal/mol, and rmsd value=0

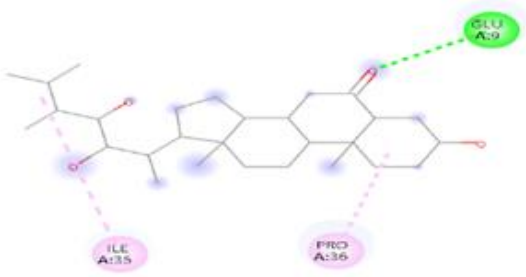


Figure 4.14

➢ **Docking of Quercetin with the A42R protein**

- Interaction diagram showing the binding of Quercetin with the target protein.
- E score =380.43 binding affinity -8kcal/mol and rmsd value=0

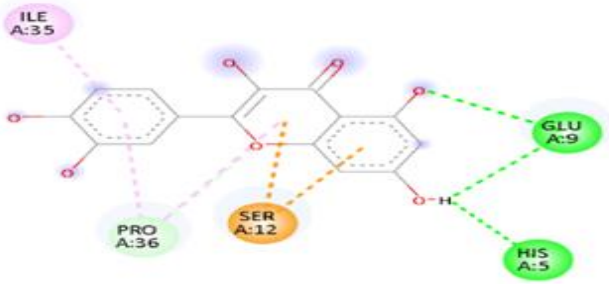


Figure 4.15

➢ **Docking of Kaempferol with the A42R protein**

- Interaction diagram showing the binding of Kaempferol with the target protein.
- binding affinity -7.9 kcal/mol and rmsd value=0

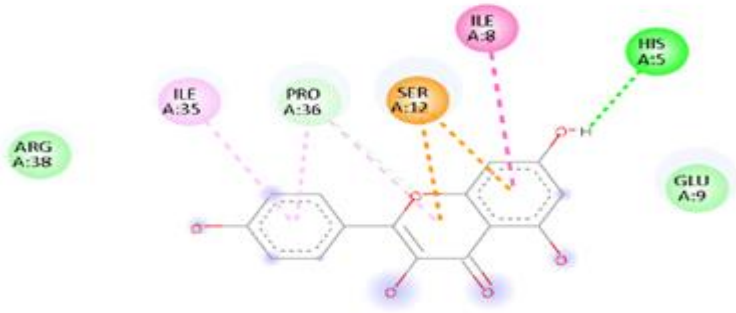


Figure 4.16

➢ **Docking of 2,2,6-trimethyl cyclohexanone with the A42R protein**

- Interaction diagram showing the binding of **2,2,6-trimethyl cyclohexanone** with the target protein.
- binding affinity -5.5 kcal/mol and rmsd value=0

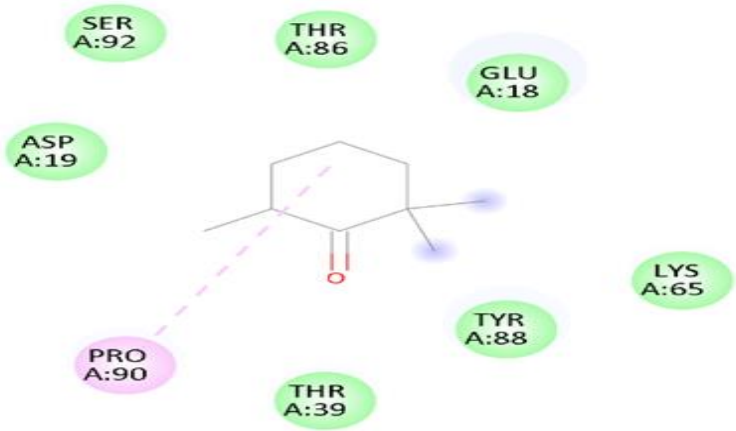


Figure 4.17

➢ **Docking of Myristic acid with the A42R protein**

- Interaction diagram showing the binding of Myristic acid with the target protein.
- binding affinity –5.4 kcal/mol and rmsd value=0

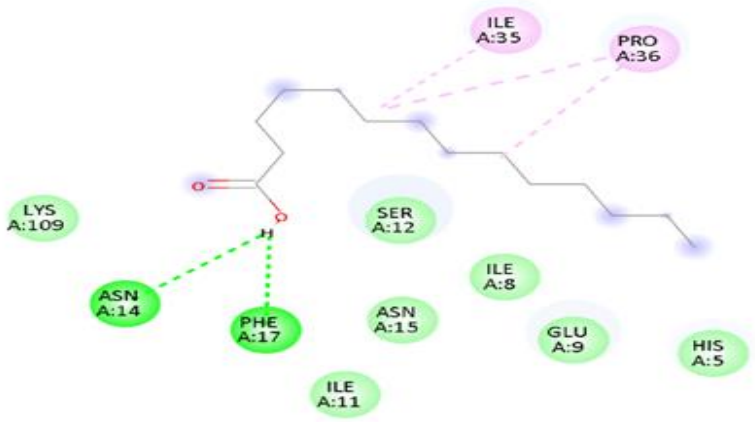


Figure 4.18

From the above results, we can predict that Brassinolide is the most favorable candidate among all other bioactive compounds used in the study, showing the efficiency to inhibit the protein A42R found in monkeypox virus, as it has the highest binding affinity of -8.7 kcal/mol highest among all.

BIOACTIVE COMPOUND	BINDING AFFINITY (Kcal/mol)
Brassinolide (A)	-8.7
Teasterone (B)	-8.6
Typhasterol (C)	-8.6
Quercetin (E)	-8.0
Kaempferol (F)	-7.9
2,2,6-trimethyl cyclohexanone (M)	-5.5
Myristic acid (N)	-5.4

Table 4.1 - Phytochemicals and Their Corresponding Calculated Binding Affinities.

DISCUSSION

In this dissertation, we used the molecular docking simulation technique to find the most potent bioactive compound for the treatment of monkeypox infection. As mentioned earlier, the *Catharanthus roseus* has been employed to treat various illnesses in humans like diabetes mellitus, cancer, hypertension, and this plant also exhibits anti-inflammatory, antithrombotic, antioxidant, anti-allergic, and vasodilatory effects, and cardio-protective properties. Having various health benefits, *Catharanthus roseus* was selected in this study for its rich phytochemical profile and its potential to yield bioactive compounds capable of targeting the A42R protein that found in monkeypox virus. Through molecular docking simulation, we can predict various bonds contributing to stabilizing the interactions. Lower docking scores show more favourable binding interactions, reflecting stronger affinity and greater stability of the ligand–protein complex. Brassinolide, a phytochemical found in *Catharanthus roseus* and various other plants, also shows the highest binding score with the targeted protein A42R among all other compounds used in this study, i.e., -8.7kcal/mol and binding interaction energy E=718.52, which suggests that brassinolide can be a potential inhibitor of protein A42R essential for proliferation and distribution of virus. Cidofovir, taken as a reference inhibitor of A42R protein of Human Monkeypox Virus (hMPXV) with showed -6 Kcal/mol binding affinity. It exhibits several unfavourable bonds while binding to the target protein, i.e., A42R protein, showing 5 phytochemicals out of the 7 phytochemicals used have performed better in comparison to the cidofovir, an already present treatment option for monkeypox infection in market. The names of those five phytochemicals are brassinolide, teasterone, typhasterol, Quercetin, and kaempferol.

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PUBLICATION:

Title of Paper - Structure-based computational investigation of Bioactive compounds from *Camellia sinensis* as a drug candidate for Human Monkeypox Virus (MPXV).

Author Names - Khushi, Eshita, Dr. Navneeta Bharadvaja.

Name of Conference – Second International Conference on Emerging Technologies in Science & Engineering (ICETSE)- 2025 organised by Akshaya Institute of Technology and Technically Co-Sponsored by Hinweis Research.

Date of Conference – 19th & 20th June 2025.
Indexing – Scopus Indexed
Status of paper – acceptance received
Date of acceptance – 30th April, 2025
Date of Camera-Ready Submission and Registration- 20 May, 2025

Gmail **Khushi <khushisingh251021@gmail.com>**

Re: submission of conference paper registration fees

5 messages

Khushi <khushisingh251021@gmail.com> 3 May 2025 at 12:34
To: ICETSE 2025 <icetse2025@gmail.com>

Respected conference chair
Registration step is done along with payment of required registration fees . Please find the attached payment slip.

PAPER ID -706

paper title:Structure-based computational investigation of Bioactive compounds from Camellia sinensis as a drug candidate for Human Monkeypox Virus.

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> > Respected conference chair,
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> > I am writing to respectfully request your consideration for the submission of my paper titled "Structure-based computational investigation of Bioactive compounds from Camellia sinensis as a drug candidate for Human Monkeypox Virus (MPXV)" in Second International Conference on Emerging Technologies in Science and Engineering (ICETSE) to be held on june 19-20,2025 at Akshaya institute of technology,tumkur karnataka. .
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> > I fully acknowledge the importance of deadlines and sincerely apologize for any inconvenience this may cause. The completed manuscript is attached herewith, and I would be most grateful if you could kindly consider it for review.
> >
> > Thank you very much for your time and understanding.
> >
> > warm regards,
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> > khushi
> >
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(Formerly Delhi College of Engineering)
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PLAGIARISM VERIFICATION

Title of the Thesis: In Silico Investigation of phytochemicals derived from Catharanthus roseus as potential therapeutics agents targeting the H42R protein of the human monkeypox virus

Total Pages: 63 Name of the Scholar: Eshita

Supervisor (s)

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