Exploring Natural Compounds as Inhibitors of Monkeypox Virus Cysteine Proteinase

Prashant Kumar Tiwari[1], Mandeep Chouhan[1], Riya Nalwa[1], Vivek Dhar Dwivedi[2], Sanjay Kumar[1]
[1]Department of Life Sciences, SSBSR, Sharda University, Greater Noida, Uttar Pradesh India
[2]Bioinformatics Research Division, Quanta Calculus, Greater Noida, India

Abstract

Monkeypox is a serious viral illness that is rarely seen but is spread from person to person and from animals to humans. The Cysteine proteinase, an essential enzyme involved in the replication of the monkeypox virus (MPXV), is one of many possible therapeutic targets for MPXV. The primary function of this enzyme is to cleave the precursor polyprotein into the distinct proteins required for viral assembly. Researchers are actively engaged to develop potential drugs that can inhibit the proteinase and stop the spreading of the MPXV. In this study, virtual screening, molecular docking, molecular dynamics simulation, and free binding energy calculations were used in order to explore the potential of 569 phytochemicals from a different variety of plants that could inhibit the proteinase of the MPXV. Based on the docking score, the top four compounds (Unii-CQ2F5O6yiy, Lithospermic acid, Kaempferol, and Rhamnocitrin) displayed higher binding energy values ranging from -9.5 to -7.4 kcal/mol and were used for further analysis. Out of these, Unii-CQ2F5O6yiy displayed the minimum binding score of -9.5 kcal/mol, indicating the highest binding to the proteinase. Unii-CQ2F5O6yiy and Kaempferol had the most stable and consistent RMSD with <4.8 Å. In order to confirm the stability of the chosen four phytochemical complexes, MM/GBSA binding free energy calculations were carried out. This result suggested, lowest binding free energy values of -54.89±6.48 kcal/mol for Unii-CQ2F5O6yiy as compared to the native ligand TTP-6171 binding energy (ΔG_{Bind} = -44.91±2.89 kcal/mol). Hence, Unii-CQ2F5O6yiy could be used as potential antiviral agents for further experimental validation against MPXV.

Introduction

Monkey pox virus (MPXV) is a viral disease that specifically affects animals including monkeys, rodents, and other mammals. It was seen that it can be transmitted in humans, and caused a viral disease known as monkey pox. MPXV disease was first identified in 1958 in a group of monkeys and primarily found in the Central and West African regions, particularly in remote parts of the rainforest (Rani J., 2022). There are several ways of transmission of MPXV from animals to humans, which is a subject of great interests for researchers to stop its transmission Some animals, such as rodents, dogs, and non-human primates, can transmit this virus to humans. It was observed that MPXV initiates infection in animals through respiratory routes, later animals infects other mammals using its respiratory droplets. It also spreads rapidly through vertical transmission from a mother to her offspring (Lum et al., 2022).

Poxvirus is an extremely unique virus that replicates its own nucleic acid or genes in the host cytoplasm and utilizes the host machinery for their transcription and translation. The proteins produced during translation process, interfere the synthesis of host proteins, lead to alteration of cellular environment of host. Among these proteins, Cysteine proteinase (I7L core proteinase) is a crucial for various stages of the poxvirus life cycle (Zephyr et al., 2021). Additionally, Cysteine proteinases, help in uncoating the virus during entry, and releasing the genome into the host's cytoplasm. Furthermore, it has been shown that Cysteine proteinases involve in the processing and maturation of viral proteins, by cleaving larger precursor proteins into smaller functional proteins during viral replication. Apart from this, this proteinase also interfere with the function of host immune system by degrading several essential components of the immune system, including cytokines, chemokines, and proteins involved in the inflammatory response (Donnelly et al., 2011). Due to their ability to perform all these essential functions, Cysteine proteinases can be very promising therapeutic targets in the management of this virus. The inhibitors that inhibit proteases have shown promising results in certain other viral diseases such as HIV. TTP-6171 was discovered as an inhibitor of I7L. Currently, Fosdagrocorat and Lixivaptan are identified as inhibitors for MPXV that target Cysteine proteinase (Dubey et al., 2023). Also, Alandijany et al., discovered two drugs, Omadacycline and Minocycline after the screening of tetracycline antibiotics against MPXV cysteine proteinase (Alandijany et al., 2023).

In this study, a comprehensive computational approach has been implemented to identify those compounds that effectively target MPXV proteinase. The structure of MPXV proteinase was not known previously, so a model of it was generated, after employing molecular dynamics simulation, the protein's most likely structure was further improved. Many phytochemicals were screened against this model protein, and 4 phytochemicals were selected under 100 ns molecular dynamics, to determine the binding affinity and stability of the hit compounds against MPXV proteinase, the dynamic characteristics of the protein-ligand complexes were investigated. This analysis involved studying parameters such as RMSD.

Methodology

The primary sequence of the Cysteine proteinase target protein in FASTA format was obtained using the gene accession number NP_536495.1 from the GenPept database.

Using this sequence, its 3D structure was modelled via Alphafold Colab v2.1.0, that allows prediction of 3D structure of protein.

The best model was validated by PROCHECK.

In order to further verify this structure, molecular dynamics (MD) simulation was carried out to check the stability of the modelled protein.

CASTp i.e., Computed Atlas of Surface Topography of proteins was utilized to predict binding pocket of viral core Cysteine proteinase.

The 3D structures of phytochemicals of selected plants (*Melissa officinalis* (Lemon balm), *Ocimum tenuiflorum* (Tulsi), *Mentha arvensis* (Wild mint), *Mentha piperita* (Peppermint), *Pogostemon cablin* (Patchouli)) were downloaded from IMPAAT in SDF format using MTiOpenScreen.

Redocking using Chimera-AutoDock Vina plugin setup.

Protein-Ligand Molecular Dynamics (MD) Simulation.

The same protocol was followed for the reference complex Cysteine proteinase- TTP-6171.

Results and Discussion

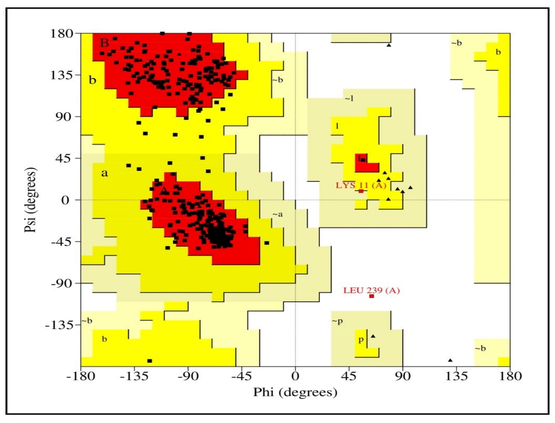

Figure 1. Ramachandran plot showing residues in favoured, allowed and disallowed regions.

Table 1. List of top four selected virtual screened phytochemical compounds against Cysteine proteinase of *Monkeypox virus.*

S. No.	IMPAAT ID	Phytochemical Name	Phytochemical Origin	Binding Score	Redocking Score
1	IMPHY004249	Unii-CQ2F5O6yiy	Ocimum tenuiflorum	-9.3	-9.5
2	IMPHY006793	Lithospermic acid	Mentha piperita	-8.8	-8.9
3	IMPHY004388	Kaempferol	Ocimum tenuiflorum	-7.5	-7.5
4	IMPHY004479	Rhamnocitrin	Melissa officinalis	-7.4	-7.4

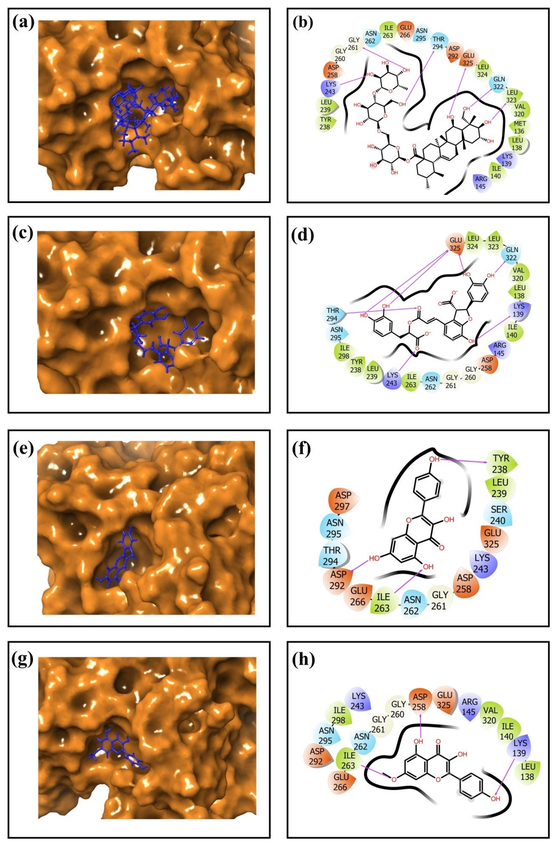

Figure 4. 3-Dimensional and 2-Dimensional docked complexes poses of the selected phytochemical compounds, i.e., (a, b) Unii-CQ2F5O6yiy, (c, d) Lithospermic acid, (e, f) Kaempferol and (g, h) Rhamnocitrin, showing binding on the active site of the Cysteine proteinase. Whereas in 2-Dimensional structures, H-bond formation (pink arrows), hydrophobic interaction (green), polar residue (blue), negative residual interaction (red), glycine (grey) and salt bridge (red and blue) interactions are logged for docked complexes of Cysteine proteinase with selected phytochemical compounds.

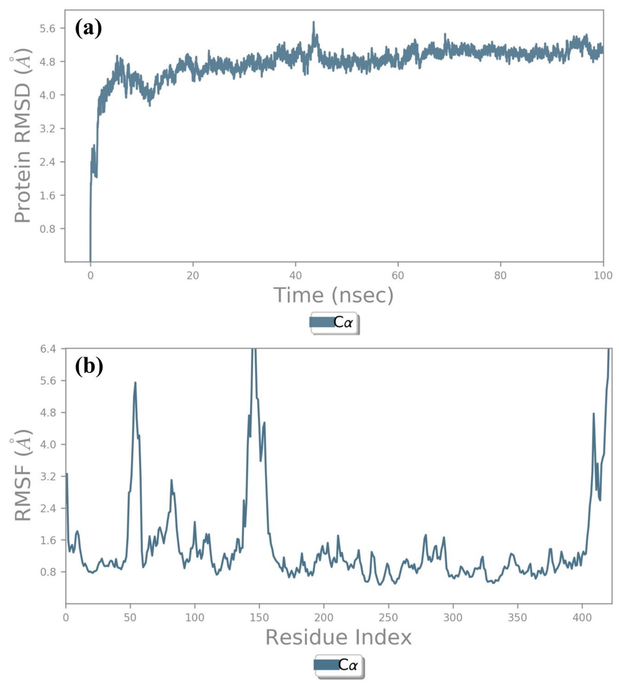

Figure 2. (a) RMSD plot for the backbone atoms of apo protein (b) RMSF plot generated for the apo protein during 100 ns molecular dynamics simulation interval.

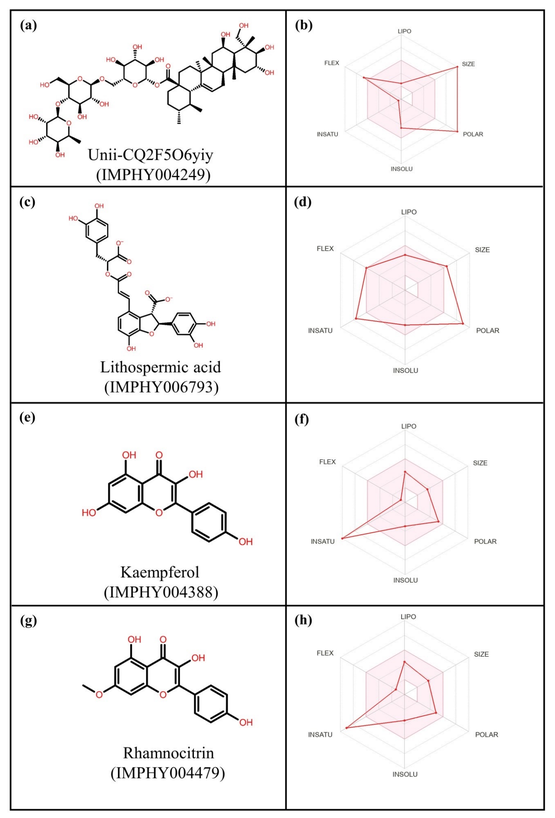

Figure 3. 2-Dimensional structures and ADME profiling of the selected phytochemical compounds, i.e., (a, b) Unii-CQ2F5O6yiy, (c, d) Lithospermic acid, (e, f) Kaempferol and (g, h) Rhamnocitrin.

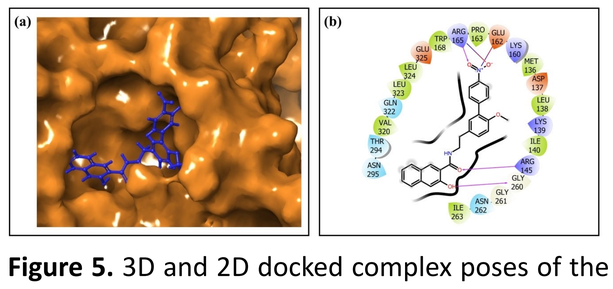

Figure 5. 3D and 2D docked complex poses of the reference complex, i.e., proteinase-TTP-6171 complex.

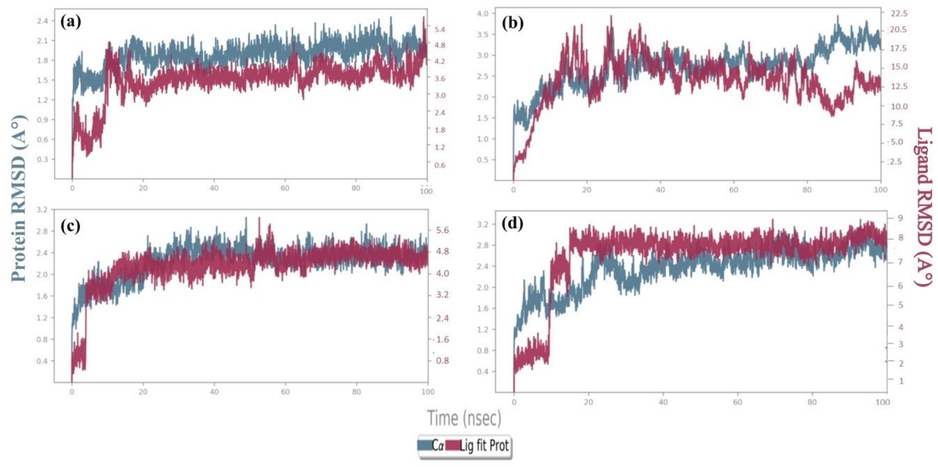

Figure 6. RMSD plots for the backbone atoms of Cysteine proteinase and selected phytochemical compounds, i.e., (a) Unii-CQ2F5O6yiy, (b) Lithospermic acid, (c) Kaempferol and (d) Rhamnocitrin, fit on selected target protein were extracted from 100 ns MD simulation trajectories of different docked complexes.

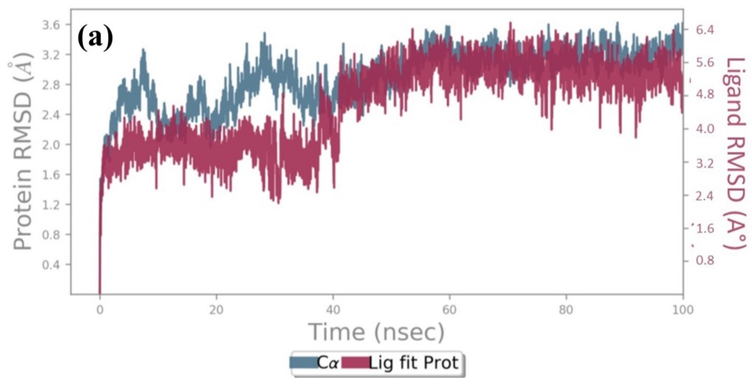

Figure 7. RMSD plot for the backbone atoms of cysteine proteinase in complex with the reference molecule, i.e., TTP-6171 during 100ns molecular dynamics simulation interval.

Conclusion

Monkeypox is an emerging infectious disease that has garnered increasing attention due to its potential to cross species barriers, posing a threat to both animals and humans. The virus can infect from its animal hosts, which serve as reservoirs, to humans. Once established in human populations, it can then spread from person to person. The proteinase of the monkeypox virus (MPXV) is a key player in the viral replication process. Therefore, the proteinase is recognized as a primary drug target to inhibit the growth of MPXV. Through in silico study, we, identified top four compounds such as Unii-CQ2F5O6yiy, Lithospermic acid, Kaempferol and Rhamnocitrin, which displayed strong binding inhibition against Cystein proteinase of MPXV. Hence, these phytochemicals may be utilized as drugs in the management of MPXV. However, further experimental validation of these compounds is required under *in vitro* and *in vivo* condition of MPXV to assess their drug potential.

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