Chronicles of Biomedical Sciences
ISSN:3065-923X
Homepage: https://cbsciences.us/index.php/cbs

In Silico Characterization and Structural Validation of VP39 Methyltransferase as a Potential Antiviral Target against Monkeypox Virus

Rana Mateen[1].

[1]University of Management and Technology, Lahore, Pakistan.

ORIGINAL ARTICLE

Received on: May 02, 2025.
Accepted on: July 18, 2025.
Published on: August 01, 2025.
Keywords: Antiviral agents; Methyltransferase; Molecular docking; Monkeypox virus; Protein structure; Simulation.

Corresponding author: Rana Mateen
mateenibb@yahoo.com

ABSTRACT

Background: Monkeypox virus (MPXV) is a zoonotic double-stranded DNA virus of the *Poxviridae* family. With increasing global outbreaks, especially the 2022 epidemic, MPXV has emerged as a public health concern. Despite its threat, no specific antiviral drugs have been approved. The viral protein VP39 methyltransferase (MTase) plays a vital role in immune evasion, mRNA capping, and viral replication, making it a promising therapeutic target.
Objective: To analyze and validate the 3D structure and physiochemical properties of VP39 MTase (PDB ID: 8cer) to evaluate its potential as a drug target for anti-MPXV therapies.
Methods: The 3D crystal structure of VP39 MTase was retrieved from the Research Collaboratory for Structural Bioinformatics (RCSB) Protein Data Bank. Structural validation was performed using Error Recognition and Analysis Tool (ERRAT), verify 3D, and PROCHECK via the SAVES v6.1 server. Physiochemical characteristics were assessed using the ExPASy ProtParam tool.
Results: The ERRAT analysis revealed a 94.39% overall quality factor. Verify 3D indicated 74.91% of residues had an averaged 3D-1D score ≥ 0.1. The Ramachandran plot showed 93.5% of residues in the most favored regions. The protein has a molecular weight of 38.89 kDa, theoretical pI of 9.47, GRAVY score of -0.395, and an aliphatic index of 88.65, suggesting it is hydrophilic and thermostable.
Conclusion: VP39 MTase demonstrates strong structural validity and favorable physiochemical properties, supporting its role as a potential drug target for MPXV. These findings justify further in vitro, in vivo, and molecular dynamics studies for antiviral drug development.

Citation: Mateen R. In Silico characterization and structural validation of VP39 methyltransferase as a potential antiviral target against monkeypox virus. Chron Biomed Sci. 2025;2(3):53. Available from: https://cbsciences.us/index.php/cbs/article/view/53.

Introduction

Monkeypox infection, caused by monkeypox virus (MPXV), is an emerging zoonotic disease that is transmitted from animals to humans [1]. It is characterized by systematic illness and pustular rash that can range from mild to fatal [2]. MPXV is a double stranded DNA virus belongs to poxviridae family. The variola virus, responsible for small pox, is genetically similar to MPXV. During an outbreak in monkeys in 1958, the name monkeypox originate from initial discovery of virus in a Danish laboratory. After its first discovery from monkeys, it was named as MPXV. From the Democratic Republic of Congo in 1970, the first human infection out of six cases was reported in an infant (9 months old) [3]. Various potential hosts of MPXV have been identified but the exact natural reservoir is still uncertain. Rope squirrels, tree squirrels, rodents, non-human primates and rats are including in susceptible species to MPXV [4]. In people outside of Africa, the United States reported the first MPXV outbreak in 2003 and it was related to contact

with infected pet prairie dogs. Due to recent travel to sub-Saharan Africa (SSA), several nations have recently reported MPXV cases including Israel in 2018, UK in 2018-22 and Singapore in 2019 [5]. MPXV mainly seen in Central and West African countries but has spread to various other countries both in and outside of Africa. In 11 African countries, MPXV cases of human have been reported since 1970 with medium age of 31 years [6]. WHO declared the Public Health Emergency of International Concern (PHEIC) on 23rd July 2022 due to an ongoing global monkeypox outbreak.

Transmission of MPXV caused by direct contact with infected animals or humans especially body fluids such as saliva and respiratory droplets. Contact with contaminated areas increases the transmission rate among population such as eating and touching the contaminated object. During the current epidemic, MPXV is universal in males than females due to the possibility of transmission through men who have sex with men (MSM) [5]. Through bites or scratches from infected animals, MPXV can also be transmitted. Most MPXV cases are associated with sexual contact between individuals. Most MPXV cases have been reported among bisexual and homosexual men in the past two years [1]. The prodromal stage begins after the virus spread in the body through secondary viremia. This phase shows swollen lymph nodes and skin rashes with high risk of transmission. After onset of fever and swollen lymph nodes, rash develops after 1-3 days in a typical case of monkeypox infection [7].

It has been reported that, up to 10% cases of MPXV disease have been associated to cause death. Infection with MPXV do not yet have a specific therapy according to Centers for Disease Control and Prevention (CDC). For the FDA approved drug Tecovirimat (TPOXX), an animal model was used in a clinical trials. According to CDC report, this drug is safe but human treatment outcomes remain uncertain [8]. As a result, insilico techniques have shown effectiveness and evolved into powerful tool and drug discovery and disease research. A study has already concluded that Hispidin demonstrated strong potential as a phytochemical inhibitor of VP39 MTase, exhibiting high binding affinity and favorable electronic characteristics. These findings offer a promising foundation for future in vitro, in vivo, and molecular dynamics investigations aimed at developing novel anti-MPXV therapies [9]. The current study suggests the VP39 methyltransferase (MTase) protein for the development of effective antiviral drugs for MPXV inhibition. This protein acting as a protective shield against host attacks for viral RNA. Through modifying cap structure of viral mRNA, VP39 MTase enables successful infection within host cells and play role in immune evasion translation and replication inhibition [10].

Due to role of Crystal structure of VP39 MTase in viral replication and immune evasion, it serves as an attractive anti-MPXV drug target. In this study, we aim to validate the protein structure to evaluate the accuracy of three-dimensional structure of VP39 MTase and analysis of protein to ensure the high resolution and structural data for the purpose of drug discovery.

Methods

3D structure retrieval

3D structure was selected on the base of experimental resolution and sequence coverage. From RCSB PDB, the crystal structure of VP39 MTase (PDB ID: 8cer) was retrieved. Only X-ray crystallography protein structure was utilized to ensure excellent resolution at 2.60Å and desirable sequence ranging from 1-297 amino acid residues. Crystal structure from X-ray studies highlight where ligand bind and protein confirmation. It may help to reveal protein shape and binding sites [11].

Structure validation and quality assessment

To evaluate the accuracy of three-dimensional structure of protein VP39 MTase, several validation techniques were utilized. Three tools were employed to ensure the quality of VP39 MTase by using online SAVES V6.1 server. Validation tools assessed the quality of protein structure. ERRAT, Verify 3D and PROCHECK are the three programs of SAVES server.

ERRAT server was used to analyze the structural efficiency of protein. The protein structure that was established by X-ray crystallography can be validated by ERRAT program. ERRAT belong to verification server SAVES helps to detect the structural flaws in the 3D protein model. By using the atomic interaction, ERRAT evaluated the non-bonded interaction between several types of atoms to determine the model's reliability and precision. In the plot, these tools identify the allowed regions and most favored regions.

For the target sequence, the established model of 3D structure was also verified by structure validation servers. Verify 3D evaluate how well each residue based on the surrounding environment. Verify 3D analyzed each amino acid for environment compatibility. Residue propensities give the final model score based on PDB format. To compare the model to existing structures and to check the residue compatibility, verify 3D was utilized. In Verify 3D, score above zero suggest suitable structural environment. Best structure has been selected based on the high scores. With its own amino acid sequence (1D), the match of an atomic model (3D) is assessed. And with the parameters derived from database of experimental structure, their quality score is predicted [12].

To visualize the amino acid dihedral distributions, PROCHECK provided the Ramachandran plot. Since their introduction in PROCHECK, Ramachandran plot have been used for the validation of protein's backbone confirmation. Ramachandran plot is a key quality metric used in validation of protein's model. Through Ramachandran plot, the quality of predicted protein structure is often analyzed. In a polypeptide, each amino acid has its own unique phi and psi angles. Hence, on Ramachandran plot, each residue can be plotted representing phi and psi angles with x, y and z coordinates respectively.

When shifting from chain to secondary structure, polypeptide will adopt a certain torsion angle. This change results in the creation of shapes like alpha helices or beta sheets in Ramachandran plot. This method enables us to identify and verify secondary structure within a constrained region on the plot. In the plot, these tools identify the allowed regions and most favored regions. The yellow area in the graph indicate the allowed region whereas the red area shows the most favored regions [13].

Physiochemical properties analysis

To examine the analysis of physiochemical properties of target VP39 MTase, ExPASY ProtParam tool was used which include following parameters like Molecular weight, GRAVY score (grand average of hydropathicity), number of amino acid residues, atomic formula, aliphatic index, number of positively and negatively charged residues, log P, predicted half-life, theoretical *pI* and iso-electric point [14].

The contribution of aliphatic side chains to the overall amino acid composition of a protein quantify by aliphatic index. A protein carries no net charge at its isoelectric point which helped us to identify whether it was acidic or basic in nature. By dividing the total hydropathy of amino acids by total residues in the protein, GRAVY score is calculated. Half-life indicates the time required for a substance to decrease to half its original amount. To compare the hydropathy of signal peptides, average overall hydropathy (GRAVY) was used [12].

Results

Structure verification

The 3D structure of VP39 MTase was retrieved from Protein Databank (PDB ID: 8cer) and purified with the help of three programs from structure analysis and verification server SAVES V6.0. These are ERRAT, Verify 3D and PROCHECK in Table 1.

ERRAT analysis shows the 94.39% overall quality factor of VP39 MTase. This may be applied in many fields such as drug designing and target identification because ERRAT results showing that this model has good quality. The prediction accuracy for protein residues analyzed by overall quality factor. ERRAT performed the quality assessment helps to locate the problematic regions in the predicted structure. VP39 MTase in the pdb format was submitted as an input in the ERRAT program that generates a residue level assessment and quality factor in the form of plot shown in Figure 1.

Above 90% quality factor indicate high (good) resolution structure. In terms of percentage error value per residue, the plot provides the residue wise insight.

74.91% residues have averaged 3D-1D score ≥ 0.1 showed by Verify 3D analysis illustrating that the structure of VP39 MTase is quite compatible. From atomic coordinates of the experimentally solved structures, the 3D profile of protein structure is calculated. Each residue's average 3D-1D score are shown in a graphical plot. VP39 MTase of MPXV required in pdb format as an input in this Verify 3D program that generates 3D-1D score for each residue as shown in Figure 2.

93.5% in most favored regions shown by Ramachandran plot in the PROCHECK, 6.5% residues in additional allowed regions and 0.0% residues in disallowed region. A well-modeled protein shows that most of solid blue square residues should lie in red shaded region marked as most favorable region, orange-shaded region shows the favored region and the yellow- shaded areas indicated the generously allowed region. The pdb file format of VP39 MTase was required as an input in this PROCHECK server to assess the quality of protein structure as shown in Figure 3.

Analysis of physiochemical properties

Physiochemical characteristics of VP39 MTase was analyzed by ExPASY ProtParam tool. The predicted protein has 38886.98 molecular weight and 297 amino acids. The theoretical PI of target protein is shown to be 9.47. The total number of positively charged residues (Arg + Lys) is 47 and 34 is total number of negatively charged residues (Asp + Glu).

$C_{1764}H_{2762}N_{472}O_{493}S_{13}$ is the molecular formula of VP39 MTase. -0.395 is the GRAVY score showing that the target protein is hydrophilic. The negative value of GRAVY score also indicate that protein exhibit non-polar nature. The protein is thermostable indicated by aliphatic index because it is seen to be around 88.65 that is little high. Protein exhibit 30 hours half-life in mammalian reticulocyte cell in-vitro due to methionine as N-terminal residue. These physiochemical properties are illustrated in Table 2.

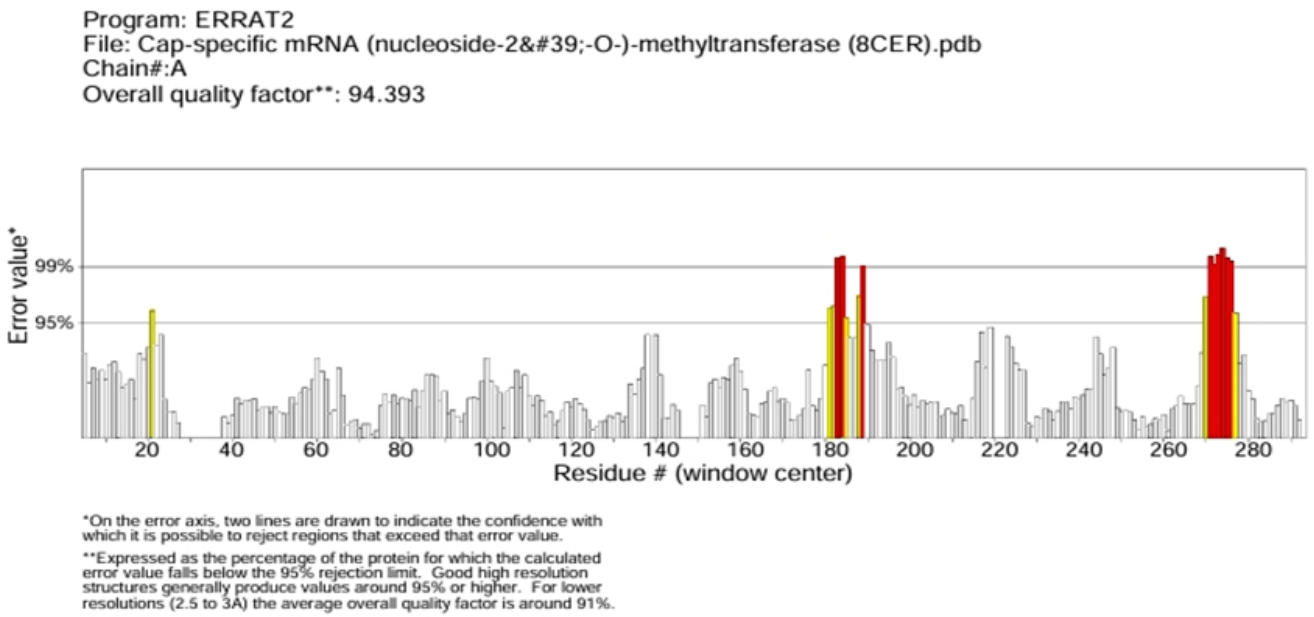

Figure 1. Structure validation result of VP39 MTase (8cer) by ERRAT

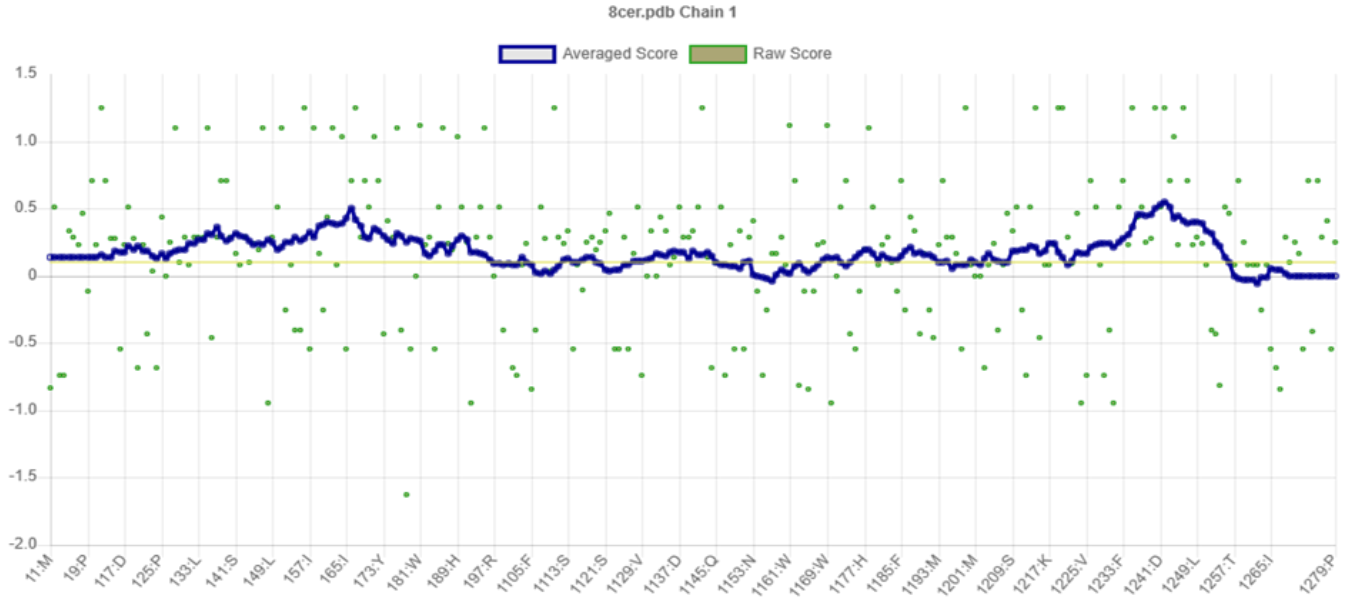

Figure 2. Structure validation of VP39 MTase (8cer) by *Verify* 3D

Figure 3. Structure validation of VP39 MTase (8cer) by Ramachandran plot of PROCHECK

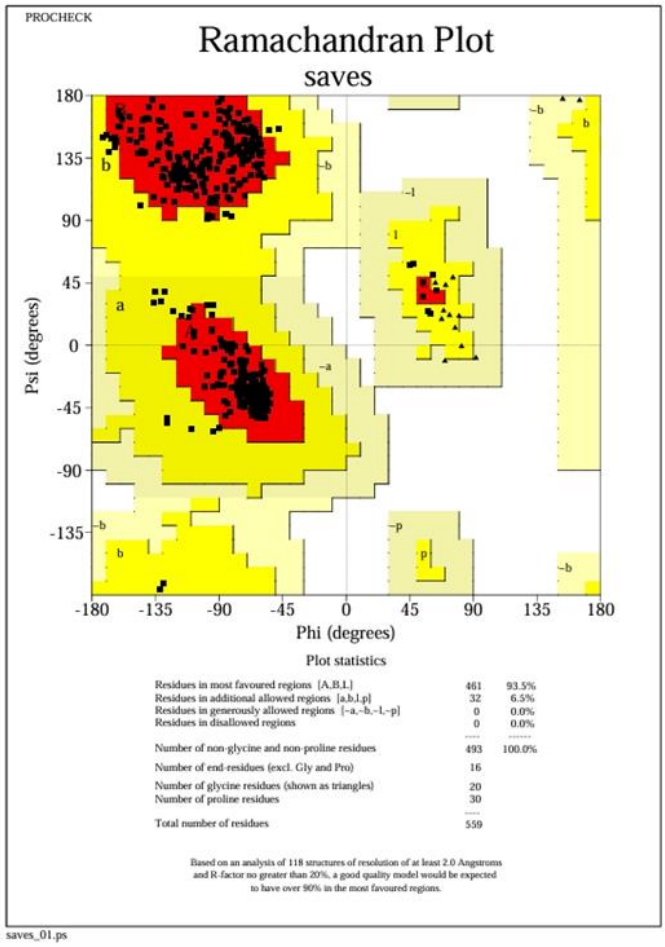

Table 1: Results of program used for crystal structure verification of VP39 MTase protein

Programs for structure validation	VP39 MTase protein (8cer)
ERRAT	Overall quality factor was 94.393
Verify 3D	74.91% residues had averaged 3D-1D score > 0.1
Ramachandran plot (PROCHECK)	93.5% residues in most favored region. 6.5% in allowed regions. 0.0% in disallowed region.

Table 2: Physiochemical parameters of VP39 MTase protein of MPXV computed by using ProtParam

Parameters	VP39 MTase protein (8cer)
Mol. Weight	38886.98
No. of amino acids	297
Theoretical Pi	9.47
Instability index (II)	50.20
No. of negatively charged residues (Asp + Glu)	34
No. of positively charged residues (Arg + Lys)	47
Aliphatic index	88.65
Grand average of hydropathicity (GRAVY)	-0.395
Estimated half-life	30h (mammalian reticulocytes, in vitro)
Atomic composition	$C_{1764}H_{2762}N_{472}O_{493}S_{13}$

Discussion

From the Poxviridae family or Orthopoxvirus genus, human MPXV is double stranded DNA virus. It is a zoonotic viral disease. In 1970 in DRC, first human case of MPXV was recorded in a child [15]. The disease has been endemic to Central and West Africa ever since its discovery. MPXV cases reported among humans that were transmitted from local wildlife. Over the past 20 years, the number of human MPXV cases has increased exponentially [16]. In United State in 2003, the endemic was linked to prairie dogs that were infected by Gambian pouched rats.

The WHO received the reports of 28 suspected cases and 92 confirmed cases between May 13, and May 21, 2022 in non-endemic countries. Most cases are involved in the men who have sex with men (MSM). MPXV circulation have been linked with human-human transmission [17]. Due to ongoing global monkeypox outbreak on 23 July 2022, WHO declared the Public Health Emergency of International Concern (PHEIC) [18].

After secondary viremia, symptoms begin leading to short prodromal phase (e.g, fever) before lesion appears. At this time, infected patients have ability to transmit virus among humans. Fever, headache, fatigue, are the initial symptoms of monkeypox disease. Mucosal lesions develop in the mouth after one to two days [19]. During 2022 outbreak, most patients developed the rash with papules, vesicles in the anal, oral or genital regions that long from 7-10 days. [20]. Computational analysis technique for drug discovery helps to provide more rapid solutions for MPXV treatment because there is not drug has been specific for monkeypox prevention [21]. VP39 MTase was chosen on the base of their role in viral replication and immune evasion [11]. Because of its role in DNA synthesis, transcription and

modification of viral proteins, viral enzyme can be considered as a promising target for treatment. VP39 MTase (8cer) escape the host immunity [22] To identify the potential inhibitor for this enzyme, our study aimed to analyze and validate the protein structure of VP39 MTase for drug discovery.

From Protein Databank (PDB), crystal structure of VP39 MTase was retrieved on the base of fine experimental resolution, number of amino acids and X-ray diffraction. Then structure validation was done by SAVES v6.1 server. And physiochemical characteristics were visualized by ExPASY ProtParam tool. All this analysis showed that the VP39 MTase is of good quality protein and is most effective in drug discovery against monkeypox disease.

Conclusion

Finding potential inhibitors by using virtual screening is cheap and less time consuming and has become one of the modern drug development techniques. Against monkeypox disease, VP39 MTase (8cer) was selected as a receptor due to its role in viral replication. In this study, protein structure was selected on the base of X-ray diffraction and sequence coverage from PDB and then purified by using ERRAT, Verify 3D and PROCHECK programs of online SAVES v6.1 server to check their validity. Then physiochemical properties of VP39 MTase was examined with the help of online ExPASY ProtParam tool. All these results indicate that protein structure is most favored and good quality protein to meet needs of effective antiviral target against MPXV for drug discovery.

Authors' contributions

ICMJE criteria	Details	Author(s)
1. Substantial contributions	Conception, OR	1
	Design of the work, OR	1
	Data acquisition, analysis, or interpretation	1
2. Drafting or reviewing	Draft the work, OR	1
	Review critically for important intellectual content	1
3. Final approval	Approve the version to be published	1
4. Accountable	Agree to be accountable for all aspects of the work	1

Acknowledgement

None

Funding

This research study received no specific grant from any funding agency.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

The Ethics Review Committee of University of Management and Technology, Lahore approved the study. Informed consent was taken from all volunteer participants.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

References

[1]. Lu J, Xing H, Wang C, Tang M, Wu C, Ye F, et al. Mpox (formerly monkeypox): Pathogenesis, prevention and treatment. Signal Transduction and Targeted Therapy. 2023;8(1):458.

[2]. Beer EM, Rao VB. A systematic review of the epidemiology of human monkeypox outbreaks and implications for outbreak strategy. PLoS Negl Trop Dis. 2019;13(10):e0007791.

[3]. Rampogu S, Kim Y, Kim S-W, Lee KW. An overview on monkeypox virus: Pathogenesis, transmission, host interaction and therapeutics. Front Cellul Infect Microbiol. 2023;13:1076251.

[4]. Soheili M, Nasseri S, Afraie M, Khateri S, Moradi Y, Mortazavi SMM, et al. Monkeypox: virology, pathophysiology, clinical characteristics, epidemiology, vaccines, diagnosis, and treatments. J Pharm Pharm Sci. 2022;25:297-322.

[5]. Anwar F, Haider F, Khan S, Ahmad I, Ahmed N, Imran M, et al. Clinical manifestation, transmission, pathogenesis, and diagnosis of monkeypox virus: a comprehensive review. Life. 2023;13(2):522.

[6]. Hraib M, Jouni S, Albitar MM, Alaidi S, Alshehabi Z. The outbreak of monkeypox 2022: An overview. Ann Med Surg. 2022;79:104069.

[7]. Letafati A, Sakhavarz T. Monkeypox virus: A review. Microb Pathog. 2023;176:106027.

[8]. Shuvo PA, Roy A, Dhawan M, Chopra H, Emran TB. Recent outbreak of monkeypox: Overview of signs, symptoms, preventive measures, and guideline for supportive management. Int J Surg. 2022;105:106877.

[9]. Aftab A, Mateen R, Afzal MS. In-silico analysis of potential phytochemicals against VP39 methyltransferase of monkeypox virus. Chron Biomed Sci. 2025;2(2):51.

[10]. Thai QM, Phung HT, Pham NQA, Horng JT, Tran PT, Tung NT, et al. Natural compounds inhibit Monkeypox virus methyltransferase VP39 in silico studies. J Biomol Struct Dyn. 2024:1-9.

[11]. Hashim HO, Al-Shuhaib JM, Mohammed MK, Al-Shuhaib MBS. Targeting monkeypox virus

methyltransferase: virtual screening of natural compounds from middle-eastern medicinal plants. Mol Biotechnol. 2024;67:3194-216.
[12]. Raen R, Islam MM, Islam R, Islam MR, Jarin T. Functional characterization and structural prediction of hypothetical proteins in monkeypox virus and identification of potential inhibitors. Mol Diver. 2024;29(1):1589-617.
[13]. Sobolev OV, Afonine PV, Moriarty NW, Hekkelman ML, Joosten RP, Perrakis A, et al. A global Ramachandran score identifies protein structures with unlikely stereochemistry. Structure. 2020;28(11):1249-58.
[14]. Gasteiger E, Gattiker A, Hoogland C, Ivanyi I, Appel R, ExPAS AB. The proteomics server for in-depth protein knowledge and analysis. Nucleic Acids Res. 2003;5(10):3784-8.
[15]. Von Pressentin KB, Kaswa R, Murphy S, Nair A. A review of published research in the South African Family Practice-A clarion call to action. S Afr Fam Pract. 2023;65(4):a5777.
[16]. Petersen E, Kantele A, Koopmans M, Asogun D, Yinka-Ogunleye A, Ihekweazu C, et al. Human monkeypox: epidemiologic and clinical characteristics, diagnosis, and prevention. Infect Dis Clin North Am. 2019;33(4):1027-43.
[17]. Kumar S, Subramaniam G, Karuppanan K. Human monkeypox outbreak in 2022. J Med Virol. 2023;95(1):e27894.
[18]. Saied AA, Dhawan M, Metwally AA, Fahrni ML, Choudhary P, Choudhary OP. Disease history, pathogenesis, diagnostics, and therapeutics for human monkeypox disease: a comprehensive review. Vaccines. 2022;10(12):2091.
[19]. Zahmatyar M, Fazlollahi A, Motamedi A, Zolfi M, Seyedi F, Nejadghaderi SA, et al. Human monkeypox: history, presentations, transmission, epidemiology, diagnosis, treatment, and prevention. Front Med. 2023;10(1):1157670.
[20]. Mitjà O, Ogoina D, Titanji BK, Galvan C, Muyembe JJ, Marks M, et al. Monkeypox. Lancet. 2023;401(10370):60-74.
[21]. Naveed M, Shabbir MA, Ain Nu, Javed K, Mahmood S, Aziz T, et al. Chain-engineering-based de novo drug design against MPXVgp169 virulent protein of monkeypox virus: A molecular modification approach. Bioengineering. 2022;10(1):11.
[22]. Hassan AM, Gattan HS, Faizo AA, Alruhaili MH, Alharbi AS, Bajrai LH, et al. Evaluating the binding potential and stability of drug-like compounds with the monkeypox virus VP39 protein using molecular dynamics simulations and free energy analysis. Pharmaceuticals. 2024;17(12):1617.