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# Evolution, pathogenesis, host interactions and therapeutic strategies against monkeypox virus

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**Published in**
Frontiers in Cellular and Infection Microbiology
Frontiers in Public Health

ISSN 1664-8714
ISBN 978-2-8325-3801-2
DOI 10.3389/978-2-8325-3801-2

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# Evolution, pathogenesis, host interactions and therapeutic strategies against monkeypox virus

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**Citation**

Khattak, S., Hoque, M., Singh, S., eds. (2023). *Evolution, pathogenesis, host interactions and therapeutic strategies against monkeypox virus*. Lausanne: Frontiers Media SA. doi: 10.3389/978-2-8325-3801-2

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frontiers | Frontiers in Cellular and Infection Microbiology

TYPE Editorial
PUBLISHED 12 October 2023
DOI 10.3389/fcimb.2023.1303598



OPEN ACCESS

EDITED AND REVIEWED BY
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RECEIVED 28 September 2023
ACCEPTED 02 October 2023
PUBLISHED 12 October 2023

CITATION
Hoque M, Singh S and Khattak S (2023) Editorial: Evolution, pathogenesis, host interactions and therapeutic strategies against monkeypox virus.
*Front. Cell. Infect. Microbiol.* 13:1303598.
doi: 10.3389/fcimb.2023.1303598



# Editorial: Evolution, pathogenesis, host interactions and therapeutic strategies against monkeypox virus

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KEYWORDS

**monkeypox (MPX), orthopox virus, epidemiology, public health concerns, diagnosis, treatment**

Editorial on the Research Topic
Evolution, pathogenesis, host interactions and therapeutic strategies against monkeypox virus

Considering the current global crisis of COVID-19, another viral pandemic might exacerbate concerns as well as the healthcare and socioeconomic repercussions. The present outbreak of monkeypox on a worldwide scale is unprecedented. Monkeypox is a zoonotic viral disease caused by a double-stranded DNA virus MPXV of the orthopox genus. This was first identified as a pox-like viral disease in monkeys in 1958, and the first case of human monkeypox was detected in the Democratic Republic of the Congo in 1970. Since then, occasional cases and minor outbreaks have been reported in non-endemic countries, with their re-emergence in 2022. On July 23, 2022, the World Health Organization (WHO) declared monkeypox a global public health emergency.

A perspective review by Shah et al. delineates the journey of monkeypox since the first human case, focusing on the reasons for the growing instances and methods to prevent the virus from spreading. The evolution of MPXV is governed by positive Darwinian selection that drives the adaptation of host interaction (Zhan et al.). The study using 156 coding genes encompassing more than 95% of the MPXV genome isolated from 1,500 samples found that the genes involved in host interaction and host determination (MPXVgp004, 010, 012, 014, 044, 098, 178, 188, and 191) evolved under positive Darwininan selection. Three recently reported missense substitution mutations (T/A426V in MPXVgp010, A423D in MPXVgp012, and S105L in MPXVgp191) were found to be critical for the virus' adaptation to humans. Thus, monitoring the mutational landscape of the viral genes, particularly those undergoing positive selection for predicting viral transmission and virulence, is crucial.

As there are not many treatment options available for monkeypox disease, understanding the mechanisms of transmission and symptoms might help prevent the disease's spread. Common disease symptoms include fever, swollen lymph nodes, muscular pains, headache, backache, and weariness. The appearance of rashes with blisters and crusts follows these symptoms. Although

the symptoms typically develop 10 to 14 days after infection, they can appear up to 21 days. One to four days following the prodrome, there is a severe, deep-seated, vesicular, or pustular rash with central distribution. The face is most commonly afflicted, followed by the palms, soles, oral mucous membrane, and occasionally the genitalia and conjunctivae (Khattak et al.; Rampogu et al.). A meta-analysis by Rani et al. confirms that skin lesions contain the viral DNA that raises the risk of infection and potential transmission via direct skin-to-skin contact. The virus can spread to people by contact with the body fluids, skin lesions, and droplets of infected animals. Additionally, eating undercooked meat, being exposed to contaminated fomites, infected animals, eating bush meat, or wild game may all result in animal-to-human transmission. Human-to-human transmission can also occur when patients share the same household and consumables, or through healthcare contact (Rampogu et al.).

Identifying a disease at the right time is crucial for prevention and treatment. There are several methods available for the diagnosis of MPXV that include assays for IgM and IgG, Enzyme-Linked Immunosorbent Assay (ELISA), Polymerase Chain Reaction (PCR), electron microscopy, virus isolation, Immunofluorescence antibody test, and histopathology (Khattak et al.). Unfortunately, most of these techniques are equivocal and cannot distinguish MPXV disease from other poxvirus infections. However, an accurate diagnosis can be made via PCR analysis. A DNA oligonucleotide microarray that involves the TNF receptor gene crmB has been designed as an additional rapid method for identifying orthopoxviruses (Khattak et al.).

Fast-track therapeutic development is crucial for combating a disease with a high transmission rate. To this end, computational drug discovery and repurposing of existing drugs could be a viable tool for developing therapies against MPXV. In an attempt to identify a potential drug against the virus, Ajmal et al. performed *in silico* screening of 9000 FDA-approved compounds from the DrugBank database against MPXV protein thymidylate kinase. The researchers found three compounds that might bind and inhibit the viral target: DB16335, DB15796, and DB16250, with DB16335 being proposed as a possible medication that could help prevent MPXV. In separate research, the potential of curcumin derivatives as MPXV and smallpox virus inhibitors was investigated using molecular docking and dynamic simulations (Akash et al.). They identified twelve possible natural curcumin compounds with antiviral potential. Al Mashud et al. conducted a similar *in silico* investigation to evaluate the ability of O-rhamnosides and Kaempferol-o-rhamnosides derivatives to inhibit MPXV and Marburg virus. They discovered that the compounds L07, L08, and L09 exhibited better affinity for the target protein than the FDA-approved antiviral drug Cidofovir, suggesting their potential for antiviral treatment against MPXV and Marburg virus. However, before these molecules can be utilized as drugs, they must be thoroughly investigated in real-world situations and put through clinical trials.

Vaccines are the most potent protective measures against viral infections. The smallpox vaccines are effective against MPXV. The ACAM2000, a newer-generation smallpox vaccination, has received FDA approval for treating MPXV (Khattak et al.). However, the earlier generation of ACAM2000 may also be used off-label for the same purpose. Vaccinia immune globulin may be alternative post-exposure preventive measures if smallpox vaccination is unavailable. Translation of a novel drug or vaccine from laboratory to clinic requires time and should be tested in clinical trials. According to the study by Alorfi et al., only 10 interventional trials are registered at ClinicalTrials.gov. Of these, 4 trials were registered in Europe, 3 in America, and 3 in Africa. Forty percent of the registered trials were conducted for the JYNNEOS vaccine and 30% for Tecovirimat. The effectiveness and safety of the medications and vaccinations used to treat MPXV must thus be evaluated immediately through large-scale randomized clinical trials. Global concerted efforts must be made continuously to prepare for another epidemic.

## Author contributions

MH: Writing – original draft, Writing – review & editing. SS: Writing – review & editing. SK: Writing – original draft, Writing – review & editing.

## Funding

The author(s) declare that no financial support was received for the research, authorship, and/or publication of this article.

## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

## Publisher's note

frontiers | Frontiers in Cellular and Infection Microbiology

TYPE Original Research
PUBLISHED 13 January 2023
DOI 10.3389/fcimb.2022.1083234



OPEN ACCESS

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SPECIALTY SECTION
This article was submitted to Virus and Host, a section of the journal Frontiers in Cellular and Infection Microbiology

RECEIVED 28 October 2022
ACCEPTED 16 December 2022
PUBLISHED 13 January 2023

CITATION
Zhan X-Y, Zha G-F and He Y (2023) Evolutionary dissection of monkeypox virus: Positive Darwinian selection drives the adaptation of virus–host interaction proteins.
*Front. Cell. Infect. Microbiol.* 12:1083234.
doi: 10.3389/fcimb.2022.1083234



# Evolutionary dissection of monkeypox virus: Positive Darwinian selection drives the adaptation of virus–host interaction proteins

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The emerging and ongoing outbreak of human monkeypox (hMPX) in 2022 is a serious global threat. An understanding of the evolution of the monkeypox virus (MPXV) at the single-gene level may provide clues for exploring the unique aspects of the current outbreak: rapidly expanding and sustained human-to-human transmission. For the current investigation, alleles of 156 MPXV coding genes (which account for >95% of the genomic sequence) have been gathered from roughly 1,500 isolates, including those responsible for the previous outbreaks. Using a range of molecular evolution approaches, we demonstrated that intra-species homologous recombination has a negligible effect on MPXV evolution. Despite the fact that the majority of the MPXV genes (64.10%) were subjected to negative selection at the whole gene level, 10 MPXV coding genes (MPXVgp004, 010, 012, 014, 044, 098, 138, 178, 188, and 191) were found to have a total of 15 codons or amino acid sites that are known to evolve under positive Darwinian selection. Except for MPXVgp138, almost all of these genes encode proteins that interact with the host. Of these, five ankyrin proteins (MPXVgp004, 010, 012, 178, and 188) and one Bcl-2-like protein (MPXVgp014) are involved in poxviruses' host range determination. We discovered that the majority (80%) of positive amino acid substitutions emerged several decades ago, indicating that these sites have been under constant selection pressure and that more adaptable alleles have been circulating in the natural reservoir. This finding was also supported by the minimum spanning networks of the gene alleles. The three positive amino acid substitutions (T/A426V in MPXVgp010, A423D in MPXVgp012, and S105L in MPXVgp191) appeared in 2019 or 2022, indicating that they would be crucial for the virus' eventual adaptation to humans. Protein modeling suggests that positive amino acid substitutions may affect protein functions in a variety of ways. Further study should focus on revealing the biological effects of positive

amino acid substitutions in the genes for viral adaptation to humans, virulence, transmission, and so on. Our study advances knowledge of MPXV's adaptive mechanism and provides insights for exploring factors that are responsible for the unique aspects of the current outbreak.

KEYWORDS

**monkeypox virus, positive Darwinian selection, virus–host interaction proteins, ankyrin, adaptation, host range**

## Introduction

The emerging and ongoing outbreak of monkeypox (hMPX) in 2022 has thus far caused more than 82,021 confirmed cases in 110 countries worldwide and is a serious global threat (Centers for Disease Control and Prevention, 2022). As a zoonotic pathogen to humans, the monkeypox virus (MPXV) belongs to the genus *Orthopoxvirus*, which also contains other infectious agents, such as the Vaccinia virus (VACA), the Cowpox virus, and the Smallpox virus (Petersen et al., 2014). In the environment, wild squirrels, wild-living sooty mangabeys, and Gambian giant rats may be natural hosts for MPXV, as indicated by the isolation and the high seroprevalence of the virus in these animals (Radonić et al., 2014; Falendysz et al., 2017), while humans may be the accidental host for MPXV (Farasani, 2022). A recent study speculated on a prevailing hypothesis about the hMPX outbreak in 2022: a single imported case, amplified through one or more super-spreader events because the current 2022 outbreak strains are similar to the strain that caused an outbreak in 2018 to 2019 in Nigeria and traveled to Singapore (Yong et al., 2020; Isidro et al., 2022).

Many studies have shown that the 2022 outbreak of hMPX may be linked to a new lineage, which did not appear before (Isidro et al., 2022; Luna et al., 2022). MPXV has undoubtedly evolved for several decades in its natural hosts, which act as a gene melting pot, unwittingly educating the virus to be more adaptable to humans. With changing epidemiology and increased human-to-human transmission in the 2022 outbreak, it appears that MPXV has become more adaptable to humans, making it a major challenge (Bunge et al., 2022; Kmiec and Kirchhoff, 2022; Velavan and Meyer, 2022; Li et al., 2022). Understanding the evolution of MPXV may therefore provide clues to the key episode of greater adaptation to humans. Wang et al. found that 10 proteins in the MPXV are more prone to mutation, and 24 nonsynonymous mutations were discovered in the 2022 isolates as compared to the 2018 isolates (Wang et al., 2022). A genomic and structural analysis suggests that six mutations in proteins involved in host–pathogen interaction in all MPVX isolates during 2022 may favor viral fitness (Benvenuto et al., 2022). An MPXV mutational study also reveals that the host APOBEC3 plays a role in viral evolution as well as indicators of potential MPXV human adaptation in ongoing microevolution (Isidro et al., 2022). Based on the protein structure study, Kannan et al. indicated that two novel mutations (L108F in F8L and G9R in E4R) could be potential contributing factors to the 2022 outbreak (Kannan et al., 2022). It is unknown whether these MPXV mutations are advantageous traits from the perspective of molecular evolution. Germline or genetic mutations leave behind heritable changes, and natural selection acts on such variations within populations by eliminating deleterious mutations and fixing advantageous ones (Desai and Fisher, 2007; Gregory, 2009). MPXV has about 190 protein-coding genes, which have various functions during the infection (Shchelkunov et al., 2002; Lum et al., 2022). It is necessary to analyze changes in all codons of these genes by comparing MPXV gene alleles from existing isolates to determine the evolutionary impact on the MPXV and reveal the possible advantage of the mutations over the current outbreak isolates. Positive selection, a kind of Darwinian natural selection, is the most crucial evolutionary mechanism (Tiwary, 2022). By using positive selection, the virus could accumulate beneficial mutations to overcome challenging environmental conditions and adapt better to the new host (Asif et al., 2022; Lu et al., 2022). Thus, identifying genes that evolved under positive natural selection is a central goal in studies of molecular evolution for the virus (Venkat et al., 2018). In addition, homologous recombination, also known as intragenic recombination, is another key evolutionary mechanism that can generate genetic variation, which is tested by natural selection, and as such, it also plays an important role in fueling adaptive evolution in the virus (Jouet et al., 2015; Perez-Losada et al., 2015; Tiwary, 2022). To date, over 1,000 MPXV genomes have been sequenced. This can accelerate both genome-wide and single-gene studies on the MPXV. Given that little is known about the evolutionary forces mentioned above acting upon MPXV at the single-gene level, the current study aims to frame the underlying patterns in MPXV evolution at the single-gene level, as well as investigate the current outbreak from

an evolutionary standpoint. We incorporate a variety of molecular evolution algorithms to identify MPXV genes that are promoted by intragenic recombination, and, in particular, positive selection. Those codons and MPXV genes that experience positive selection may play key roles in favor of viral survival or human-to-human transmission in the context of the current outbreak, and thus be a potential keystone in the extraordinary 2022 hMPX outbreak. As a result, identifying these genes and codons is crucial for future research. It may provide evolutionary clues for exploring the unique aspects of the current outbreak regarding transmission dynamics, particularly its unprecedented rapid expansion and enhanced and sustained human-to-human transmission.

# Materials and methods

## MPXV strains and study design

About 1,500 sequenced MPXV isolates were enrolled in the study. MPXV genes with lengths more than 300 bp were selected for study since shorter genes lack sufficient variation/alleles for recombination and selection analysis. A total of 156 protein-coding genes were selected for study, accounting for approximately 82% and >95% of MPXV genes in terms of number and nucleotide length, respectively. The details of these genes are shown in Supplementary Table 1. The coding genes of MPXV isolate MPXV-USA2003_099_Rope_Squirrel (GenBank accession no. MT903348) were set as references. Nucleic acid sequences of a single gene of MPXV were retrieved by the NCBI Basic Local Alignment Search Tool (BLAST) (https://blast.ncbi.nlm.nih.gov/Blast.cgi) using reference gene sequences as a query. The BLAST program was configured to use Standard databases (nr) of Nucleotide collection (nr/nt) on 25 September 2022, the organism was set as MPXV (taxid:10244), and the algorithm was set as default parameters by increasing the maximum target sequences to 5,000.

## MPXV gene sequence and phylogenetic analysis

The alignment of about 1,500 MPXV isolates downloaded from BLAST was manually checked for integrity. Those truncated gene sequences were removed. Gene sequences were then re-aligned by MEGA X software using Muscle (codons) algorithms (Kumar et al., 2018). Allele profile analyses were performed by using DnaSP 6.12.03 (Rozas et al., 2017). Three to 55 alleles of these genes were obtained (Supplementary dataset). The most appropriate nucleotide substitution model for each coding gene was determined by the model finder module of MEGA X and using the Akaike Information Criterion (AIC) (Posada and Buckley, 2004). An unrooted phylogenetic tree of these genes was constructed using MEGA X, with evolutionary history inferred using the Neighbor-Joining (NJ) method and an appropriate nucleotide substitution model obtained by the model finder algorithm (Hasegawa et al., 1985). The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1,000 replicates) is shown next to the branches. The evolutionary distances were computed using the Tamura 3-parameter method and are in the units of the number of base substitutions per site (Tamura, 1992). A minimum spanning network (MSN) was constructed by PopART (http://popart.otago.ac.nz) (Bandelt et al., 1999; Leigh and Bryant, 2015), using the alignment of the coding genes to visualize the relationships among gene alleles within MPXV.

## Intra-species homologous recombination analysis

The allele sequences of the protein-coding genes of MPXV were screened by RDP5 to detect intragenic recombination (Martin et al., 2015). Six methods named RDP (Martin and Rybicki, 2000), GENECONV, BookScan (Martin et al., 2005), MaxChi (Smith, 1992), Chimaera (Posada, 2002), and SiScan (Gibbs et al., 2000) that were implemented in the RDP5 were utilized. Recombination was defined as those gene alleles that were found to have recombination events identified by at least three of the methods. All six methods used the same settings: treating sequences as linear, setting statistical significance at $p < 0.05$, and using Bonferroni correction for multiple comparisons. Recombination was also confirmed by the reticulate network tree by observing the side edges in the reticulate network, which commonly arise from recombination, using the SplitsTree5 software (Huson and Bryant, 2006).

## Evolutionary selection analysis

To identify selection pressure operating on genes at the gene or codon level, we used the Maximum Likelihood (ML) method with a visual tool of the codeML software program (Bielawski et al., 2016), named EasyCodeML (Gao et al., 2019). Many codon substitution models were used not only for measuring the average divergence nonsynonymous (dN) and synonymous (dS) ratio of a gene's substitution, which is commonly used in evolutionary genetic studies and denoted dN/dS, also known as ω, but also for identifying positive selection at the codon level of a gene. First, the topologies of NJ trees for each gene allele were

generated by MEGA X, as mentioned above, for the subsequent selection analysis. Multiple seed values were used to fit the model. Codon substitution models include M0 (one ratio, one ω for all sites, indicating an average ω for a whole gene), M1a (nearly neutral, two classes of sites, defined $\omega 0 < 1$ or $\omega 1 = 1$), M2a (positive selection, allows three site classes including negative, $\omega 0 < 1$; neutral, $\omega 1 = 1$; and positive, $\omega 2 > 1$), M3 (discrete, allows unconstrained discrete distribution of ω among sites), M7 (β, fit to a β distribution for ω among sites), and M8 (β and $\omega > 1$, fit to a β distribution with an extra rate that allows $\omega = 1$). They were typed into null models (M0, M1a, and M7) and positive selection models (M3, M2a, and M8) (Yang et al., 2000). Three nested models, including the M3 vs. M0, the M2a vs. M1a, and the M8 vs. M7 were compared using likelihood ratio tests (LRTs) to assess the best fit of codons. If one of the three nested models showed an LRT $p < 0.05$ (which implies the rejection of null models, also known as the rejection of all codons with $\omega \leq 1$), the genes were defined as positive selection ones at the codon level. Then, Bayes empirical Bayes (BEB) and Naive Empirical Bayes (NEB) methods were used to identify codons that evolved under positive selection based on a posterior probability of more than 0.90. The average ω values of each gene, which indicate the whole gene selection level (average ω of all codons), were obtained by the M0 model. One exception is MPXVgp059, for which only three alleles in this gene could be found and the ML method could not be used for the calculation (≥4 alleles of the gene are required for using the ML method). Thus, the Nei-Gojobori method implemented in the MEGA X was utilized to estimate the dN/dS of the gene (Luna et al., 2022). Because recombination may impair the ML method's accuracy in detecting positive selection at the codon level, we used topologies of NJ trees of the gene alleles that exclude the recombinant for positive selection analysis. Fast, Unconstrained Bayesian AppRoximation (FUBAR) (Murrell et al., 2013), a method implemented in the Hyphy package that is based on a Markov chain Monte Carlo (MCMC) routine that ensures robustness against model misspecification by averaging over a large number of predefined site classes, was used to validate the results obtained by the ML method.

## Mapping of positively selected sites to structure models of proteins

The three-dimensional structure of those positive selection genes was modeled using the Phyre server (Kelley et al., 2015). The positive selection sites were mapped onto the structure and visualized by PyMOL (http://www.pymol.org/) (DeLano, 2002).

## Statistical analysis

Continuous variables were compared using the nonparametric Mann–Whitney *U* test. We estimated the frequency rates in each category for categorical variables. The proportions for categorical variables were compared using the chi-square test or Fisher's exact test (when the data were limited). Statistical significance was defined as $p < 0.05$. GraphPad Prism 8 (GraphPad Software) was used for graphing. Statistical analyses were performed using SPSS 25.0 (IBM software).

# Results

## Intragenic recombination rarely acts on the MPXV

Among the 156 analyzed genes, only two genes were found to have undergone a single recombination event during evolution, which generated only one recombinant allele for each gene (Table 1 and Figure 1). These genes were MPXVgp090 and

TABLE 1 Intragenic recombination among the gene sequences of monkeypox protein-coding genes by using six different methods implemented in the RDP software.

| Recombination events | Recombinant alleles | Major parent[#] | Minor parent[$] | Detection methods implemented in RDP software[^] | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | RDP | GENECONV | Bootscan | Maxchi | Chimaera | SiSscan |
| **MPXVgp090** | | | | | | | | | |
| 1 | NC_003310* | N/A | HM172544 | N | N[a] | N | Y[b] | Y | Y |
| **MPXVgp182** | | | | | | | | | |
| 1 | ON929062 | Unknown | AY741551 | N | Y | Y | Y | Y | Y |

* The allele sequence names are shown as their representative isolates' genome NCBI accession numbers.
[#] Major parent: parent allelic sequences contribute to the larger fraction of the sequence.
[$] Minor parent: parent allelic sequences contribute a smaller fraction of the sequence.
[^] Recombination events detected by more than two methods are shown.
[a]N indicates recombination events that were not detected by the selected method.
[b]Y indicates recombination events that were detected by the selected method.
N/A indicates not available.

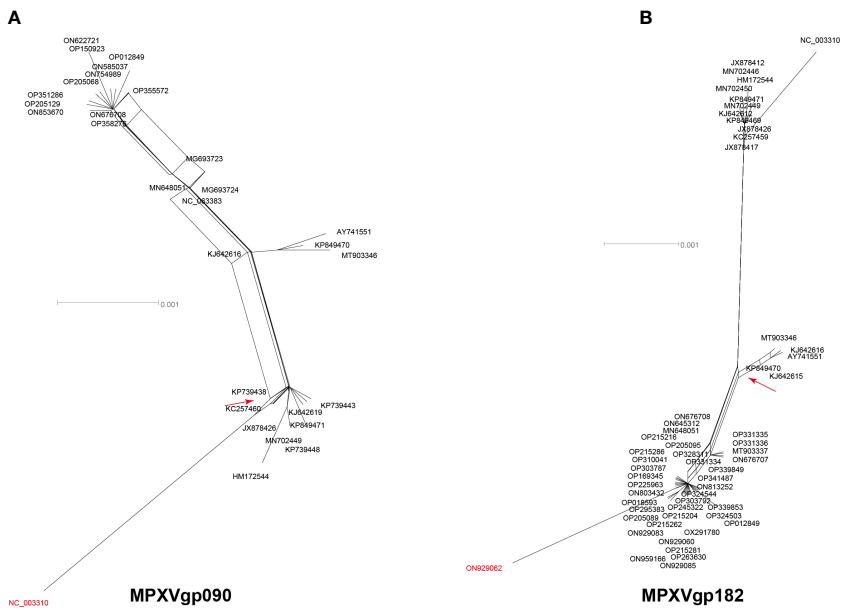


FIGURE 1
Neighbor-net phylogenetic network shows the relationship among the MPXVgp090 **(A)** and MPXVgp182 **(B)** alleles. The relation between and within alleles is illustrated by splits, representing simultaneously both grouping in the data and evolutionary distances between taxa, highlighting potential conflicting signals or alternative phylogenetic histories (recombination) in the gene molecular evolution. Allele names are shown as their representative MPXV isolate names, shown as GenBank accession numbers, and those that are marked in red indicate that they are recombinants. The network's internal nodes indicate hypothetical ancestral alleles, while the edges conform to reticulate events like recombination. The red arrow points to a representative reticulate event. Recombination events that were identified by three or more methods were selected and numbered. The parents' names of the recombinant were indicated according to the RDP5 analysis (see Table 1).

MPXVgp182, which functioned as RNA polymerase subunit and surface glycoprotein of the virus. The discovery of only one recombination event and one recombinant in the two genes suggests that intragenic recombination on the MPXV is rare.

## Whole gene level negative selection is the main force driving the evolution of MPXV

At the whole gene level, most (>94%) MPXV protein-coding genes experienced negative or neutral selection. It should be noted that 11 genes, namely, MPXVgp036, MPXVgp050, MPXVgp062, MPXVgp067, MPXVgp071, MPXVgp099, MPXVgp111, MPXVgp114, MPXVgp119, MPXVgp123, and MPXVgp164, experienced extremely purifying selection with $\omega < 0.1$. Detailed information on these genes is shown in Supplementary Table 2. In contrast, nine genes, namely, MPXVgp015, MPXVgp016, MPXVgp024, MPXVgp030, MPXVgp033, MPXVgp131, MPXVgp133, MPXVgp156, and MPXVgp182, experienced positive selection at the whole gene level with an average $\omega > 1.5$ (Figures 2A, B and Supplementary Table 2). There are 100 genes with ω values less than 0.5, accounting for 64.10% of all, indicating that negative selection is the main force driving the evolution of MPXV (Figure 2B).

## Adaptive evolution has a particular impact on genes involved in virus–host interactions, as well as the extraordinary ankyrin genes

By analyzing all the 156 MPXV genes using three paired LRTs, we found that ten genes, namely, MPXVgp004, MPXVgp010, MPXVgp012, MPXVgp014, MPXVgp044, MPXVgp098, MPXVgp138, MPXVgp178, MPXVgp188, and MPXVgp191, had alternative models (M3, M2a, and M8) that were significantly better fit ($p < 0.05$) than the relevant null models (M0, M1a, and M7), indicating that some codons of these genes were subjected to strong positive selection (Table 2). The codon-level positive selection operating these genes was also verified by the FUBAR algorithm, and identical results were obtained (Table 3 and Supplementary Table 3). Detailed information of the 10 genes, including the gene names, lengths, functions, amino acid substitution profiles, and nucleotide mutation profiles of these codons, is shown in

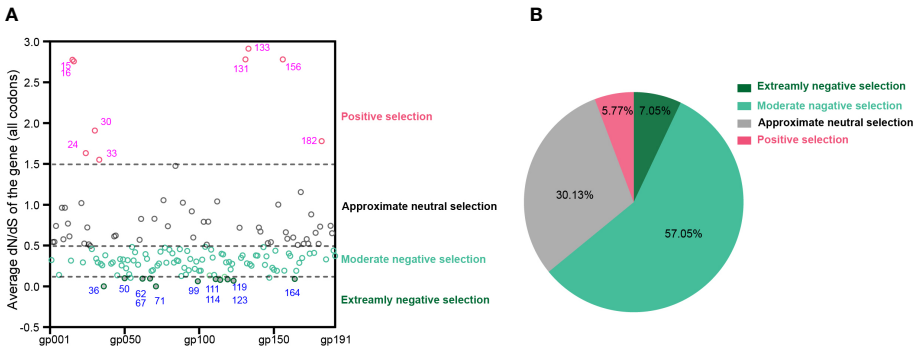


FIGURE 2
Uniform selective pressure at all sites of an MPXV coding gene. **(A)** The distribution of MPXV genes under different selection pressures. The genes are shown as their protein product names (e.g., MPXVgp001 and MPXVgp002). The blue font shows MPXV genes that have been subjected to extremely negative selection, while the magenta font shows those that have been subjected to positive selection at the whole gene level. Data are shown as dots with median, 25th, and 75th percentile lines. **(B)** The percentage of MPXV genes that are under different levels of uniform selection pressure.

TABLE 2 Log-likelihood values and parameter estimates for the MPXV genes (positive selection ones are shown).

| Gene/ Model | *nP^* | *lnL* | Estimates of parameters | LRT *p*-value | Positive sites (amino acid sites of the proteins) |
|---|---|---|---|---|---|
| **MPXVgp004** | | | | | |
| M3 (discrete) | 33 | −1808.429290 | $p0$ = 0.94660, $p1$ = 0.00031, **$p2$ = 0.05308**, ω0 = 0.0000, ω1 = 0.00000, **ω2 = 16.10807** | 0.0531 | **4 ***, 239***** |
| M0 (one ratio) | 29 | −1813.099765 | ω0 = 0.74079 | | Not allowed |
| M2a (selection) | 32 | −1808.429325 | $p0$ = 0.94967, $p1$ = 0.00146, **$p2$ = 0.05288**, ω0 = 0.0000, ω1 = 1.0000, **ω2 = 16.14577** | **0.0275** | **4 ***, 239***** |
| M1a (neutral) | 30 | −1812.022877 | $p0$ = 0.54762, $p1$ = 0.45238<br>ω0 = 0.0000, ω1 = 1.0000 | | Not allowed |
| M8[a] (beta & ω) | 32 | −1808.429296 | $p0$ = 0.94692, $p$ = 0.00500, $q$ = 1.3444<br>**$p1$ = 0.05308, ω=16.10854** | **0.0203** | **4 *, 239*** |
| M7 (beta) | 30 | −1812.328061 | $p$ = 0.020616, $q$ = 0.00783 | | Not allowed |
| **MPXVgp010&** | | | | | |
| M3 (discrete) | 37 | −2804.209085 | $p0$ = 0.90531, $p1$ = 0.00269, **$p2$ = 0.09200**, ω0 = 0.0000, ω1 = 0.0000, **ω2 = 11.83474** | **0.03044** | **258 ***, 426 ***, 637 ***** |
| M0 (one ratio) | 33 | −2809.414812 | ω0 = 0.96227 | | Not allowed |
| M2a (selection) | 36 | −2804.209082 | $p0$ = 0.90800, $p1$ = 0.0000, **$p2$ = 0.09200**, ω0 = 0.0000, ω1 = 1.0000, **ω2 = 11.83473** | **0.01093** | **258, 426, 637 (all Pr<0.90)** |
| M1a (neutral) | 34 | −2808.725356 | $p0$ = 0.35210, $p1$ = 0.64790<br>ω0 = 0.0000, ω1 = 1.0000 | | Not allowed<br>Not allowed |
| M8[a] (beta & ω) | 36 | −2804.209075 | $p0$ = 0.90800, $p$ = 0.00500, $q$ = 1.09878<br>**$p1$ = 0.09200, ω=11.83425** | **0.01090** | **258*, 426 *, 637*** |
| M7 (beta) | 34 | −2808.728483 | $p$ = 0.03361, $q$ = 0.01507 | | Not allowed |

*(Continued)*

TABLE 2 Continued

| Gene/ Model | *nP*^ | *lnL* | Estimates of parameters | LRT *p*-value | Positive sites (amino acid sites of the proteins) |
|---|---|---|---|---|---|
| **MPXVgp012** | | | | | |
| M3 (discrete) | 47 | −2587.302496 | *p*0 = 0.01201, *p*1 = 0.98568, ***p*2 = 0.00231**, ω0 = 0.64897, ω1 = 0.65105, **ω2 = 118.65649** | **0.02853** | **423** *** |
| M0 (one ratio) | 43 | −2592.718210 | ω0 = 0.76806 | | Not allowed |
| M2a (selection) | 46 | −2587.302497 | *p*0 = 0.99769, *p*1 = 0.0000, ***p*2 = 0.00231**, ω0 = 0.65096, ω1 = 1.00000, **ω2 = 118.62822** | **0.00852** | **423** *** |
| M1a (neutral) | 44 | −2592.067453 | *p*0 = 0.40911, *p*1 = 0.59089 ω0 = 0.0000, ω1 = 1.0000 | | Not allowed |
| M8[a] (beta & ω) | 46 | −2590.180238 | *p*0 = 0.87687, *p* = 0.00500, *q* = 2.27606 ***p*1 = 0.12313, ω=6.59688** | 0.1447 | **423**** |
| M7 (beta) | 44 | −2592.113143 | *p* = 0.01724, *q* = 0.00500 | | Not allowed |
| **MPXVgp014[@]** | | | | | |
| M3 (discrete) | 35 | −682.492062 | *p*0 = 0.00307, *p*1 = 0.97918, ***p*2 = 0.01776**, ω0 = 0.12777, ω1 = 0.12780, **ω2 = 13.16697** | **0.03837** | **153** *** |
| M0 (one ratio) | 31 | −687.554557 | ω0 = 0.31554 | | Not allowed |
| M2a (selection) | 34 | −682.492098 | *p*0 = 0.98224, *p*1 = 0.0000, ***p*2 = 0.01776**, ω0 = 0.12780, ω1 = 1.00000, **ω2 = 13.16703** | 0.09360 | **153** *** |
| M1a (neutral) | 32 | −684.860852 | *p*0 = 0.80994, *p*1 = 0.19006 ω0 = 0.0000, ω1 = 1.0000 | | Not allowed |
| M8[a] (beta & ω) | 34 | −682.894600 | *p*0 = 0.94323, *p* = 0.00500, *q* = 2.67398 ***p*1 = 0.05677, ω=6.36999** | 0.08159 | **153**** |
| M7 (beta) | 32 | −685.400602 | *p* = 0.02169, *q* = 0.06924 | | Not allowed |
| **MPXVgp044** | | | | | |
| M3 (discrete) | 43 | −2674.58825 | *p*0 = 0.0000, *p*1 = 0.99817, ***p*2 = 0.00183**, ω0 = 0.0000, ω1 = 0.49337, **ω2 = 162.92105** | **0.006599** | **203***** |
| M0 (one ratio) | 39 | −2681.702701 | ω0 = 0.62132 | | Not allowed |
| M2a (selection) | 42 | −2674.588251 | *p*0 = 0.99817 *p*1 = 0.0000, ***p*2 = 0.00183**, ω0 = 0.49325, ω1 = 1.0000, **ω2 = 169.91661** | **0.002770** | **203***** |
| M1a (neutral) | 40 | −2680.477136 | *p*0 = 0.55617, *p*1 = 0.44383 ω0 = 0.0000, ω1 = 1.0000 | | Not allowed |
| M8[a] (beta & ω) | 42 | −2677.568823 | *p*0 = 0.93841, *p* = 0.00500, *q* = 2.07484 ***p*1 = 0.06159, ω=11.07608** | **0.04588** | **203**** |
| M7 (beta) | 40 | −2680.650537 | *p* = 0.05271, *q* = 0.06315 | | Not allowed |
| **MPXVgp098** | | | | | |
| M3 (discrete) | 51 | −3498.007392 | *p*0 = 0.0000, *p*1 = 0.99640, ***p*2 = 0.00360**, ω0 = 0.0000, ω1 = 0.19605, **ω2 = 52.77541** | **0.02852** | **3**, 543**** |
| M0 (one ratio) | 47 | −3503.423302 | ω0 = 0.30333 | | Not allowed |
| M2a (selection) | 50 | −3498.007388 | *p*0 = 0.99640, *p*1 = 0.0000, ***p*2 = 0.00360**, ω0 = 0.19607, ω1 = 1.0000, **ω2 = 52.78524** | 0.05269 | **3**, 543**** |

*(Continued)*

TABLE 2 Continued

| Gene/ Model | *nP*^ | *lnL* | Estimates of parameters | LRT *p*-value | Positive sites (amino acid sites of the proteins) |
|---|---|---|---|---|---|
| M1a (neutral) | 48 | −3500.950791 | $p0 = 0.76240$, $p1 = 0.23760$<br>$\omega0 = 0.0000$, $\omega1 = 1.0000$ | | Not allowed<br>Not allowed |
| M8[a] (beta & ω) | 50 | −3498.646308 | $p0 = 0.95761$, $p = 0.00500$, $q = 1.69693$<br>**$p1 = 0.04239$, $\omega=7.54946$** | 0.0665 | **3*, 543*** |
| M7 (beta) | 48 | −3501.356812 | $p = 0.00500$, $q = 0.00694$ | | Not allowed |
| **MPXVgp138** | | | | | |
| M3 (discrete) | 99 | −2270.364472 | $p0 = 0.0000$, $p1 = 0.94970$, **$p2 = 0.05030$**, $\omega0 = 0.0000$,<br>$\omega1 = 0.09662$, **$\omega2 = 6.38119$** | **<0.00001** | **205 ***** |
| M0 (one ratio) | 95 | −2285.601424 | $\omega0 = 0.37838$ | | Not allowed |
| M2a (selection) | 98 | −2270.365494 | $p0 = 0.94967$, $p1 = 0.00008$, **$p2 = 0.05025$**, $\omega0 = 0.09666$, $\omega1 =$<br>$1.0000$, **$\omega2 = 6.38466$** | **0.003318** | **205 ***** |
| M1a (neutral) | 96 | −2276.073841 | $p0 = 0.77775$, $p1 = 0.22225$<br>$\omega0 = 0.0000$, $\omega1 = 1.0000$ | | Not allowed<br>Not allowed |
| M8[a] (beta & ω) | 98 | −2270.377125 | $p0 = 0.94987$, $p = 0.57961$, $q = 4.61694$<br>**$p1 = 0.05013$, $\omega=6.38213$** | **0.00116** | **205 ***** |
| M7 (beta) | 96 | −2277.135432 | $p = 0.00666$, $q = 0.02262$ | | Not allowed |
| **MPXVgp178** | | | | | |
| M3 (discrete) | 55 | −3234.202713 | $p0 = 0.0000$, $p1 = 0.99683$, **$p2 = 0.00317$**, $\omega0 = 0.00000$,<br>$\omega1 = 0.52285$, **$\omega2 = 62.79922$** | **0.03911** | **689 ***** |
| M0 (one ratio) | 51 | −3239.242595 | $\omega0 = 1.77937$ | | Not allowed |
| M2a (selection) | 54 | −3234.202647 | $p0 = 0.99683$, $p1 = 0.00441$, **$p2 = 0.01728$**, $\omega0 = 0.32017$, $\omega1 =$<br>$1.00000$, **$\omega2 = 14.74808$** | **0.01757** | **689 ***** |
| M1a (neutral) | 52 | −3238.244122 | $p0 = 0.48293$, $p1 = 0.51707$<br>$\omega0 = 0.0000$, $\omega1 = 1.0000$ | | Not allowed |
| M8[a] (beta & ω) | 54 | −3235.876765 | $p0 = 0.91074$, $p = 0.00500$, $q = 2.8176$<br>**$p1 = 0.08926$, $\omega=7.93303$** | 0.07363 | **689 **** |
| M7 (beta) | 52 | −3238.485516 | $p = 0.04221$, $q = 0.03779$ | | Not allowed |
| **MPXVgp188** | | | | | |
| M3 (discrete) | 33 | −1809.049451 | $p0 = 99767$, $p1 = 0.0000$, **$p2 = 0.00233$**, $\omega0 = 0.64737$,$\omega1 =$<br>$5.47829$, **$\omega2 = 435.15062$** | 0.1201 | **239***** |
| M0 (one ratio) | 29 | −1812.707933 | $\omega0 = 0.74175$ | | Not allowed |
| M2a (selection) | 32 | −1809.049133 | $p0 = 0.99767$, $p1 = 0.0000$, **$p2 = 0.00233$**, $\omega0 = 0.64736$, $\omega1 =$<br>$1.00000$, **$\omega2 = 435.07177$** | **0.03831** | **239***** |
| M1a (neutral) | 30 | −1812.311139 | $p0 = 0.1842$, $p1 = 0.58158$<br>$\omega0 = 0.0000$, $\omega1 = 1.0000$ | | Not allowed |
| M8[a] (beta & ω) | 32 | −1810.938155 | $p0 = 0.91350$, $p = 0.00500$, $q = 1.42513$<br>**$p1 = 0.08650$, $\omega=9.57356$** | 0.2446 | **N/A** |
| M7 (beta) | 30 | −1812.346467 | $p = 0.02986$, $q = 0.01303$ | | Not allowed |

*(Continued)*

TABLE 2 Continued

| Gene/ Model | *nP*^ | *lnL* | Estimates of parameters | LRT *p*-value | Positive sites (amino acid sites of the proteins) |
|---|---|---|---|---|---|
| **MPXVgp191**[$] | | | | | |
| M3 (discrete) | 39 | −1100.910355 | $p0$ = 0.00305, $p1$ = 0.95835, **$p2$ = 0.03860**, ω0 = 0.0000, ω1 = 0.0000, **ω2 = 12.49552** | **0.01762** | **86 ***, 105 ***** |
| M0 (one ratio) | 35 | −1106.892432 | ω0 = 0.37267 | | Not allowed |
| M2a (selection) | 38 | −1100.910163 | $p0$ = 0.96140, $p1$ = 0.0000, **$p2$ = 0.03860**, ω0 = 0.000, ω1 = 1.0000, **ω2 = 12.49516** | **0.02696** | **86***, 105***** |
| M1a (neutral) | 36 | −1104.523584 | $p0$ = 0.76009, $p1$ = 0.23991<br>ω0 = 0.0000, ω1 = 1.0000 | | Not allowed<br>Not allowed |
| M8[a] (beta & ω) | 38 | −1101.255770 | $p0$ = 0.96133, $p$ = 0.00500, $q$ = 2.25829<br>**$p1$ = 0.03867, ω=12.46335** | **0.04273** | **86*, 105*** |
| M7 (beta) | 36 | −1104.408649 | $p$ = 0.00705, $q$ = 0.01079 | | Not allowed |

^p denotes the number of parameters in the ω distribution; lnL denotes the log-likelihood; ω is the ratio of dN/dS, and LRT p-value denotes the value of the chi-square test. Positive selection parameters are shown in bold, where p2 indicates the proportion of positive sites and ω2 indicates average ω of the positive selection sites obtained by the models; positive selection sites were identified using Bayes empirical Bayes (BEB) methods under the M8 model or by Naive Empirical Bayes (NEB) or Empirical Bayes methods under M3 and M2a models.

[&] Although M2a vs. M1a yields an LRT $p < 0.05$, the posterior probabilities for the potential positive selection sites 258, 426, and 637 are all less than 0.90.

[@] The C-terminal of MPXVgp014 varies among different alleles and is shown as "DDDR", "DDDDE", or DDDDDDDDR/D, and so on, resulting in a variation in the number of amino acid residues of the protein (153 to 158) among alleles, and the site nomenclature was based on the 2022 outbreak allele ON645312.

[$] MPXVgp191 has two distinct protein profile groups, one of which encodes an additional six amino acid sequence "EDDEVS" between sites 67 and 68 (e.g., allele MT903348). The position of the proteins' positive sites is shown here as the position in the 2022 outbreak gene alleles.

The posterior probabilities (Pr) ≥ 0.90, (Pr) ≥ 0.95, and Pr ≥ 0.99 are indicated by *, **, and ***, respectively.

Table 4. Interestingly, with the exception of MPXVgp098, almost all of these genes encode proteins that interact with the host (Herbert et al., 2015), serve as host cell selection for infection, host immune response regulators, or participate in virion maturation. The most intriguing finding is that half of these genes (MPXVgp004, MPXVgp010, MPXVgp012, MPXVgp178, and MPXVgp188) encode ankyrins, and show more significant positive selection both at the whole gene and at the codon levels (Figure 3), despite the fact that only 11 ankyrin genes were found in MPXV genome (based on NCBI genome annotation, detailed information of ankyrin genes is shown in Supplementary Table 4). Even more interesting, ankyrins and another virus–host interaction protein, MPXVgp014, a Bcl-2-like protein, have been demonstrated to play roles in determining poxviruses' host range.

## Phylogeny revealing a novel viral clade with positive amino acid substitutions similar to early-emerging isolates

The phylogeny of MPXV genes that underwent codon-level positive selection revealed that the majority of them consisted of three main clades, with those alleles only found in isolates from 2022 being evolutionary distinct from the others (Figures 4A–J). This was very obvious in the genes, including MPXVgp010, MPXVgp044, MPXVgp098, MPXVgp138, and MPXVgp178 (Figures 4B, E–H), and could be categorized as the 2022 outbreak, the West African, and Congo clades, based on the representative isolate information. In general, we could observe a close relationship between the 2022 isolates and the 2019 (MT250197 and MG693724) or 2017 isolates (MG93725 and MG693723) based on these 10 genes (Figure 4). However, some certain unusual situations in the phylogeny of these genes should be highlighted. The MPXVgp012 alleles ON880520 and ON675438, for example, which first appeared in 2022, formed unique clades that differed significantly from the clade formed by 2022 isolates (Figure 4C). A similar result was observed on MPXVgp014, with the 2022 allele OP245336 and the 1965 allele KJ642614 forming a distinct clade (Figure 4D).

The positive amino acid substitution profiles of these 10 genes are shown by mapping the sites to the branches of the evolutionary trees. Diverse positive amino acid substitutions were found in these genes (Figure 4 and Table 4). We did observe unequal amino acid substitution profiles among the alleles that arose in 2022. Amino acid profiles of 258S and 258L could both be observed in the MPXVgp010 genes (Figure 4B), 543A and 543T could be observed in the MPXVgp098 gene (Figure 4F), and 689C, 689H, and 689R could all be observed on the MPXVgp178 of 2022 MPXV isolates (Figure 4H).

We found that most of the amino acid substitutions (12/15, 80%) occurred several decades before the current outbreak (Figures 5A, B). Those alleles with positive amino acid substitutions that could be found in the 2022 outbreak isolates

TABLE 3 Parameter estimates for protein-coding genes of monkeypox virus and positive selection sites detected by Fast Unconstrained Bayesian Approximation methods implemented in the HyPhy package.

| Genes/positive selection sites | α | β | Bayes factor [β > α] | Posterior Pr [β > α] |
|---|---|---|---|---|
| **MPXVgp004** | | | | |
| 4 | 4.292 | 33.92 | 15.721 | 0.930 |
| 239 | 4.574 | 34.006 | 14.538 | 0.925 |
| **MPXVgp010** | | | | |
| 258 | 3.367 | 32.228 | 19.401 | 0.944 |
| 426 | 3.015 | 31.287 | 21.225 | 0.949 |
| 637 | 3.955 | 35.52 | 18.474 | 0.941 |
| **MPXVgp012** | | | | |
| 423 | 6.031 | 36.63 | 11.016 | 0.900 |
| **MPXVgp014#** | | | | |
| 153 | 4.099 | 38.139 | 21.07 | 0.947 |
| **MPXVgp044** | | | | |
| 203 | 3.897 | 41.5 | 27.855 | 0.958 |
| **MPXVgp098** | | | | |
| 3 | 3.063 | 30.57 | 21.573 | 0.943 |
| 543 | 3.052 | 29.582 | 20.548 | 0.941 |
| **MPXVgp138** | | | | |
| 205 | 3.039 | 22.398 | 18.529 | 0.933 |
| **MPXVgp178** | | | | |
| 689 | 3.53 | 36.696 | 25.246 | 0.955 |
| **MPXVgp188** | | | | |
| 239 | 4.579 | 35.345 | 15.533 | 0.929 |
| **MPXVgp191#** | | | | |
| 86 | 2.272 | 29.772 | 27.652 | 0.959 |
| 105 | 3.402 | 31.009 | 18.047 | 0.939 |

α indicates (dS) value and β indicates (dN) value.
*Cutoff value for posterior Pr in FUBAR was set at 0.90 based on the research reported by Ben Murrell et al. [Mol. Biol. Evol. 30(5):1196–1205 doi:10.1093/molbev/mst030].
#Due to some gaps that were generated in the process of coding gene alignment, the nomenclature of these sites is corrected to be consistent with the codeML method.

include A4V and L239W of the MPXVgp004 (1968 and 1979), L258S and E637K in the MPXVgp010 (1965), D153R in the MPXVgp014 (1970), Y203C in the MPXVgp044 (1968), T3A and T543A in the MPXVgp098 (1971 and 1968), and H205R in the MPXVgp138 (1965), which occurred about 40 to 50 years before (Figure 5B). Only three amino acid substitutions arose recently (in 2019 or 2022), with A423D in MPXVgp012 and S105L in MPXVgp191 appearing in isolates from the 2022 outbreaks (Figure 5B), indicating a potential role for further human adaptation of these substitutions for the virus.

## Positive selection mutations have recently caused population growth and are closely related to early virus strains

A mutation at a positive selection site should benefit the individuals who bear the mutation. We postulate that mutations in positive selection genes may aid in the spread of MPXV. Some evidence has been obtained from the MSNs of the 10 gene alleles mentioned above (Figures 6A–J). Most of the genes with positive selection at the codon level (MPXVgp004, 010, 098, 138,178, and

10.3389/fcimb.2022.1083234

TABLE 4 Description of genes under positive selection at the codon level.

| Gene product (based on the annotation of MT903348) | Gene | Gene (VACV nomenclature) | Sequence length (aa) | Substitution profiles (aa) (relative to the 2022 outbreak isolates) | Positive mutation profiles () | Description | Function | Virus–host protein interactions | Ref. |
|---|---|---|---|---|---|---|---|---|---|
| MPXVgp004 | D1L | N/A | 437 | G4V, A4V; L239W | 11G>T, 11C>T; 716T>G | Ankyrin | Host range | Y# | (Herbert et al., 2015) |
| MPXVgp010 | D7L | N/A | 659 | L258S; A426V, T426V; E637K | 773T>C; 1276A>G, 1277C>T; 1909G>A | Ankyrin | Host range | Y | (Spehner et al., 1988; Herbert et al., 2015) |
| MPXVgp012 | D9L | C9L | 630 | A423D | 1268C>A | Ankyrin | Host range | Y | (Herbert et al., 2015) |
| MPXVgp014* | D11L | C6L | 153 to 158 | E153R; D153R | 457G>A, 458A>G; 457G>A, 458A>G, 459T>A | Bcl-2-like protein, IFN-beta inhibitor | Suppression of host immune response, host range | Y | (Gonzalez and Esteban, 2010) |
| MPXVgp044 | C18L | F12L | 635 | Y203C | 608A>G | EEV maturation protein | Virion association, wrapping membrane, interaction with host KLC2 | Y | (Laliberte and Moss, 2010) |
| MPXVgp098 | E1R | D1R | 845 | T3A; T543A | 7A>G; 1627A>G | mRNA capping enzyme subunit | Catalyzes the three steps of mRNA capping and regulates gene transcription | N# | (De la Peña et al., 2007) |
| MPXVgp138 | A28L | A26L | 507 to 515 | H205R | 614A>G | Component of IMV surface tubules | Participate in the binding of MVs to the cell surface | Y | (Moss, 2016) |
| MPXVgp178 | B17R | B20R | 787 to 789 | C689R; H689R | 2065T>C; 2066A>G | Ankyrin | Host range | Y | (Herbert et al., 2015) |
| MPXVgp188 | N4R | N/A | 437 | L239W | 716T>G | Ankyrin | Host range | Y | (Herbert et al., 2015) |
| MPXVgp191$ | J3R | C23L | 246 to 252 | N86T; S105L | 257A>C; 314C>T | Chemokine binding protein | Modulate host immune response | Y | (Townsend et al., 2013; Townsend et al., 2017) |

* The number of MPXVgp014 amino acid residues was not the same (153 to 158) among different alleles, and the length was calculated using the 2022 outbreak allele ON645312.
$ MPXVgp191 has two distinct protein profile groups, one of which encodes an additional six amino acid sequence "EDDEVS" between sites 67 and 68 (e.g., allele MT903348). The position of the proteins' positive sites and the DNA mutation profiles are shown here as the position in the 2022 outbreak gene alleles.
# Y is the abbreviation of "Yes", which means the existence of virus–host protein interactions in these proteins, whereas N indicates the opposite.
N/A indicates not available.

188) displayed an evident star structure for the alleles of 2022 outbreak isolates with alleles MT250197, which originated in 2019, in the center. MPXVgp012 and MPXVgp191 showed a star structure with alleles from the 2022 outbreak isolates in the center (Figures 6C, J). However, only one mutation could be observed among the central alleles (OP295383 of MPXVgp012 and OP451016 of MPXVgp191) and MT250197 in these genes (Figures 6C, J). The putative positive selection mutation was found in the central alleles of these MSNs of the eight genes (except for MPXVgp014 and MPXVgp044), indicating a recent population expansion (Figures 4, 6). Furthermore, a close relationship has been observed in MPXVgp004, MPXVgp014, MPXVgp098, MPXVgp138, and MPXVgp178 between the alleles with positive selection mutations in the 2022 outbreak isolates, and those that arose several decades ago (Figures 6A, D, F, G, H). There is only one linking step between the ancestral alleles of the 2022 outbreak isolates and those with identical positive selection mutations that arose several decades ago (Figures 6A, F–H).

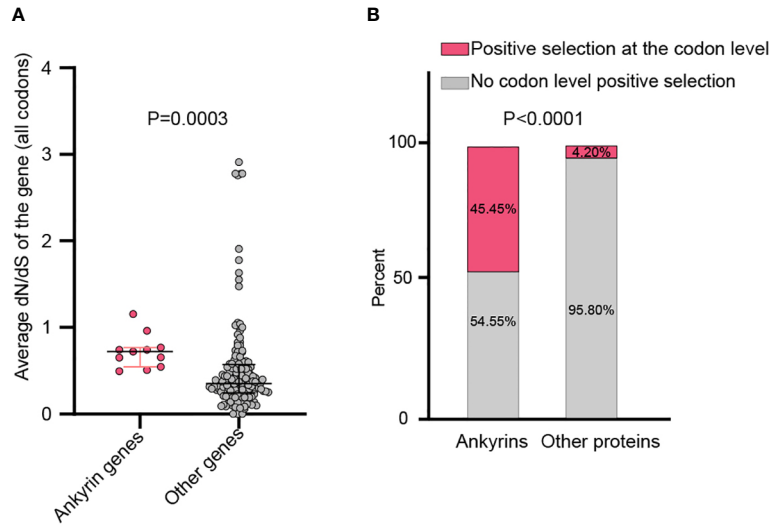


FIGURE 3
Whole gene and the codon-level positive selection on the ankyrin genes of MPXV. **(A)** Average dN/dS between all the 11 MPXV ankyrin genes and other MPXV genes. Data are shown as dots with 25th, 50th, and 75th percentile lines. The Mann–Whitney *U* test was used for statistical analysis. **(B)** Percentage of ankyrin genes, or other types of genes under positive selection. Chi-square test was used for statistical analysis.

## Positive selection mutations may affect protein functions in a variety of ways

The three-dimensional structure of those positive selection genes was modeled using the Phyre server and is shown in Figures 7A–H, for predicting the effects of amino acid substitutions on protein function. Positive amino acid substitutions in the ankyrin proteins MPXVgp004, MPXVgp012, and MPXVgp188 all occur in the α-helix of the ankyrin repeat, indicating that amino acid substitution in positive selection sites can significantly change the α-helical propensities (Figures 7A, C, G). The amino acid residue alanine at site 4 of the MPXVgp004 is often substituted by glycine, while the residue leucine at site 239 is often substituted by tryptophan (Figures 4A, 7A). All these substitutions may significantly influence the stability of the helical structure of the ankyrin repeat. Similar results could often be observed in the substitution of aspartic acid for alanine at site 423 of MPXVgp012 and the substitution of tryptophan for leucine at site 239 of MPXVgp188 (Figures 7C, G). In contrast, we did not find those positive amino acid substitutions in the α-helix structures of MPXVgp010 and MPXVgp178 (Figures 7B, F). For MPXVgp010, the Phyre server only yields a part of a three-dimensional structure, where only site 258 could be mapped and located on the loop or linker between the ankyrin repeat (Figure 7B). Therefore, we employed SMART (Simple Modular Architecture Research Tool), a web resource (https://smart.embl.de) for the identification and annotation of protein domains and the analysis of protein domain architectures to characterize the positive sites of MPXVgp010 for further study. All three positive sites were not found in the ankyrin repeat but could be located on the loop or linker between the ankyrin repeat (sites 258 and 426). It should be noted that site 637 was included in the PRANC (Pox proteins Repeats of Ankyrin-C terminal) domain (Figure 7B), which appears to be related to the F-box domain and may play roles in modulating diverse cellular responses to viral infection by ubiquitin-mediated degradation. Although the MPXVgp178 positive selection site was not discovered in the α-helix of ankyrin repeat, the cystine-to-arginine substitution may significantly increase the stability of α-helix partly formed by 612D and 644E, as additional hydrogen bond (H-bond) could be formed by the C689R substitution (Figure 7F).

For those non-ankyrin proteins, including MPXVgp014 and MPXVgp044, positive selection sites could not be mapped to the three-dimensional structure, as the Phyre server only yielded a partial structure not containing those sites (e.g., residues 2 to 141 for MPXVgp014 and 223 to 601 for MPXVgp044). Based on the simple principle that if the amino acid substitutions significantly changed the properties of these residues (e.g., acidic amino acid changed to a basic amino acid) (Figure 4D), it could influence the distant residue from the changed position (Montalibet et al., 2006), causing the functional change in the three-dimensional structure. This could explain the functional change of D153R and E153R in the MPXVgp014 (Figure 4D). However, the role of the Y203C substitution in MPXVgp044 deserves further research.

We could only obtain part of the three-dimensional structure from the Phyre, which lacks residues 535 to 548 of MPXVgp098 where site 543 is included. One H-bond was

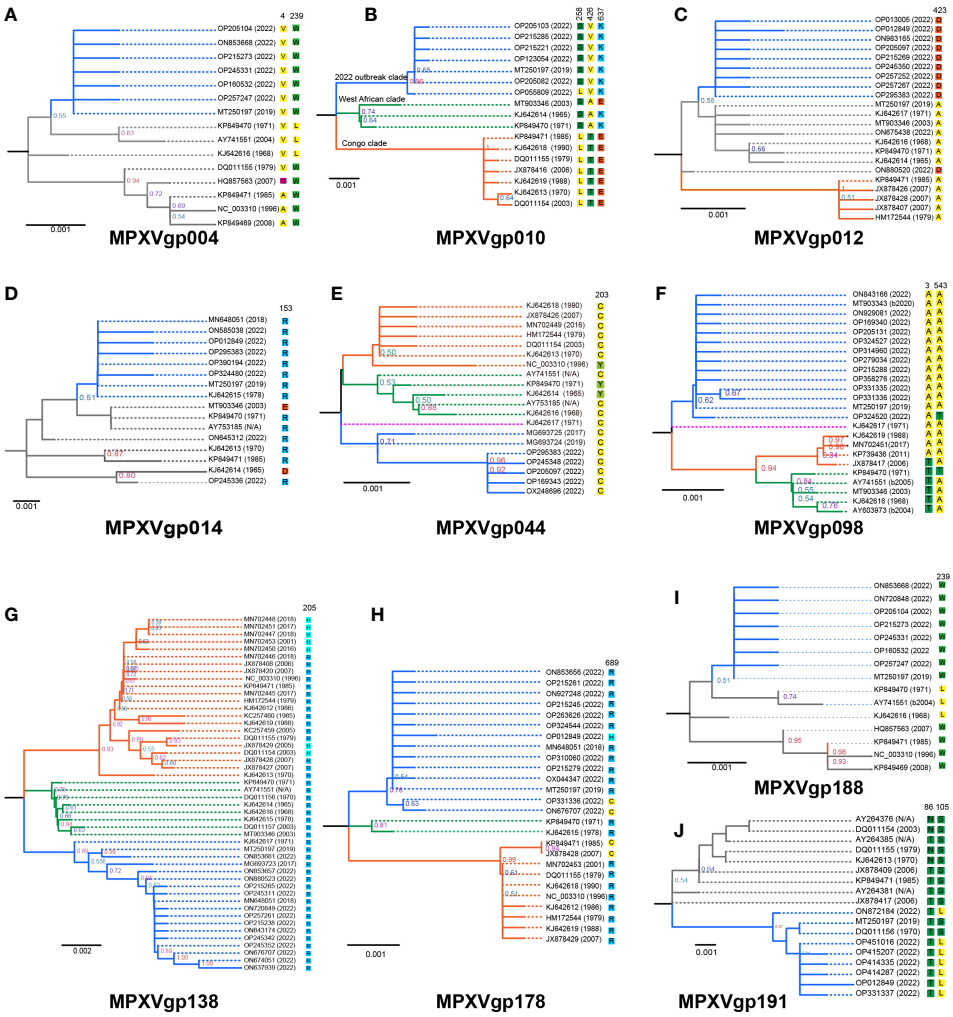


FIGURE 4
Phylogeny of MPXV genes evolved under positive selection at the codon level and amino acids at the positive selection sites in the 10 MPXV genes. Those genes that were identified as positively selected genes at the codon level include **(A)** MPXVgp004, **(B)** MPXVgp010, **(C)** MPXVgp012, **(D)** MPXVgp014, **(E)** MPXVgp044, **(F)** MPXVgp098, **(G)** MPXVgp138, **(H)** MPXVgp178, **(I)**. MPXVgp188, and **(J)** MPXVgp191. Interior branch numbers represent bootstrap values and are indicated when the values are greater than 0.5. Allele names were marked as their representative isolate names, shown as GenBank accession numbers, and the earliest possible year for the allele to arise is shown. Each allele's amino acid substitution in positive selection sites is depicted. The isolate ON880520 MPXVgp012 gene has seven nucleotides (overlap amino acid residues 835 to 839) that were not obtained by sequencing and are shown as "N", and these nucleotides were filled with the most identity nucleotides. Each allele of the 10 genes was re-BLASTed to determine the first time they appeared in an isolate based on the annotation. Branches of the same color are clustered into a clade. The blue clade represents the "2022 outbreak clade", while the orange and blue represent the "Congo and West African clades" as shown on MPXVgp010 as an example. The purple clade represents a singleton of alleles that make up an additional clade. The gray branches indicate that the branch topology did not allow them to be precisely classified into the three clades mentioned above. N/A denotes that the year the representative isolate arose could not be determined. Based on the analysis of the isolates harboring these alleles, B2020, B2004, and B2005 indicate that these alleles arose no earlier than 2020, 2004, or 2005.

reduced after T3A substitution (Figure 7D). H205R could be found as a substitution in the positive selection site of MPXVgp138. The sites were discovered to be in an α-helix (Figure 7E). The stability of the α-helix containing the substitution may be influenced by H205R, as conformational changes in the α-helix may be caused by the substitution due to arginine's longer side chain. Unlike the other nine proteins, the mapping of the MPXVgp191 positive selection sites (sites 86 and 205) revealed that they were all located in the β-sheet (Figure 7H).

## Discussion

Consisting of ~197 kb with ~190 non-overlapping coding genes, the double-stranded DNA genome of MPXV is

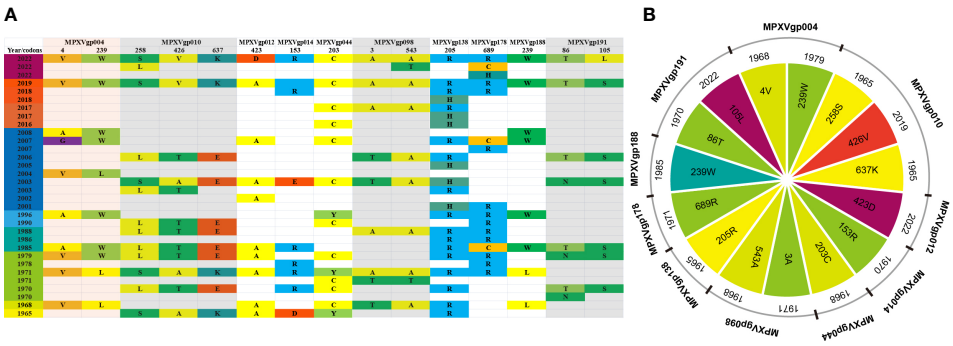


FIGURE 5
Amino acid substitution profiles of the 10 positive selection genes in a time scale. **(A)** The amino acid sequences at the 10 MPXV genes' positive selection sites evolved over time. The genes that encode proteins with more than one positive selection sites are highlight with a light color. **(B)** The earliest year when the positive amino acid substitutions arose.

responsible for various biological characteristics of the virus and plays a key role in viral host preference/range, host immunomodulation, and viral pathogenicity (Luna et al., 2022). Detailed characterization of the evolution patterns of these coding genes may aid in a thorough understanding of the mechanism underlying the recent emergence of MPXV cases in several regions and outbreaks in multiple countries (Chakraborty et al., 2022). The unusually enhanced human-to-human transmission of hMPX among patients has been discovered in the 2022 outbreak, indicating that the 2022 outbreak isolates have better host adaptation to humans, but how this happens is still unknown. We discovered that MPXV was not a virus whose evolution was frequently driven by intra-species homologous recombination, despite the fact that some poxviruses have high rates of recombination, resulting in the recurrent emergence of tandem gene duplications (Esposito et al., 2006; Sasani et al., 2018), and even this evolution mechanism was proposed in MPXV (Gigante et al., 2022). Only two genes (MPXVgp090 and MPXV182), which functioned as RNA polymerase subunit and surface glycoprotein, were found to have recombinants supported by both the RDP and splits tree.

The ratio of substitution rates, denoted dN/dS, is still a reliable measure to detect proteins undergoing adaptation, which is used to infer the direction and magnitude of natural selection acting on protein-coding genes (Kryazhimskiy and Plotkin, 2008). More than 60% of the MPXV coding genes have an overall average dN/dS < 0.5, indicating that purifying selection is the primary evolutionary direction for MPXV. Eleven of these genes, in particular, were found to have an extremely negative selection (very low dN/dS), implying improved functional stability (Escorcia-Rodríguez et al., 2022), which could also be deduced from the functional annotation of these genes, including DNA- or telomere-binding proteins, virion core proteins, transcription factors, and enzymes (Supplementary Table 2). In contrast to these genes favored by extremely purifying selection at the whole gene level, nine MPXV genes, some of which participate in virus–host interaction, including the kelch-like protein (MPXVgp015) (Kochneva et al., 2005), the IL-1 receptor antagonist (MPXVgp016) (Weaver and Isaacs, 2008), the alpha amanatin target protein (MPXVgp024) (Gonzalez and Esteban, 2010), and the caspase-9 (apoptosis) inhibitor (MPXVgp033) (Suraweera et al., 2020), have significantly high dN/dS (>1.5), implying whole gene level positive selection operating these genes or the relaxation of negative selection (Lin et al., 2019). The dN/dS ratio in an amino acid-coding sequence alignment has been extensively used to identify individual codons/sites evolving under positive selection, which could uncover whose signal was "masked" by averaging across the whole sequence, reveal, and speculate functional changes in these sites (Bloom, 2017; Zhan and Zhu, 2018; Zhan et al., 2020). This is particularly important for studying pathogens' adaptive evolutionary and biological mechanisms for defending against unfavorable environments or promoting host adaptation, transmission, and pathogenicity (Bloom, 2017; Zhan and Zhu, 2018; Velazquez-Salinas et al., 2020; Zhan et al., 2020; Emam et al., 2021). In the present study, 10 MPXV genes were identified as positive selection genes at the codon level and the positive amino acid substitution profiles were uncovered. Most of these genes are involved in virus–host interaction, and some, such as those ankyrin genes (MPXVgp004, MPXVgp010, MPXVgp012, MPXVgp178, and MPXVgp188) and Bcl-2-like genes (MPXVgp014) are likely involved in host range determination (Spehner et al., 1988; Perkus et al., 1990; Opgenorth et al., 1992; Haller et al., 2014). A subsequent study revealed that positive

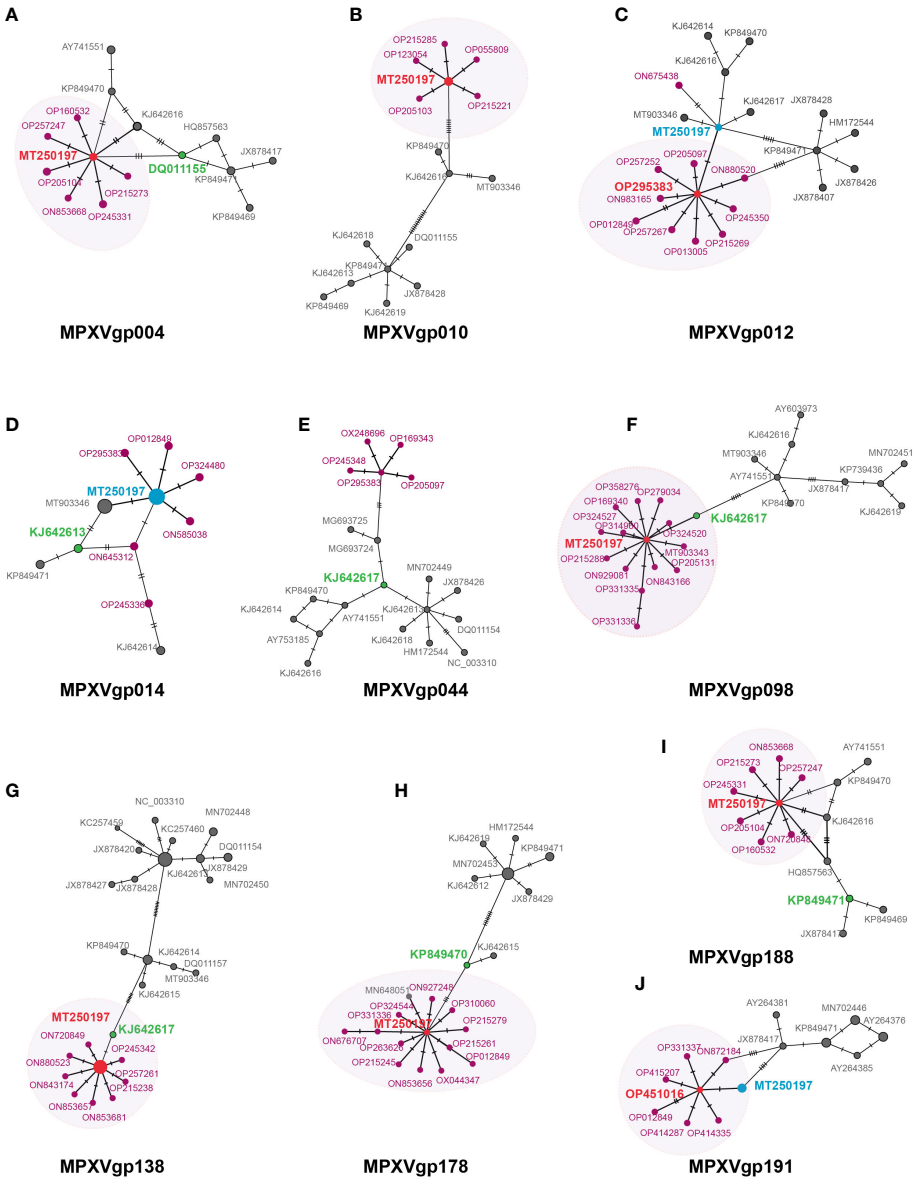


FIGURE 6
Minimum spanning network of MPXV genes under positive selection at the codon level. Those genes that were identified as positively selected genes including **(A)** MPXVgp004, **(B)** MPXVgp010, **(C)** MPXVgp012, **(D)** MPXVgp014, **(E)** MPXVgp044, **(F)** MPXVgp098, **(G)** MPXVgp138, **(H)** MPXVgp178, **(I)**. MPXVgp188, and **(J)** MPXVgp191 are shown. Haplotype (allele) diversity was obtained from about 1,500 MPXV isolates worldwide. Each oblique line linking between alleles (allele name is shown as its representative isolate NCBI accession numbers) represents one mutational difference. The ancestral allele, or root of the network, is labeled with red, and the represented allele name is marked red. The dark red nodes indicate alleles that arose in the 2022 outbreak isolates. The oval box with a light shadow shows the star structure of the MSNs. For MPXVgp014, MPXVgp138, MPXVgp178, and MPXVgp191, some alleles were identified as the same by popART, which removes the gaps of the alignment, and these alleles show as larger circles. For those alleles, MT250197 is not shown as an obvious center of the star structure and is marked light blue. Those alleles that have positive selection mutations that first arose about 40 to 50 years ago are marked green.

selection was more likely to occur in those host range genes (Figure 3), indicating that positive selection in these genes at the given codons may be beneficial to human-to-human transmission in the current outbreak. Aside from genes that determined host ranges, MPXVgp044, which interacts with the host kinesin light chain, wraps the host cell membrane and plays roles in the extracellular enveloped virus (EEV) maturation (Laliberte and Moss, 2010; Carpentier et al., 2015), and MPXVgp138, which is a component of IMV surface tubules, participates in the binding of MVs to the cell surface (Moss, 2016); enzyme MPXVgp098 and one of the host immune modulators, MPXVgp191, were also found to be subjected to

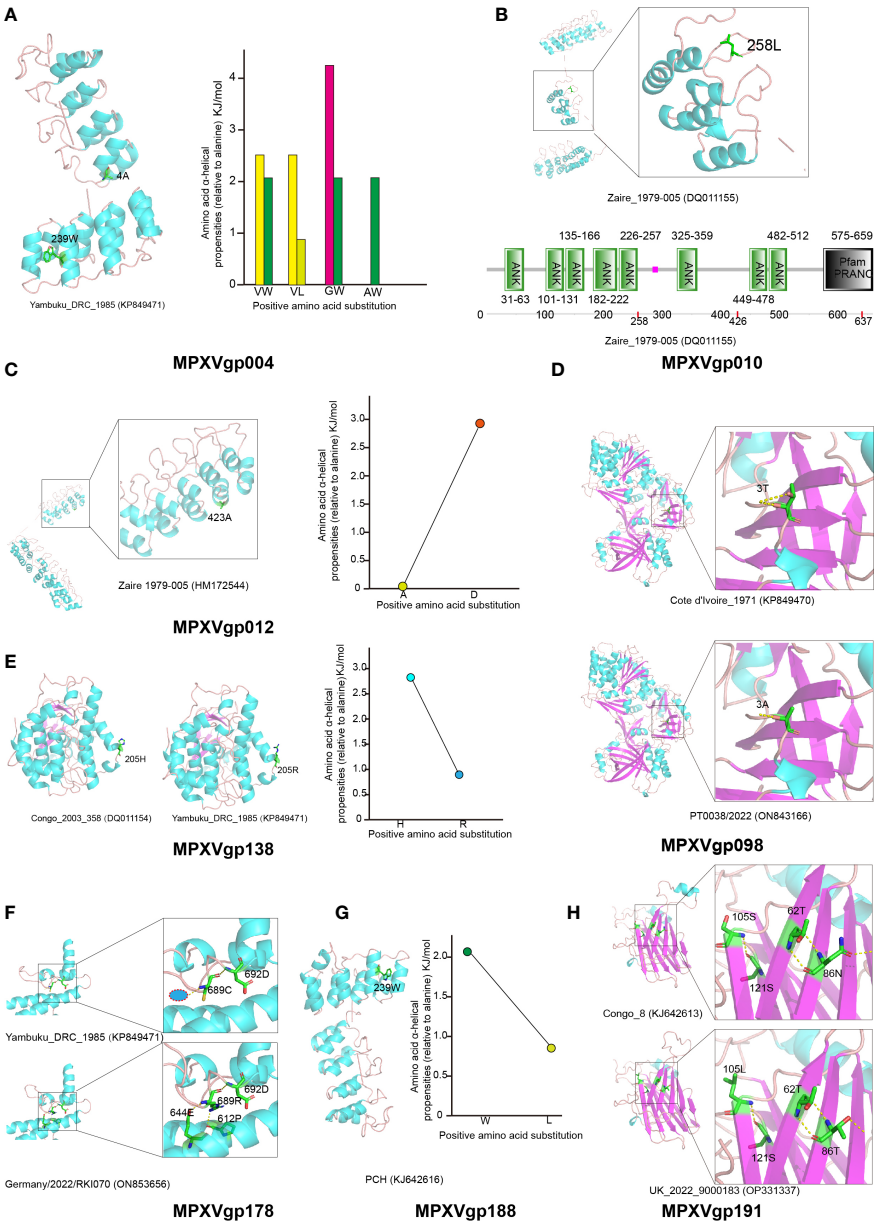


FIGURE 7
Structure of MPXV proteins that are under positive selection and potential influence of amino acid substitutions in positive selection sites. Secondary structure elements of proteins encoded by positively selected genes are colored in cyan (helix), purple (sheet), and orange (loop), respectively. The amino acid residues that undergo positive selection are shown as sticks. **(A)** Left: The protein structure of MPXVgp004 is shown using strain Yambuku_DRC_1985 as a reference. Right: Amino acid α-helical propensities among different substitution profiles are shown. **(B)** Up: The protein structure of MPXVgp010 is shown using strain Zaire_1979-005 as a reference. Down: Annotation of protein domains and the domain architectures of MPXVgp010. Predictive domains and amino acid residue ranges of each domain are shown. The red dots indicate the location of positive selection sites. **(C)** Left: The protein structure of MPXVgp012 (part) is shown with strain Zaire 1979-005 as a reference. Right: Amino acid α-helical propensities of substitution A423D. **(D)** Up: The protein structure of MPXVgp098 is shown with strain Cote d'Ivoire_1971 as a reference and amino acid residue 3T is shown as a stick. Down: The protein structure of MPXVgp098 is shown using strain PT0033/2022 as a reference and amino acid residue 3A is shown as a stick. Yellow dotted lines indicate H-bonds. The T3A substitution leads to a decrease of H-bond, which may interact with the small subunit of mRNA capping enzyme. **(E)** Left: Protein structures of MPXVgp138 with different amino acid residues at the positive selection sites. Right: Amino acid α-helical propensities among different substitution profiles are shown. **(F)** Comparing MPXVgp178 protein structures with different amino acid residues (689C and 689R) at positive selection sites. The Phyre server could not model MPXVgp178 allele with 689H; thus, it was not shown. Amino acid residues of those that interact with residue 689 are shown as sticks. H-bonds are shown as yellow dotted lines. A red circle wrapped in blue indicates a putative water molecule. **(G)** Left: The protein structure of MPXVgp188 is shown using strain PCH as a reference and amino acid residue 239W is shown as a stick. Right: Amino acid α-helical propensities of substitution (W239L) are shown. **(H)** Comparing MPXVgp191 protein structures with different amino acid residues (86N, 105S and 86T, 105L) at positive selection sites. Amino acid residues of those that interact with residues 86 and 105 are shown as sticks. H-bonds are shown as yellow dotted lines.

positive selection, indicating that substitutions in these genes and the subsequent functional changes may also contribute to the current outbreak (De la Peña et al., 2007).

In terms of genome architecture, MPXV has historically been classified into two major clades (West African and Central African, also known as Congo clades) with mortality rates of <1% and about 10%, respectively (Ladnyj et al., 1972; Simpson et al., 2020). To date, five major hMPX outbreaks have been documented, occurring in 1970, 1996, 2003, 2018, and 2022, respectively (Luna et al., 2022). We discovered that alleles of half of the positively selected genes were clustered into three main clades based on the phylogenetic analysis. Each clade correlates with the different epidemiological hMPX outbreaks, lending support to a new proposal for MPXV classification that divides isolates into three clades (Luna et al., 2022). Despite this, the phylogenetic incongruence among some of these genes suggested possible genetic drift, gene duplication, horizontal gene transfer, or lineage sorting in the evolutionary history of the MPXV population (Jeffroy et al., 2006; Som, 2015). The alleles from the most recent 2022 outbreak isolates were mostly clustered with those originating from the 2018–2019 Nigeria outbreaks, indicating MPXV's continuous evolution and divergence. However, due to the lower frequency of human-to-human transmission, the majority of the positive selection force is relatively weak, which is consistent with monkeypox as a zoonotic disease with limited human-to-human transmission (Parker et al., 2007). We also discovered that the 2022 isolates still possess alleles that lack putative positive amino acid substitutions in some genes. Site 689 in MPXVgp178 of OP331336 and ON676707, and site 258 in MPXVgp010 of OP0558509, for example, remain unmutated, indicating that a set of MPXV isolates is responsible for the 2022 outbreak, as well as the ongoing selection pressure operating these genes. This emphasizes the significance of tracking the mutation profiles of the MPXV isolates in the current outbreak, which was being done by other researchers (Benvenuto et al., 2022; Gigante et al., 2022; Isidro et al., 2022; Kannan et al., 2022; Wang et al., 2022). While the MSNs of the 10 genes indicate a close relationship between the 2022 outbreak isolates and the 2019 isolate (e.g., MT250197), the starburst pattern with one allele (2019 isolate MT250197 or 2022 isolates OP295383 and OP451016) in the center and many other alleles surrounding the central allele suggests a signature of rapid population expansion and the possibility of the initial effect (Bubac and Spellman, 2016). The discovery of only one step link between the alleles of 2022 isolates and those with positive selection mutations that arose several decades ago further suggests that the more adaptive and circulated alleles are more prevalent in the natural reservoir.

Many amino acid substitutions/mutations occurred among alleles of the 10 genes under codon-level positive selection. Amino acid substitution, for example, occurred in 10 of 437 sites of MPXVgp004, which was five times the number of positive selection sites. Mutations in the amino acid sequence allow proteins to acquire new functions. However, only those mutations at the positive selection sites may significantly improve the survival of or be beneficial to microorganisms with mutated alleles, and thus be fixed. In this study, 15 sites in MPXV's 10 proteins were subjected to strong positive selection (Table 4 and Figures 5A, B). Some of the three-dimensional structures of those positive selection genes with the positive sites could not be modeled using the Phyre server, because the characterization of poxviral proteins is unfortunately scarce. Most of the positive selection sites are located in the α-helix, which comprises the convex surface "back" of ankyrin proteins' stacked repeats (sites 4 and 239 for MPXVgp010 and site 239 for MPXVgp188), indicating that the mutation may influence host-range selection (Li et al., 2010). We also found a significant change of amino acid α-helical propensities by the amino acid substitution in the positive selection sites of some genes (MPXVgp010, MPXVgp012, and MPXVgp188), as well as a more H-bond in the MPXVgp178 after a C689R substitution, indicating a potential change in the stability of the ankyrin repeat α-helix in these genes after substitutions (Kumar et al., 2000). Unlike ankyrin proteins, the amino acid substitutions in MPXVgp098 and MPXVgp191 were found in the loop or β-sheet. MPXVgp191 is a chemokine binding protein that moderates host immune response; lacking this gene in the poxviruses may increase disease severity (Townsend et al., 2013; Townsend et al., 2017; Lum et al., 2022), so we supposed that the amino acid substitutions on the two positive selection sites (N86T and S105L) may influence MPXV virulence. As a result, S105L became one of the mutations that drew the attention of the UK Health Security Agency (2022). The MPXVgp098 gene encodes an mRNA capping enzyme subunit that is important in catalyzing the three steps of mRNA capping and regulating virus gene transcription (De la Peña et al., 2007). It is believed that it had no interactions with host proteins (De la Peña et al., 2007). The T543A mutation, according to Benvenuto et al., may stabilize the mRNA capping protein MPXVgp098 (Benvenuto et al., 2022). The T3A mutation, on the other hand, may impair the stabilization of the N-terminal extension to the catalytic site and the precise positioning of the methyl-donor in MPXVgp098 (De la Peña et al., 2007). Because RNA triphosphatase and guanylyl-transferase activities of MPXVgp098 might be influenced by a decrease of one H-bond when T3A substitution occurs, we supposed that the positive amino acid substitution may have an effect on transcription initiation in the host cells (Grimm et al., 2021). However, further biological experiments are required to validate our hypothesis.

## Conclusions

Incorporating intensive molecular evolution research, we discovered that intra-species homologous recombination occurred rarely among the MPXV genes, whereas 10 genes that were mostly associated with virus–host interaction, particularly those that determine the virus's host range, the ankyrin genes, and a Bcl-2-like gene, were found to have codon-level positive selection, which may aid the virus's

adaptation to humans in the context of the 2022 outbreak. Positive selection amino acid substitution profiles of the 10 genes were uncovered and mapped to these genes' phylogenetic trees. Interestingly, the majority of the positive mutations (12 of 15, 80%) were discovered in the virus isolates from several decades ago, but spread across almost all alleles of the 2022 outbreak isolates. The 2022 outbreak alleles are closely related to those that emerged several decades ago and have since experienced population expansion, implying that these genes have been continuously adapted and that a reservoir of the more adaptation alleles has circulated in its natural reservoir (e.g., some susceptible animals). The three or, more precisely, the two never-before-seen positive mutations (A423D in MPXVgp012 and S105L in MPXVgp191) may play a special role for MPXV, allowing it to spread more easily among people. hMPX has already accelerated human-to-human transmission. Monitoring the gene mutational landscape, particularly identifying positive selection mutations after the virus's widespread transmission among humans, is crucial for predicting changes in virulence and transmissibility. Our research on the molecular evolution of MPXV at a single-gene level will fill some knowledge gaps about the virus and provide clues for the unusual emergence of the current outbreak.

## Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/Supplementary Material.

## Author contributions

X-YZ and YH conceived and designed the study. X-YZ and GZ analyzed the data. YH verified the data. X-YZ made the data interpretation. X-YZ wrote the manuscript. X-YZ revised the manuscript. All authors contributed to the article and approved the submitted version.

## Funding

This research was supported by the National Natural Science Foundation of China (grant number 31870001) and Shenzhen Science and Technology Innovation Commission Fund (Project Nos. JCYJ20210324122802006 and JCYJ20220530144811025) to X-YZ.

## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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## Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcimb.2022.1083234/full#supplementary-material

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frontiers | Frontiers in Cellular and Infection Microbiology

TYPE Review
PUBLISHED 06 February 2023
DOI 10.3389/fcimb.2022.1088471



OPEN ACCESS

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SPECIALTY SECTION
This article was submitted to Virus and Host, a section of the journal Frontiers in Cellular and Infection Microbiology

RECEIVED 03 November 2022
ACCEPTED 30 December 2022
PUBLISHED 06 February 2023

CITATION
Khattak S, Rauf MA, Ali Y, Yousaf MT, Liu Z, Wu D-D and Ji X-Y (2023) The monkeypox diagnosis, treatments and prevention: A review.
*Front. Cell. Infect. Microbiol.* 12:1088471.
doi: 10.3389/fcimb.2022.1088471



# The monkeypox diagnosis, treatments and prevention: A review

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The world is currently dealing with a second viral outbreak, monkeypox, which has the potential to become an epidemic after the COVID-19 pandemic. People who reside in or close to forest might be exposed indirectly or at a low level, resulting in subclinical disease. However, the disease has lately emerged in shipped African wild mice in the United States. Smallpox can cause similar signs and symptoms to monkeypox, such as malaise, fever, flu-like signs, headache, distinctive rash, and back pain. Because Smallpox has been eliminated, similar symptoms in a monkeypox endemic zone should be treated cautiously. Monkeypox is transmitted to humans primarily via interaction with diseased animals. Infection through inoculation via interaction with skin or scratches and mucosal lesions on the animals is conceivable significantly once the skin barrier is disrupted by scratches, bites, or other disturbances or trauma. Even though it is clinically unclear from other pox-like infections, laboratory diagnosis is essential. There is no approved treatment for human monkeypox virus infection, however, smallpox vaccination can defend counter to the disease. Human sensitivity to monkeypox virus infection has grown after mass vaccination was discontinued in the 1980s. Infection may be prevented by reducing interaction with sick patients or animals and reducing respiratory exposure among people who are infected.

KEYWORDS

**monkeypox (MPX), epidemiology, diagnosis, treatment, public health concerns**

**Abbreviations:** Monkeypox virus, MPXV; Monkeypox, MPX; Vaccinia immune globulin, VIG; World Health Organization's, WHO; United Kingdom, UK; Inverted terminal repeats, ITRs; Open reading frames, ORF; Internal mature virus, IVM; Extracellular-enveloped virus, EEV; Cell-associated virions, CEVs; Inner encapsulated virus, IEV; Trans-Golgi network, TGN; Dense particles, DPs; Sexually transmitted infection, STI; Men who have sex with men, MSM; Democratic Republic of the Congo, DRC; Nigeria Center for Disease Control, NCDC; Center for Disease Control, CDC; Cowpox virus, CPXV; Orthopoxvirus, OPVs; Vaccinia Virus, VACV; A-type inclusion body protein, ATIP; A-type inclusion, ATIs; Enzyme-linked immunoassay, ELISA; Polymerase Chain reaction, PCR; restriction-length fragment polymorphism, RFLP; extracellular-envelope protein gene, B6R; next-generation sequencing, NGS; Human immunodeficiency virus, HIV.

## 1 Introduction

The monkeypox virus (MPXV) causes monkeypox (MPX), an uncommon viral illness. MPXV is an Orthopoxvirus genus Poxviridae family member. Vaccinia, variola, and cowpox viruses are other members of this genus that may infect people (CDC, 2021a). There are signs similar to those of smallpox patients, but they are less severe. MPX is an infectious disease transmitted from animals to humans. Since the eradication of smallpox in 1980, MPX has become one of the most prevalent orthopoxviruses, especially in tropical forests. MPX is widespread throughout Central and West Africa (WHO, 2019). Fever, swelling lymph nodes, muscle aches, and fatigue are common symptoms. A rash with blisters and crusts then follows it. Although they typically occur one to two weeks after infection, MPX symptoms can appear up to 21 days after someone is exposed (Fenner et al., 1988a; Khodakevich et al., 1988; Di Giulio and Eckburg, 2004). There is an intense deep-seated, vesicular or pustular rash with a central spread 1-4 days after the prodrome. The scratches are highly defined and commonly umbilicate or become adherent, eventually resulting in scabs, and the rash can spread. Several current cases started with vaginal and perianal lesions but no particular temperature or other prodromal indications.

As a result, instances may be misdiagnosed as more frequent conditions like varicella-zoster or sexually transmitted. However, MPX could have a larger prevalence of sensitive people (McCollum and Damon, 2014; Durski et al., 2018; Petersen et al., 2019a). MPXV spreads through bushmeat, animal bites or scrapes, contaminated products, body fluids, or personal interaction with a septic individual. Numerous rodents in Africa have been infected by the virus (WHO, 2021). MPX disease may be diagnosed by extracting MPXV DNA from a patient's blood sample and growing it in a viral culture (Moore and Zahra, 2021). The illness resembles chickenpox in appearance (McCollum and Damon, 2014). Vaccination against smallpox defends against infection (Yinka-Ogunleye et al., 2018). Currently, there is no established, safe therapy for MPXV illness. Antiviral drugs, vaccinia immune globulin (VIG), and smallpox vaccination can or have been utilized in the United States of America (USA) to control MPX infections. Cidofovir and brincidofovirin's effectiveness in treating MPX is unknown. Studies in animals and *in vitro* have shown that both are effective against poxviruses (CDC, 2021b).

First human cases were documented in 1970 in the Democratic Republic of the Congo (DRC) (Bunge et al., 2022). In the West and Central Africa region, MPX still occurs infrequently despite the World Health Organization's (WHO's) 1980 declaration that Smallpox had been eradicated (WHO, 2016). A pet store that imported mice from Ghana in 2003 caused an epidemic in the United States (CDC, 2018; Yinka-Ogunleye et al., 2018). From October 2017 to February 2018, the recent MPX pandemic in Nigeria was catastrophic to the healthcare system (Fowotade et al., 2018). In the current MPX incident, a UK resident who arrived in Nigeria on April 20, 2022, visited Lagos and Delta State before departing Lagos on May 3, 2022, and landing in the UK on May 4, 2022, was involved (Muanya and Onyedika-Ugoeze, 2022). Several experts, including specialists, were unable to identify what caused the epidemic early on. In September 2017, the following MPX occurrence in Nigeria, the country regularly reported isolated viral cases from across all states, as per Nigeria Center for Disease Control (NCDC) (2022). 22 states reported 558 cases and eight deaths between September 2017 and April 30, 2022.There are 46 suspects, and fifteen confirmed cases from seven states had been registered with no recorded death (Nigeria Centre for Disease Control (NCDC), 2022). For its virulence, which is just next to the Variola virus, the source of Smallpox, with a 10% rate of death, MPXV is a potential biological warfare weapon (McCollum and Damon, 2014) like SARS-CoV-2 (Khattak et al., 2021a; Khattak et al., 2021b; Ullah et al., 2021; Ahmad et al., 2022; Khattak et al., 2022a).

Consequently, clinicians and the general population must be informed about its diagnosis, management, and control. This review will examine the present state of knowledge on human MPX, focusing on epidemiology features, diagnosis, prevention, clinical aspects, and therapy. Furthermore, the increasing number of MPX cases in non-endemic countries has made it a public health threat for other countries like Pakistan (Khattak et al., 2022b). The situation requires disease surveillance at a country level, and timely detection and notification of suspected cases are essential for effective preventive measures.

## 2 MPXV structure, genome and morphology

The morphology of MPXV shows that virions are ovoid or brick-shaped particles encased by geometrically corrugated lipoprotein outer membrane, sharing the same physical traits as other orthopoxviruses. The size estimates for MPXV have been confirmed to be 200 to 250 nm (Cho and Wenner, 1973). The outer membrane protects the membrane-bound and tightly packed core, double-stranded DNA genome, transcription factors, and enzymes. The core is characterized as a biconcave owing to an electron microscopy obsession artifact and has an adjacent body on either side (Jahrling et al., 2007; Odom et al., 2009).

The genomes of PVXM are composed of 197 kb of linear double-stranded DNA (Kugelman et al., 2014) that is intrinsically joined across both ends *via* the inverted terminal repeats (ITRs), consisting of tandem repeats hairpin loop as well as several open reading frames (ORF) and palindromic hairpins. Even though MPXV is a DNA virus, it spends its entire life cycle in the cytoplasm of infected cells. The MPXV genome encodes all proteins essential for viral DNA replication, transcription, virion assembly, and egress. Housekeeping function genes are preserved across Orthopoxvirus (OPVs) and found in the genome's central area. In contrast, virus-host interaction genes are less conserved and located in the termini area (Cho and Wenner, 1973; Esposito and Knight, 1985; Takemura, 2001; Jahrling et al., 2007; Boyle and Traktman, 2009; Remichkova, 2010). Intracellular mature virus (IVM) and extracellular-enveloped virus (EEV) are the two kinds of infective virions formed by Vaccinia Virus (VACV) (and most likely MPXV). IVM is released when cells are lysed, but EEV is produced when cells come into contact with actin tails, leading to the virus's quick long-distance spread within the host body. Whereas the traits listed above are unique to VACV, they are most likely shared through all OPVs (Remichkova, 2010). Cell-associated virions (CEVs) are produced when an inner encapsulated virus (IEV) travels to the edge of the cell and fuses with the plasma membrane,

remaining connected to the cell's surface. Cell-to-cell communication is essentially the responsibility of CEVs. IEV is produced when IMV is surrounded by a double membrane formed by an initial endosomal component (Jahrling et al., 2007) or the trans-Golgi network (TGN) (Schmelz et al., 1994). Aside from IEV exocytosis, another route for EEV production is IMV budding across the plasma membrane (Meiser et al., 2003). Virion morphogenesis in the prototype VACV may be erroneous, leading in non-infectious dense particles (DPs) (Meiser et al., 2003; Okeke et al., 2006), However, no reports of this for MPXV have yet been made. Additionally, contrast certain CPXV strains where IMVs are covered by A-type inclusions (ATI) (Shida et al., 1977; Okeke et al., 2006). MPXV does not generate ATIs or sequester IMVs within A-type inclusion (ATIs) due to the A-type inclusion body protein (ATIP) gene truncation (Figure 1) (Howard et al., 2010).

# 3 Epidemiology of MPX disease

## 3.1 Outbreak in Africa

Since humans were first infected with the virus through direct contact with diseased animals thousands of years ago, MPX has likely been present in Sub-Saharan Africa for thousands of years (Weinstein et al., 2005). There is currently no information about MPXV's reservoir. Though, there is evidence that monkeys, like humans, are accidental hosts. The reservoir will likely be one or more mice or monkeys found in central Africa's forest (Khodakevich et al., 1988). MPX was not known as a unique infection till 1970, when the eradication of Smallpox in Zaire (DRC) showed the persistence of a smallpox-like disease in rural regions. In the worldwide purging campaign, mass immunization in Central Africa appears to have resulted in a temporary decrease in the prevalence of human MPX. However, the disease has returned due to a lack of immunization in subsequent generations and a growing reliance on hunting animals in conflict-torn regions (Weinstein et al., 2005). A total of 47 human cases of MPX have been reported in Sub-Saharan Africa from 1970 to 1979, with 38 of these occurring in the DRC and the remaining taking place in Cameroon, the Central African Republic, Gabon, Cote d'Ivoire, Liberia, Nigeria and Sierra Leone (Breman et al., 1980; WHO, 1980). The DRC cases all occurred near tropical rainforests and appeared connected to animal interaction. Seven of the 47 diseases documented were lethal. The secondary spread was shown to be the leading prospective source of contagion in four cases, with the second occurrence proportions of 7.5% between nearby family members living in the same house and 3.3% between all exposed interactions. Many cases have been recorded in the DRC since 1980 (Weinstein et al., 2005).

From 1981 to 1986, the WHO conducted a comprehensive investigation in the DRC to determine whether MPX may arise from central Africa and fill the void left by Smallpox. From 1970 to 1986, 338 of the 404 recorded African instances occurred (Jezek and Fenner, 1988). In 245 of the 338 cases, an animal source of infection was suggested, and secondary spread from a human source was proposed in the remaining 93 cases. Most victims were children, with an average age of 4.4 years. These rises in the secondary transfer rate (3 times the 9% rate for patients in the 1970s) and demography were assumed to suggest a decline in immunity once vaccination was discontinued. Only four generations of human-to-human spread were seen in the largest recorded disease chain, indicating that MPXV had a limited capacity to cause epidemics (Jezek et al., 1986). During this period, serological investigations of vaccine-naive newborns revealed that 12–15% of the children, had antibodies to MPVX. However, the majority had no history of the associated illness, showing that asymptomatic transmission also existed (Jezek and Fenner, 1988). Since the WHO monitoring program stopped in 1986, few medical research works have reported the recent incidence of human MPX. Individual 13 cases were documented in the literature between 1986 and 1992, and none were recorded between 1993 and 1995 (Heymann et al., 1998).

However, in the Kasai-Oriental region of the DRC, over 500 probable MPX cases have been identified between 1996 and 1997 (Heymann et al., 1998; Hutin et al., 2001). Even just a very few of these cases were laboratory validated. The percentage of secondary cases was substantially more significant than the previous WHO research findings (78%). The death rate was markedly lower (1-5%), showing that many were varicella cases. There were no further confirmations of suspected MPX cases till 2001, when 31 people

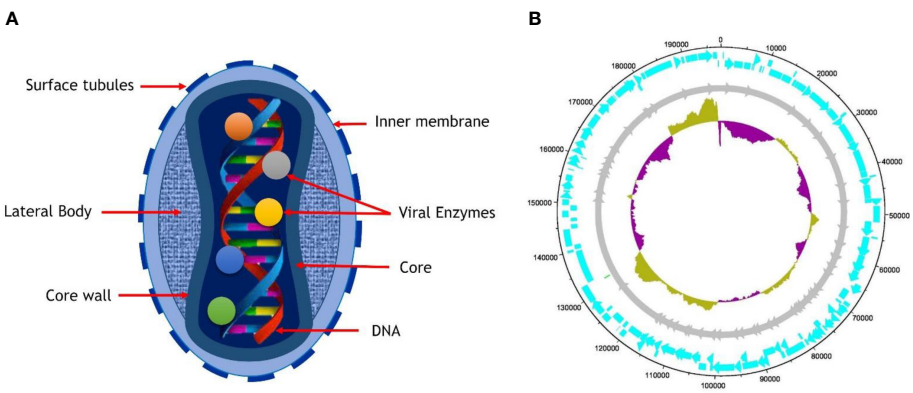


FIGURE 1
**(A)** Structure of mature virion of MPXV, **(B)** The complete genome of MPXV isolates SI2022 S7 (Genbank ID: ON838178.1) is 197652 bp long, with the length of the arrowhead representing genes (cyan) and the length of the arrow representing gene size. The dark yellow indicates that the GC content (33%) is above average, while the indigo color represents GC content below average.

with MPX were found in 7 distinct disease groups in the Equateur Province of DRC.

In the DRC, healthcare contractors monitor passive disease despite governmental unrest and inadequate resources. Their results show that MPX is more frequent than in previous studies (Kebela, 2004). The DRC Ministry of Health received reports of 1265 instances between January 1, 1998, and December 31, 2002, with specimens collected in 215. MPXV was the cause of 88 of the 215 cases, according to PCR and viral culture. Patient ages varied from 10 months to 38 years for laboratory-confirmed cases, with a mean age of 16.5 and 15.5 years. Patients made up 73.2% of the population who were over 25, while 26% were children under 10 (Kebela, 2004).

## 3.2 The outbreak in the US

During the summer of 2003, a wave of disease cases associated with MPXV were confirmed in the midwestern United States (Reed et al., 2004). MPXV was discovered for the first time in the Western World. Thirty seven of 72 human cases in an outbreak were confirmed by laboratory tests (Control, C.f.D. and Prevention, 2003; Control, C.f.D. and Prevention, 2003a; Sejvar et al., 2004). Because most infected people fell ill after associating with dogs, prairie dogs (Cynomys species) kept with rodents transported from Ghana in western Africa were assumed to be the primary source of the pandemic (Sejvar et al., 2004). Ignoring that viral infection seemed to happen *via* direct interaction with an infected dog, two patients cared for their ill children, and individual contamination could not be excluded entirely (Reed et al., 2004).

Even though no symptoms or clinical signs were observed in a new research work of 81 health care employees who contacted three people with MPX infection, one asymptomatic health care employee had laboratory proof orthopoxvirus contagion, which could be attributed to also current infection or smallpox vaccination (Fleischauer et al., 2005). There was a mild, self-limiting feverish rash disease among most African patients who contracted the disease during the US outbreak. 18 of the 69 people whose data was available were from the hospital. However, several were just admitted for isolation measures (Control, C.f.D. and Prevention, 2003a). Two patients, both youngsters, were suffering from severe clinical illness (Missouri, Ohio, and Wisconsin, 2003; Anderson et al., 2003; Control, C.f.D. and Prevention, 2003a). The first infant got severe encephalitis and needed 14 days in an intensive care unit (Missouri, Ohio, and Wisconsin, 2003; Sejvar et al., 2004). Encephalitis is a relatively unusual consequence of MPX, with only one prior report (Ježek et al., 1987; Jezek and Fenner, 1988). The second youngster was admitted to the hospital with tonsillar lymphadenopathy and agonizing symptoms of cervical and oropharyngeal pox (Anderson et al., 2003). Both kids were cured, and no one resulted in the epidemic.

Surprisingly, just one patient (a toddler) developed a broad rash comparable to earlier African patients. Many others had limited lesions on their fingers and hands due to exposure to an infected animal, which could be because inoculating a strain of MPXV through prairie dog bites results in significantly milder disease than inhaling the same strain, which is also less lethal as isolates from the Congo basin (Chen et al., 2005).

# 4 Human MPX

In 1970, a 9-month-old Zaire kid was diagnosed with MPX as a human disease (50, 51). Till now, utmost human MPX medical information originated from epidemic research in Western and Central Africa. Humans are believed to contract the virus through contact with diseased animals' body fluids or lesions. Significant breathing droplets might be transferred in extended face-to-face contact, although less effective than smallpox (Fenner et al., 1988b). Human MPX clinical characteristics are quite similar to ordinary Smallpox (Breman et al., 1980). It usually takes 10 to 14 days of incubation for patients to develop a rash after experiencing prodromal symptoms such as fever, malaise, and enlarged lymph nodes (Figure 2) (Jezek and Fenner, 1988; Di Giulio and Eckburg, 2004). Chills, sweating, backache, headache, sore throat, shortness of breath, and cough are also indications and symptoms of MPX. MPX is characterized by lymphadenopathy, Smallpox is not a common disease, being reported in 90% of unvaccinated individuals. Swelling of lymph nodes can happen in the neck, submandibular or inguinal regions (Fenner et al., 1988a). Before the typical maculopapular rash occurs, the prodromal stage usually lasts 1–3 days. The patient is contagious in the first week of the rash and should be quarantined until all scabs have been removed and throat swab PCR results are negative. Its skin lesions are 0.5–1 cm in diameter and progress from macules to papules, vesicles, and pustules, followed by scabbing, desquamation, and umbilication over 2-4 weeks (Fenner et al., 1988a). A circular spread of the rash may occur on the palms and soles of the feet, even though it appears first on the neck. Mouth and tongue lesions, as well as vaginal lesions, are also possible.

In contrast to skin wounds and lesions, individuals infected with MPXV might develop extra-cutaneous symptoms such as Pneumonitis (12% of cases), encephalitis (1%), secondary skin and soft tissue infection (19%), and ocular complications (4-5%) (Di Giulio and Eckburg, 2004).

# 5 Transmission

Contamination of animals with blood or bodily fluids; direct contact with blood or bodily fluids. Ingestion of insufficiently prepared meat from infected animals, inoculation from infected animals' mucocutaneous sores, particularly once the skin barrier is

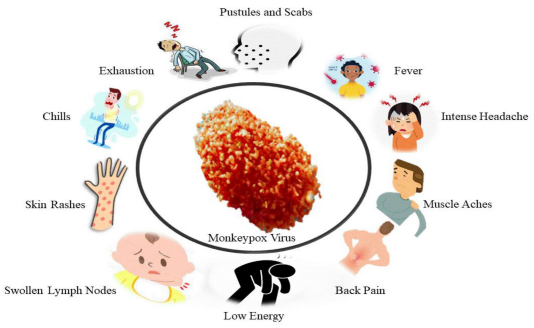


FIGURE 2
The sign and symptoms of MPX Disease.

ruptured due to bites, scratching, or other disturbances, or inoculation from mucocutaneous lesions on diseased faunae are methods for the virus to reach people (Figure 3) (Sutcliffe et al., 2012; Pal et al., 2017). Interacting diseased monkeys, rats, rabbits, Gambian giant squirrels, Porcupines, dormice, prairie dogs, and gazelles has been identified as a possible transmission mode (Pal et al., 2017). Direct physical interaction is the most common source of infection in MPX outbreaks, in small groups where people hunt and assemble. Even though no specific species have been found, rodents are studied as a reservoir. When large respiratory droplets come into touch with one another, they can transfer infection from person to person. Disease with MPX has the potential to spread through the placenta (Pal et al., 2017).

MPX infection begins with a cutaneous or respiratory epithelial infection in affected animals or people. A primary viremia results in systemic infection, followed by secondary viremia, which causes epithelia infection and ulceration in the skin and mucosa. Through oropharyngeal secretions, viruses replicate on mucosal surfaces and spread to close contacts. Even though MPXV is capable of evading host immune responses, the density of viral particles in oropharyngeal secretions, proximity and duration of contact, and viral survival affect virus transmission (Sutcliffe et al., 2012).

## 6 Reservoirs and hosts

It is believed that rodents are the primary reservoir in Africa; a study showed that some forest-dwelling rodents can be infected by Orthopoxvirus (including MPX) (Figure 3) (Khodakevich et al., 1986; Huhn et al., 2005). Serological studies show that various species, such as squirrels, nonhuman primates, and rats, are infected with MPV in the wild. Numerous observational studies in the DRC have revealed squirrels (particularly Funisciurus anerythrus) residing in farming regions as potential carriers of viruses to people living in nearby towns (Khodakevich et al., 1986). Funisciurus spp. squirrels, compared to Heliosciurus spp. squirrels (15%) and primates (8%), had a higher rate of seropositivity for MPV (24%). In February 1997, a second seroprevalence analysis conducted as a segment of the inquiry into the DRC epidemic revealed significantly greater positive proportions in these squirrels (39 to 50% in Funisciurus spp. and 50% in Heliosciurus spp. squirrels) (Hutin et al., 2001). Furthermore, this study identified serological confirmation of MPV infection in 16% of the Gambian giant rats. After contacting an ill prairie dog, a rabbit contagion (family Leporidae) at a veterinary clinic established the virus's transmissibility between North American animal species. Less is known regarding MPV and HIV co-infection (Di Giulio and Eckburg, 2004).

## 7 Diagnosis

The clinical manifestations of MPX are not the same as that of Smallpox and chickenpox. A correct assessment is crucial for treating illnesses or early detection of a potential bioterrorism occurrence. Although infections caused by parapoxviruses, e.g., bovine stomatitis and orf, can create little skin wounds and lesions identical to those recognized in the US MPX epidemic, electron microscopy clearly distinguishes them from orthopoxviruses. As FDA-approved antiviral treatment for MPX, once the illness pathogen has been recognized, the appropriate public health prevention methods are quarantine and fast ring vaccination. Due to the apparent efficiency with which direct touch and aerosol particles can transmit disease, aseptic conditions should be maintained by using respiratory measures when handling samples such as a scab. Although clinical features help differentiate poxvirus illnesses from many further causes of vesiculopustular rashes, laboratory diagnosis is essential for precise identification.

Laboratories' investigative procedures for MPX contain IgM, IgG, Enzyme-linked immunoassay (ELISA), electron microscopy, Polymerase Chain reaction (PCR), Virus Isolation, Immuno-fluorescent antibody test, and histopathologic examination. Regrettably, most of these approaches are ambiguous and cannot distinguish MPXV disease from those other poxvirus diseases. For example, lesions caused by the MPXV histologically resemble those caused by the varicella-zoster, cowpox, variola, and herpes simplex viruses, with massive fibroblast degeneration, cutaneous edema, acute inflammation, and severe spongiosis (Bayer-Garner, 2005). However, immunohistochemically examination containing monoclonal and polyclonal antibodies in contradiction of all orthopoxviruses can

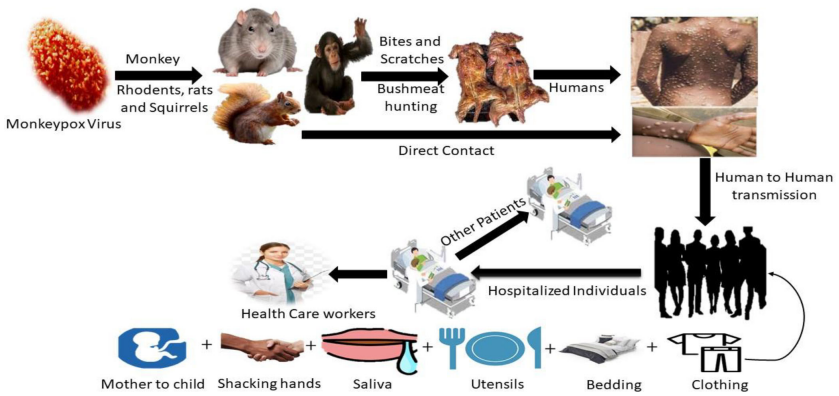


FIGURE 3
The primary host and transmission route of MPX, eating infected animals as a food source, is the major cause of animal-to-human virus transmission. Healthy people can contract the virus by intimately contacting someone who is infected.

help distinguish a herpes virus infection from a pox virus disease. In the past, electron microscopy was frequently used to aid viral diagnosis (Gentile and Gelderblom, 2005).

A laboratory with a biosafety level-3 should conduct PCR or real-time PCR (Fowotade et al., 2018). In clinical and veterinary specimens as well as in cell cultures with MPXV infection, MPXV DNA is routinely detected using real-time PCR using conserved regions of the extracellular envelope protein gene (B6R) (Li et al., 2006), DNA polymerase gene, E9L (Yinka-Ogunleye et al., 2019). Rpo18, a DNA-dependent RNA polymerase subunit, and the F3L gene (Reynolds et al., 2010; Orba et al., 2015). PCR-amplified genes or gene fragments are also examined by restriction-length fragment polymorphism (RFLP) to detect MPXV DNA (Meyer et al., 1994; Kulesh et al., 2004). However, RFLP takes time and requires viral culture. In clinical settings where speed, sensitivity, and specificity are critical, RFLP of PCR products may not be the best approach because it also requires enzyme digestion and gel electrophoresis. MPXVs and other OPVs remain best characterised by whole-genome sequencing using next-generation sequencing (NGS) methods (Radonić et al., 2014; Cohen-Gihon et al., 2020). Despite its advantages, downstream sequencing technology is expensive and requires a great deal of computer power.

In resource-constrained areas like Sub-Saharan Africa, NGS may not be the most efficient characterization technique. While real-time PCR remains the preferred approach for routine MPXV diagnosis, it must be augmented *via* field genome sequencing technology, including Oxford NanoporeMinION, to provide epidemiological interventions based on evidence-based viral genome data in real time. In resource-constrained parts of West Africa, during the outbreak of Ebola, MinION field sequencing was used effectively for genomic surveillance (Quick et al., 2016).

The MPXV incubation period is 4-21 days, according to clinical diagnosis, and a prodromal disease frequently accompanies it with various symptoms such as lymph node enlargement, fever, myalgia, headache, back pain, strong asthenia, malaise, pharyngitis, and drenching sweats. In the prodromal phase, a vesiculopustular rash appears between 1-10 days of development and spreads throughout the body during the exanthema phase. In MPXV patients, the lesions appear to be monomorphic, pea-sized, and hard, similar to smallpox lesions. Smallpox can be distinguished from MPXV by the crop-like form of the lesion and the absence of vigorous centrifugal spread. MPXV can also be distinguished from smallpox by lymphadenopathy (McCollum and Damon, 2014; Sklenovska and Van Ranst, 2018; Kabuga and El Zowalaty, 2019). It is imperative to make early identification of suspected MPX cases, even without laboratory confirmation. In a cohort of 645 patients, the clinical definition of MPX had high sensitivity (93-98%). However, the specificity is low (9-26%) (Osadebe et al., 2017; Beer and Rao, 2019).

The detection of viral antigens is accomplished by immunohistochemistry, and the detection of IgG and IgM antibodies is accomplished by ELISA. Immunochemistry analysis can be performed using polyclonal or monoclonal antibodies in contradiction of entirely OPVs to distinguish amid poxvirus and herpes virus infection. Antiviral antibody levels, as well as T-cell responses, have been shown to rise around the time of illness onset. IgM and IgG, however, can be detected in serum between five and eight days after the rash begins. An individual without a history of rash or severe illness who tests positive for IgM and IgG antibodies, an indirect MPXV diagnosis may occur. However, none of these strategies is unique to MPX (McCollum and Damon, 2014; Nasir et al., 2018; Petersen et al., 2019b; Sadeuh-Mba et al., 2019). In addition to other OPV species, IgM can be utilized to diagnose MPX infection in people who have previously been vaccinated against smallpox (Weaver and Isaacs, 2008). The IgM capture ELISA indicates recent contact to OPV (possibly MPXV in endemic areas), while the IgG capture ELISA indicates prior exposure to OPV (Karem et al., 2005; MacNeil et al., 2011). Thus, OPV antibodies in a sample indicate recent exposure by individuals who have previously received the vaccine or susceptible to spontaneous infection. In MPX-endemic areas, IgM is found in individuals who are already immunized versus smallpox.

Similarly, if accessible, electron microscopy can be used as a laboratory for identifying poxvirus contagions. It might be one of the early signs of a rash disease. Under electron microscopy, typical poxvirus virions with the characteristic morphology would be predictable to be detected. For example, during a current MPX epidemic in the United States, electron imaging revealed keratinocytes containing a substantial proportion of mature virions along with immature virions in the stage of synthesis (also called "viral factories") inside the cytoplasm (Bayer-Garner, 2005). This approach, though, cannot distinguish between orthopoxvirus species. Virus isolation and classification through different PCR methods, whether restriction fragment length polymorphism testing or amplicon sequencing, is frequently regarded as conclusive for MPXV identification (Murray et al., 2006). Furthermore, the accessibility of real-time PCR that employs panorthopoxvirus or MPXV-specific targets has expanded (Kulesh et al., 2004; Olson et al., 2004). Another quick approach for detecting orthopoxviruses has been developed; a DNA oligonucleotide microarray containing the TNF receptor gene crmB (Lapa et al., 2002).

Regardless of the epidemic, doctors should evaluate MPX in patients with a novel onset pyretic temperature and rashes if lymphadenopathy is evident. The rashes generally start on the lips and spread to the cheeks and extremities in a centrifugal pattern (including the palms and soles). PCR analysis of skin lesions or fluids yields a precise diagnosis. Such diagnostics are available exclusively at national public health labs; no commonly accessible screening is available (Centers for Disease Control and Prevention, 2022; Adalja and Inglesby, 2022).

## 8 Treatment

MPX infection is currently without a clinically validated therapy. The therapy, like with most viral diseases, is symptom control. There are, though, preventative methods that can assist avoid an epidemic.

The septic person must be isolated, keep covered lesions and wounds, and wear a mask even more than probable till all lesion crusts break off and a new skin layer form. In extreme circumstances, for exploratory usage, medicines with efficacy against orthopoxviruses in animal trials and severe vaccinia vaccination sequelae may be evaluated. There is an approved treatment for smallpox known as brincidofovir, tecovirimat, and vaccinia immunoglobulin, as well as an inhibitor of intracellular viral release known as tecovirimat and

which has shown efficacy against MPX in animals (Reddy, 2018; Bunge et al., 2022; WHO, 2022c).

Following exposure, measurement of temperature and symptoms should be done twice daily for 21 days since that is the recognized maximum incubation period for MPX. Because infectiousness co-occurs as illness starts, close contacts do not need to be separated when asymptomatic. Vaccination with the Ankara vaccine after vaccinia exposure (live, non-replicating smallpox vaccine) is advised in certain situations. Interaction among wounded skin or mucous membranes with the bodily fluids, respiratory droplets, or scabs of an infected patient is thought to be a "high risk" contact that necessitates post-exposure vaccination as soon as possible. After close connection with an MPX case, it is recommended that vaccination take place within four days after first contact with the virus, but it is possible to give the vaccination up to 14 days after that (Control, C.f.D. and Prevention, 2003b).

A vaccination containing a replication fault, the Ankara vaccine is a two-dose vaccine administered four weeks separately and has a better profile than first- and second-generation smallpox vaccination. Ankara injection, contrasting live vaccinia virus training, does not cause skin lesions or provide a danger of extensive or local transmission (McCollum and Damon, 2014). The results of medical studies confirmed that altered "vaccinia Ankara" is safe and increases antibody production in people with early or weakened immune systems, both of which are limitations of delivering live vaccinia to individuals (Petersen et al., 2019).

More detailed data and feasibility studies are needed to identify preventive MPX immunization's potential benefits and drawbacks in endemic areas. The availability of therapeutic care diagnosis and facilities limits the ability to create knowledgeable judgments on managing this deserted tropical ailment (Reynolds et al., 2017; Petersen et al., 2019). While, MPX does not have any specific treatment, such as brincidofovir, tecovirimat, vaccinia immunoglobulin, and tecovirimat, which has shown efficacy against MPX in animals (Figure 4) (Costello et al., 2022; Moore and Zahra, 2022). CMX-001, ST-246, and Cidofovir are other promising antivirals (McCollum and Damon, 2014). The US Food and Drug Organization has authorized the usage of the latter two drugs in smallpox treatment (FDA). Such therapies would be earmarked for severe cases or immunocompromised individuals and provided through a public health agency or the CDC (Centers for Disease Control and Prevention, 2022; Adalja and Inglesby, 2022).

## 9 Control and prevention

Enhanced infection control procedures, like regular screening and isolation of new infections animals, would surely benefit epidemic control (Figure 5). Proper hygiene habits are essential to prevent the virus from spreading on fomites and becoming a source of new infections. Vaccination with the vaccinia virus may be an alternative for animal protection. Because diseases have been observed in Asian monkeys mixing with African primates, these species must be maintained apart (Pal et al., 2017; Quiner et al., 2017). To avoid spreading the disease, someone infected with it should minimize interaction with animals, notably mice and animal primates (Quiner et al., 2017).

Take the following actions to prevent infection with the MPXV:

1). Avoid any animals that may be infected with the virus (animals that are sick or dead in areas where MPX occurs).
2). Avoided things that interacted with infected animals and humans.
3). While caring for the infected, use personal protective equipment, including gowns, masks, respirators, gloves, shoe covers, goggles, eye protectors, and face shields.
4). Isolate sick individuals from people who may have been infected.
5). When interacting with sick humans or animals, ensure proper hand sanitation by cleaning hands with soap or sanitizer.

Smallpox vaccinations are applicable against MPX. The FDA has approved ACAM2000, a newer-generation smallpox vaccine, for treating MPX (Hatch et al., 2013; Koenig et al., 2022; Rao et al., 2022), the older generation of ACAM2000 can also be used off-label for the same reason. Vaccinating close contacts has proven to be effective in controlling transmission of previous epidemics. As soon as feasible after probable exposure, prophylactic vaccination administration can abort or dramatically reduce infection. If smallpox vaccination is unavailable, vaccinia immune globulin is possibly an alternate post-exposure prophylactic negotiator (Centers for Disease Control and Prevention, 2022; Adalja and Inglesby, 2022). While in an epidemic, MPX viral transmission can be prevented by isolating affected animals (at least for six weeks from the day of the

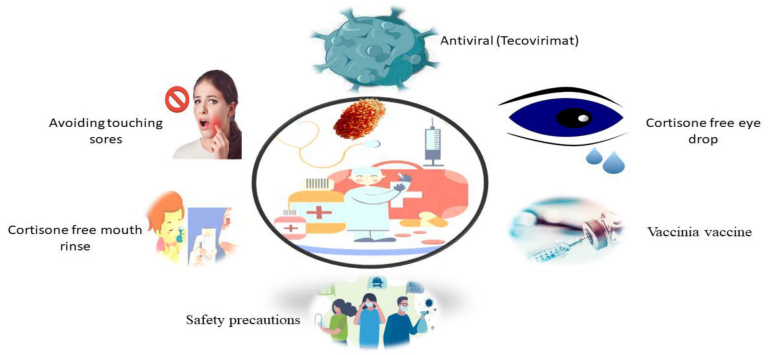


FIGURE 4
Possible treatment of MPX Virus Disease.


FIGURE 5
Preventive measures to control the MPX.

contract) and tracking their interaction. The regions where these animals were housed should be carefully decontaminated. Specific directions from the healthcare-related authority or the CDC Website must be followed.

## 10 Reasons for the re-emergence of MPX disease

Several interconnected causes have contributed to the recent comeback of infectious illnesses. Global travel and business are opening up the world to more people. The deepening of economic, political, and cultural ties emphasizes relationships between humans and other animals. These interact and engage in both accidental and intentional microbial agent transmissions. The United Kingdom reported three outbreaks of MPX between May 25 and June 15, 2021 (Reynolds et al., 2019; Okyay et al., 2022; WHO, 2022a). On May 8, 2021, a first case from Nigeria was identified. This comment might be utilized to highlight the importance of global travel assistance in virus transmission. Although inter-human transmission is limited, particularly in healthcare and the home, it causes epidemics.

Nevertheless, studies indicate that human infections will not emerge without recurring zoonotic invasions. Expanding animal-human connections increases the likelihood of infection from animals to humans (Reynolds et al., 2019; Okyay et al., 2022; WHO, 2022a). As a result, one of the essential goals in the fight against this disease is limiting virus transmission from animals to humans. Preventive methods include avoiding contact with diseased animals and ill or dead animals located in infested regions, in the case of handling materials that have come into contact with sick animals, including furniture, and segregating infected patients from others who may be diseased. Hands should be washed with soap and water after touching infected animals or humans, or utilize an alcohol-based hand sanitizer. To prevent MPX, it is important to raise awareness of risk factors and educate individuals about how to decrease their exposure to it (Sklenovska and Van Ranst, 2018).

It should be noted that the usage of smallpox vaccination may offset the virus's return. MPX, initially found in the DRC in 1970, is most likely the result of a lack of immunization following the elimination of smallpox. Concerns about the eradication of smallpox through vaccination due to the presence of the disease in the exact geographic location with varying epidemiological aspects and field outcomes (Sarwar et al., 2022). The interruption of vaccine administration may increase the number of susceptible persons. Another issue that may cause the disease to reemerge is a failure to offer vaccination to susceptible persons in places where Human immunodeficiency virus (HIV) infection is widespread. Evaluating the immunization program, identifying susceptible individuals, and assuring vaccination are vital. Though there have been isolated incidences of MPX outside African countries, insufficient work has been devoted to producing a specialized vaccine to prevent the infection (Heymann et al., 1998) concerning the recent return of infectious illnesses during an epidemic.

Invasive procedures (such as angiography labs) and therapeutic medications used in the prevention of thromboembolic events have become more widely used, as the global population grows, all lead to the average life expectancy being prolonged as technology advances. The growing global population, along with rising average life expectancy, predicts a rise in the population of sensitive person. Vaccination discontinuation, expanding population, longer life expectancy, and more worldwide relations resulting from more straightforward transportation are prospects for the re-emergence of MPX illness (Okyay et al., 2022).

## 11 Public health strategy

Health experts are currently monitoring all individuals to determine when the isolation period will end (i.e., when all scabs

have fallen off and fresh, healed skin appears). The CDC made available tecovirimat, an antiviral licensed for Smallpox but with antiorthopoxvirus action, through extended access from the strategic national stockpile (Merchlinsky et al., 2019). The CDC also made vaccination, PEP accessible to contacts who had high-risk exposures (for example, it would be dangerous to contact a patient's skin or mucous membranes unprotected or be exposed to their bodily fluids (i.e., being within 6 feet of an unmasked patient for 3 hours with no protective measure). In low or unknown-risk circumstances (for example, new health care providers), PEP is not recommended. PEP with ACAM2000 or JYNNEOS vaccinations is administered to appropriate intermediate and high-risk contacts. The interaction research is in progress; of the 13 patients who have recognized interactions, 56 are considered high risk, 117 are regarded as intermediate risk, and 235 are considered low or unknown risk. For 21 days following the last encounter, contacts should be observed for symptoms and indications of MPX. According to DNA sequencing results of the virus obtained from the Massachusetts patient, it resembles other genomes described in this European outbreak (Next strain/MPX) (Genomic epidemiology of monkeypox virus, 2022). They are linked to the 2017–2018 MPX pandemic in Nigeria. According to early statistics, as of June 6, around 800 cases of MPX have been recorded in this epidemic from 28 countries, such as the United States (Monkeypox Outbreak — Nine States, 2022).

## 12 Concerns regarding the ongoing epidemic

MPX has been documented in North America, the US, and Europe. These cases exist outside the endemic area of the virus, and are transmitted by person-to-person. Several seemingly unrelated groups have formed based on the fact that most of them come from a prevalent country. Most cases are reported in sexually transmitted infection (STI) hospitals as well as in men who have had sex with men (MSM) (WHO, 2022b). In this population, the virus may or may not transmit sexually rather than *via* skin-to-skin contact and droplet respiratory spread. In MSM clusters, the latter has been identified as a transmission mechanism for meningococcus. The most critical challenge is to determine how the epidemic started. Compared to the previous MPX epidemic outside of Africa, why is this epidemic so much more widespread and significant? There are no genetic variants that are believed to promote transmissibility, according to preliminary genetic research (Isidro et al., 2022). Case-control training and quick case examinations are now underway and are essential for understanding this problem.

In the meantime, efforts will be focused on detecting cases, diagnosing them early, tracking contracts, isolating patients, and vaccinating them after exposure. If MPX epidemics continue, medical professionals, outpatient treatment providers, critical care clinicians, dermatologists, and clinics dealing with STIs could identify additional cases.

## 13 Conclusion

It is likely that MPX has existed in Sub-Saharan Africa since humans first came into contact with diseased animals thousands of years ago. Humans can exhibit signs and symptoms that are disturbingly similar to chickenpox, Smallpox, or other vesiculopustular rashes. An outbreak requires precise and prompt laboratory identification.

MPX cases in Africa are closely associated with Smallpox cases and the population is developing antibodies deficiency as a result of the discontinuation of routine Smallpox vaccination has raised concerns that MPXV may be used as a bioweapon. As a result of these factors, MPXV, along with the variola virus and many other poxviruses, is on the NIH's highest danger list. The CDC has categorized it as a "select agent." Human travel is prevalent today, providing risk for the spread of MPX, and animals carried across borders represent an immediate danger of disease spread. Because biological warfare potential cannot be ruled out, enhanced knowledge of MPXV and related virus may improve emergency management.

## Author contributions

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

## Funding

This work was supported by the National Natural Science Foundation of China (Nos. 81802718, U1504817), the Foundation of Science and Technology Department of Henan Province, China (Nos. 192102310151, 202102310480), and the Training Program for Young Backbone Teachers of Institutions of Higher Learning in Henan Province, China (No. 2020GGJS038).

## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

## Publisher's note

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frontiers | Frontiers in Cellular and Infection Microbiology

TYPE Review
PUBLISHED 10 February 2023
DOI 10.3389/fcimb.2023.1076251



OPEN ACCESS

EDITED BY
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Henan University, China

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SPECIALTY SECTION
This article was submitted to Virus and Host, a section of the journal Frontiers in Cellular and Infection Microbiology

RECEIVED 21 October 2022
ACCEPTED 10 January 2023
PUBLISHED 10 February 2023

CITATION
Rampogu S, Kim Y, Kim S-W and Lee KW (2023) An overview on monkeypox virus: Pathogenesis, transmission, host interaction and therapeutics.
*Front. Cell. Infect. Microbiol.* 13:1076251.
doi: 10.3389/fcimb.2023.1076251



# An overview on monkeypox virus: Pathogenesis, transmission, host interaction and therapeutics

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*Orthopoxvirus* is one of the most notorious genus amongst the *Poxviridae* family. Monkeypox (MP) is a zoonotic disease that has been spreading throughout Africa. The spread is global, and incidence rates are increasing daily. The spread of the virus is rapid due to human-to-human and animals-to-human transmission. World Health Organization (WHO) has declared monkeypox virus (MPV) as a global health emergency. Since treatment options are limited, it is essential to know the modes of transmission and symptoms to stop disease spread. The information from host–virus interactions revealed significantly expressed genes that are important for the progression of the MP infection. In this review, we highlighted the MP virus structure, transmission modes, and available therapeutic options. Furthermore, this review provides insights for the scientific community to extend their research work in this field.

KEYWORDS

**monkeypox virus, replication, MPV, drugs, viral structure, transmission mode**

## 1 Introduction to viruses

Viruses are organisms that cause infectious diseases globally and their infections to humans have been observed since ancient times (Chappell and Dermody, 2015). Studies on viruses and viral diseases began in 19$^{th}$ century (Chappell and Dermody, 2015). Reports have shown that at least 219 virus species can cause infections in humans (Woolhouse et al., 2012). The first virus to be identified by Iwanovsky and Beijerinck was the tobacco mosaic virus in 1892 and the foot-and-mouth disease virus by Loeffler and Frosch in 1898 (van Kammen, 1999; Woolhouse et al., 2012; Chappell and Dermody, 2015). In humans, the first discovery was the yellow fever virus in 1901 (Woolhouse et al., 2012; Chappell and Dermody, 2015). Since then, multiple viruses have been discovered.

Several viruses have been infecting humans and posing a challenge in finding effective therapeutics (Wong et al., 2017), such as human immunodeficiency virus (HIV) (Lloyd, 1996; Simon et al., 2006) and SARS-CoV-2 (Dhama et al., 2020; Hu et al., 2021; Rampogu and Lee, 2021a). Particularly, the SARS-CoV-2 has hampered the health system, and this virus also

warrants the scientific community to be ready for future pandemics. One such infectious diseases is monkeypox, which has been recently spreading globally.

## 2 Monkeypox

Monkeypox (MP) is a zoonotic disease that demonstrates smallpox-like characteristics in humans and is related to variola virus, the causative agent of small pox (Bunge et al., 2022). Monkeypox virus (MPV) belongs to the family: Poxviridae, subfamily: chordopoxvirinae, genus: orthopoxvirus, and species: Monkeypox virus (Moore et al., 2022). Other members of this genus include cowpox, camelpox, and vaccinia (VV) (Pauli et al., 2010; McCollum and Damon, 2014; Silva et al., 2020). As on 14$^{th}$ October 2022, a total of 73,288 cases have been recorded according to https://www.cdc.gov/poxvirus/monkeypox/response/2022/world-map.htm. Distribution of MPV infection in different countries (Figure 1).

## 3 Monkeypox and smallpox

Interestingly, variola virus (VARV) and MPV are related antigenically and genetically (Shchelkunov et al., 2001; Cann et al., 2013). However, these viruses vary in the regions of their sequence for virulence and host-range factors at the genome termini (Shchelkunov et al., 2001; Cann et al., 2013). Upon comparing the VARV strain Japan 1951 (Harper, Masterseed) and MPV strains (Zaire 96-I-16), the length of MPV genome was 10,678 bp larger than Variola virus (Shchelkunov et al., 2001; Esposito et al., 2006; Cann et al., 2013). Furthermore, MPV has displayed mutations that influence the process of translation of interferon resistance genes that code for proteins C3L and E3L. The MPV genome also encodes a secreted interleukin (IL)-1β-binding protein, which is absent in VARV genomes. The absence of this vaccinia virus (VACV) - gene corresponds to amplified pathogenicity (Cann et al., 2013).

## 4 History of MPV

The advent of MPV was primarily observed in monkeys shipped from Singapore to Copenhagen in 1958 by von Magnus et al. (Cho and Wenner, 1973; Moore et al., 2022). The name monkey virus is coined after its first discovery from monkeys (Bunge et al., 2022). Approximately, 20-30% of animals have manifested clinical illness (Cho and Wenner, 1973; Moore et al., 2022). Notably, out of the 6 cases in 1970, the first human infection was reported in an infant (9 months old) from the Democratic Republic of the Congo (Ladnyj et al., 1972; Cho and Wenner, 1973; Moore et al., 2022). Four other infections were observed in children from Bouduo, Liberia aged 4-9 years (Cho and Wenner, 1973), and the other case was in a male of 24 years from Sierra Leone (Cho and Wenner, 1973). In 2003, MPV cases were recorded in the USA that is outside Africa (Bunge et al., 2022). Notably, this is caused due to animal-to- human transmission (Reed et al., 2004). Between 2018 and 2021, the MPV spread was observed in the USA, the UK, Israel, and Singapore (Mauldin et al., 2022). By 2022, MPV reached 31 countries (Xiang and White, 2022) with no travel history to endemic countries, and the virus isolates are reported as West African clade (Xiang and White, 2022).

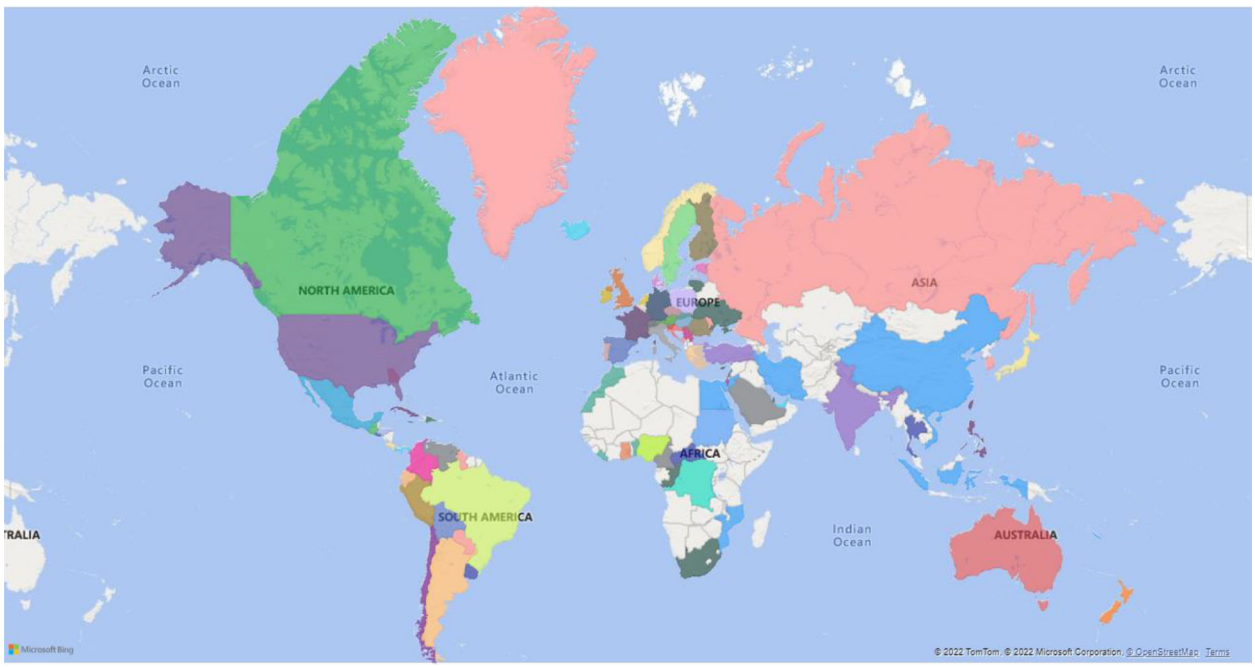


FIGURE 1
The global spread of monkey pox virus (MPV). The figure is prepared using PowerBI desktop.

## 5 Genome and structure of MPV

The MPV genome is a linear genome with a size of approximately 197 kb (196,858-base pairs) (Shchelkunov et al., 2001; Alkhalil et al., 2009) comprising ≈190 non-overlapping ORFs >180 nt in length (Kugelman et al., 2014). The central coding region sequence is positioned at ≈56000–120000 nucleotides (Kugelman et al., 2014). This conserved region is flanked by variable ends that comprise inverted terminal repeats (ITRs). The genome encodes biological machinery that is essential for the survival of the virus (Alkhalil et al., 2009). Genes in the central region are necessary for entry, self-replication, and maturation (Alkhalil et al., 2009). The less conserved terminal regions are useful for host-virus interaction (Alkhalil et al., 2009).At the ITR zone, a minimum of 4 ORFs are present (Kugelman et al., 2014).

There are two clades present in the MPV: the Congo Basin strain and West African strain (Kindrachuk et al., 2012). The Congo Basin strain is also known as the Central African strain (ZAI-96). The West African strains are SL-V70, COP-58, and WRAIR-6 (Weaver and Isaacs, 2008). These clades demonstrate dissimilar virulence (Kindrachuk et al., 2012) and are geographically, clinically, and genetically different (Kindrachuk et al., 2012). The Congo Basin strain demonstrated a case fatality rates of approximately 10% noticed in the people who are non-vaccinated than the less lethal West African MPV clade (Kindrachuk et al., 2012). Furthermore, the fatality rate differs with the strain, with 3.6% with the West African clade and 10.6% with the Central African clade (Bunge et al., 2022).

The West African strain is linked with lower transmission within the humans (Hutson et al., 2009), with a difference of 0.55-0.56% nucleotide between both the strains (Chen et al., 2005). A difference of 0.01–0.07% nucleotides is observed among the West African strains (Chen et al., 2005). A recent study conducted through shotgun metagenomics, showed that the MPV belongs to clade 3 and presumably has a single origin (Isidro et al., 2022). Another phylogenetic study was conducted on African monkeypox (Nakazawa et al., 2015). The authors examined fairly large genomic sequence data obtained from the MPV isolates across the area of their distribution to determine the relationship between the clades and among the isolates and further improve the phylogenetic analysis (Nakazawa et al., 2015).

Furthermore, the amplified virulence observed in the Central African strains is believed to be due to D14L (complement inhibitor), D10L (host range protein), B14R (interleukin [IL]-1β binding protein), B10R (apoptotic regulator), and B19R (serine protease inhibitor-like protein) genes (Estep et al., 2011) (Karumathil et al., 2018). The West African strains are devoid of D14L (Estep et al., 2011). The orthologs of D10L and B19R orthologs are conserved in both clades (Chen et al., 2005). The orthologs of ZAI-96, B10R and B14R are absent in West African strain (Chen et al., 2005). Additionally, selective repression of the host response is noticed in Central African strain demonstrated by its ability to regulate apoptosis in the host (Kumar et al., 2022).

MPV is one of the largest and most highly complex viruses (Barreto-Vieira and Barth, 2015) demonstrating a brick-shaped structure with a length of 220 - 450 nm and a width ranging from 140 - 260 nm (Hyun, 2022). It has four components: core, lateral bodies, outer membrane, and outer lipoprotein envelope (Sklenovská, 2020). The core is the central part, encompassed by core fibrils and double stranded viral DNA. This layer is encircled by a rigid structure called the palisade layer (Sklenovská, 2020). The outer membrane accommodates the palisade layer, lateral bodies and the central core (Sklenovská, 2020). The structures called the surface tubules and are present on the outer surface (Sklenovská, 2020) (Figure 2).

Typically two types of virions are noticed in the negatively stained preparations of orthopoxviruses, namely the M form (mulberry) and C form (capsule) (Jezek and Fenner, 1988). The M-type virions are undamaged, while the C forms are the damaged ones (Jezek and Fenner, 1988). In C type virions, the stain percolates the cell, exposing the outer membrane and partially exposing the internal structures (Jezek and Fenner, 1988). Clinically, the M-types is present in the vesicle fluid and the C type in dried scabs (Jezek and Fenner, 1988).

## 6 Reservoir and transmission

The common reservoir for MPV is thought to be not only monkeys (Moore et al., 2022), but also other animals such as squirrels (Khodakevich et al., 1987), and sooty mangabey. (Radonić et al., 2014). Although the rate at which the virus resides among animals is still obscure, rodents are thought to be the reservoir hosts for this virus (Nolen et al., 2015; Wilson, 2017). Infections may also occur in mice, rats, humans (Learned et al., 2005; Nolen et al., 2016; Besombes et al., 2019), and prairie dogs (Tesh et al., 2004; Moore et al., 2022). MPV transmission may occur when a healthy individual comes into contact with the skin lesions, droplets, and bodily fluids of infected animals. Partially cooked food might also be a reason for the infection (Ahmed et al., 2022). The infection may also result from to contact with contaminated fomites (Moore et al., 2022). Additionally, the infections may also result due to activities that enhance exposure to animals, such as sleeping on the ground outside and habituating near the forest (Fuller et al., 2011; Guagliardo et al., 2020). The risk of infection may also be noted when eating bushmeat or wild game (Kmiec and Kirchhoff, 2022).

In 2018, MPV transmission was observed from an infected patient to a healthcare assistant which might be due to contact with bedding that was contaminated (Vaughan et al., 2020). Human-to-human transmission has also been noted in the Democratic Republic of the Congo (Nolen et al., 2016). Transmission within humans can also occur by sharing the same household and consuming food or water from the same dish as the patient (Bunge et al., 2022). In 2022, the United Kingdom witnessed an increase in MPV cases, categorized into three different events (Vivancos et al., 2022). The first case was in Nigerian imports, the second case was a cluster in a household, and the third case was reported in men with no reported link with previous cases or the travel history (Vivancos et al., 2022). One study reported the first case of MPV transmission from a human to dog (Seang et al., 2022). A recent report showed that out of 528 infections across 16 countries, 98% were bisexual or gay, 75% of the infections were seen within the white, and 41% were positive for HIV (Thornhill et al., 2022) (Figure 3).

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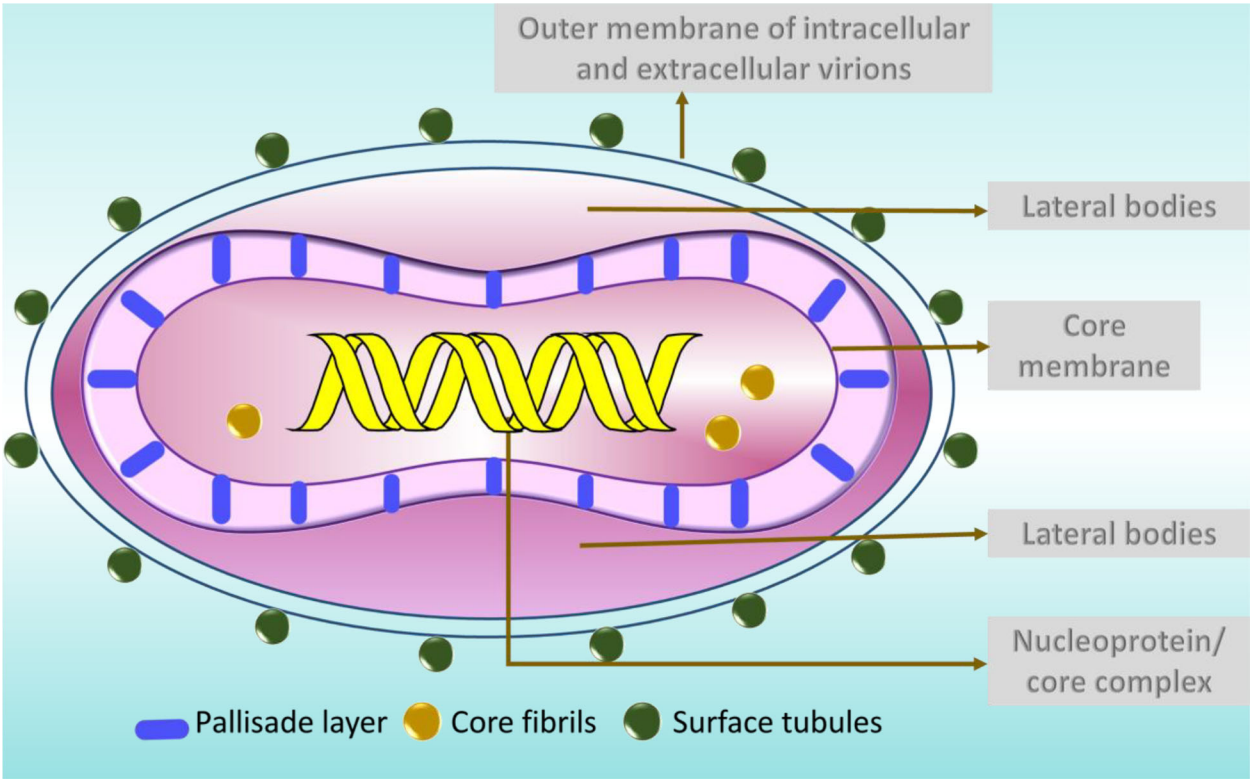


FIGURE 2
The structure of MPV particle.

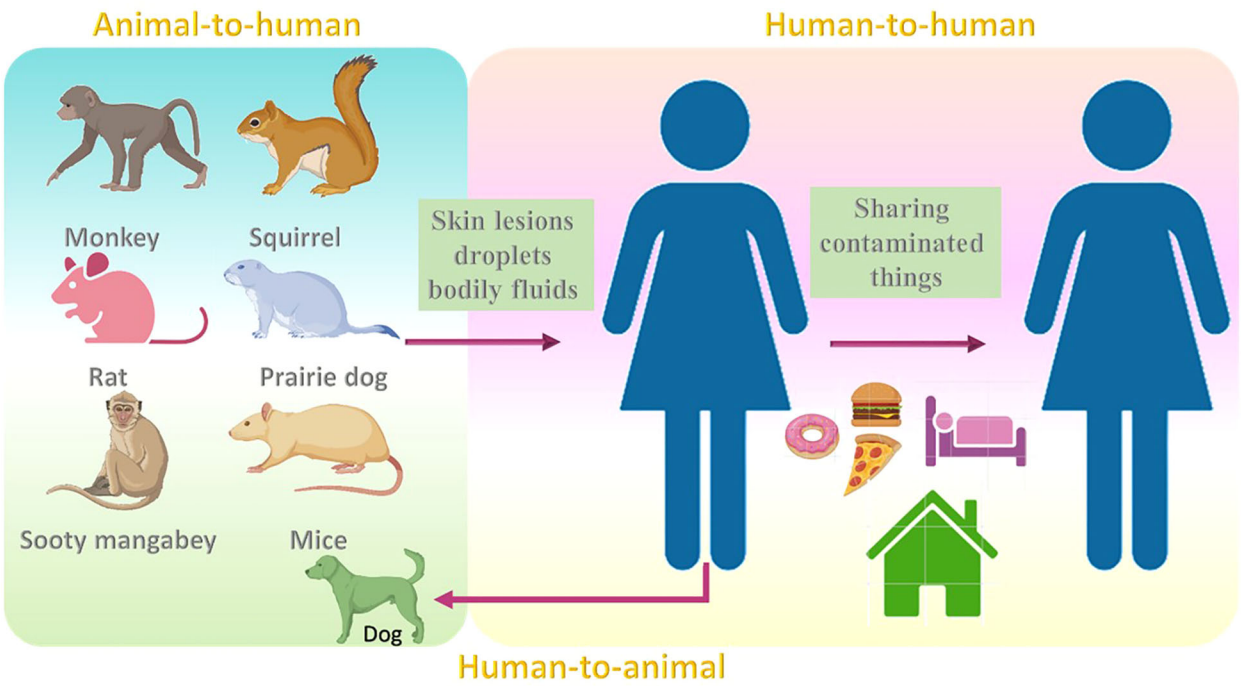


FIGURE 3
Transmission modes of MPV from animals (reservoirs) to humans and humans to animals.

## 7 Replication

To gain insight onto MPV replication, we relied on pox virus replication. Poxvirus replication occurs in the cytoplasm in specific structures known as Guarnieri bodies (Kieser et al., 2020; Kaler et al., 2022). These bodies are also called factories obtained from infecting particles (Kieser et al., 2020). The count (number) of factories depends on the multiplication rate of infection (Moss, 2013). The factory is the site where transcription translation, and assembly of virions take place (Moss, 2013).

Generally, there exists two types of infectious forms of MPV: the mature virion [MV, also called intracellular mature virions (IMV)] and the enveloped virion (EV, also called extracellular enveloped virions (EEVs)) and cell-associated enveloped virions (CEVs) (Chung et al., 2006; Moss, 2012). The MV has a single membrane, and the EV has an additional outer membrane that is cleaved before fusion. When the MV is enclosed within an endosomal membrane or trans-Golgi, it forms a triple-membrane. These are hence termed wrapped virions (WVs, also called intracellular enveloped virions (IEVs) (Moss, 2012). Unenclosed MVs remain free until cell lysis occurs (Chung et al., 2006; Moss, 2012). Both MV and EV are infectious and can spread the disease (Moss, 2012; Sklenovská, 2020). Comparatively, the stable MV transmits the infection between the host animals, while EVs with fragile external membranes are instrumental in spreading the disease within the host (Moss, 2012; Sklenovská, 2020).

The proteins facilitate the attachment of the virus to the cell, fusion of the membrane and entry into the host cell (Kaler et al., 2022). The single membrane in MV and an extra outer membrane in the EV perform the disruption prior to fusion (Kaler et al., 2022). There are a total of four viral proteins connected to the MV that help in the process of attachment of MV to the host cell (Kaler et al., 2022).

The virus attaches to the host *via* 11 to 12 non-glycosylated transmembrane proteins (4- to 43-kDa) (Moss, 2012; Kaler et al., 2022). In some pox viruses, laminin and heparine sulfate aid in the attachment (Kmiec and Kirchhoff, 2022). After infection DNA synthesis is initiated for no more than 2 hours (Moss, 2013). For replication, MV initially uncoats to gain entry into the cytoplasm (Sklenovská, 2020). The early genes are then expressed after the inactivation of the cells defence mechanisms. This inactivation is achieved by the generation of prepackaged viral proteins and the enzymatic factors (Sklenovská, 2020). Subsequently, the early messenger RNA (mRNA) is synthesized through the DNA-dependent RNA polymerase of the virus. The translated early mRNA assists another uncoating mechanism, replication of DNA, and generation of intermediate transcription factors. (Sklenovská, 2020). Then the transcription and translation of intermediate mRNA occurs to promote the late mRNAs expression. Further, the translation of late mRNAs into structural and nonstructural proteins initiates (Sklenovská, 2020). The proteins that are translated are gathered together with the concatemers of DNA that are processed in the earlier step of replication (Sklenovská, 2020). They are enclosed to form an immature virions (IMVs) that transform to MV which are devoid of external membrane and causes infection when liberated due to the disruption of the cell. (Hiller and Weber, 1985; Bray and Buller, 2004; Roberts and Smith, 2008) They then travel into the inner cell membrane aided by the microtubules and eventually fuses to from the cell-associated virions (CEVs). These trigger the actin polymerization and the development of the filaments. The CEVs exists the cell that are now called the extracellular enveloped virions (EEVs) (Roberts and Smith, 2008) (Figure 4).

## 8 Symptoms and clinical manifestations of MP

The symptoms of MP are similar to those of smallpox but are generally milder (Ligon, 2004). Once exposed to an infection, the incubation period is between 10 and 14 days and followed by a prodromal period of two days (Weaver and Isaacs, 2008). The individuals demonstrate, muscle aches, backache, headache, intense asthenia, fever between 38.5°C and 40.5°C (McCollum and Damon, 2014) with swollen lymph nodes. Often, patients experience discomfort and exhaustion (Ligon, 2004). The feature that separates monkeypox from smallpox is lymphadenopathy (Moore et al., 2022). Mucosal lesions are observed in the mouth (enanthem) (Kaler et al., 2022) after 1 or 2 days. The skin lesions appear on the face and extremities. According to the World Health Organization (WHO) the face is highly affected, as noticed in 95% of the cases. In 75% of the cases, the palms and soles are affected and in 75% of the cases the oral mucous membranes are affected. The affect is also noticed on genitalia and conjunctivae in 30% and 20% of the cases.

The lesions progress through four stages namely, the macular (development of macular lesions), popular (the lesions are a slightly raised), vesicular (the lesions are raised clearly and filled with fluid), and pustula (lesions are filled with opaque fluid and forms a depression at the Centre and remain for about 5 - 7 days) in the next 2 - 4 weeks (Moore et al., 2022). After the pustular phase, the formation of the crust starts and desquamates in the next 7 - 14 day (Moore et al., 2022). Once the crusts are fallen off, the patients are no longer infectious (Moore et al., 2022). Lesions can cause dyspigmented scars in a few instances (Weaver and Isaacs, 2008).

The distribution of the lesion is typically centrifugal and appears firm, deep, well-circumscribed and umbilicated (McCollum and Damon, 2014). Additionally, dissimilarities were observed in the morphology of lesions between vaccinated and unvaccinated people (McCollum and Damon, 2014). In individuals those who were vaccinated <20 years before the infection, the lesions were smaller and fewer, with the centrifugal spreading of the rash (McCollum and Damon, 2014). The skin of patients is firm, inflamed, and painful before the formation of crusts (McCollum and Damon, 2014) (Figure 5).

Sequelae and serious complications have also been noticed usually in unvaccinated individuals of approximately 74% compared to those in the vaccinated individuals (39.5%) (McCollum and Damon, 2014). Patients develop pulmonary distress or bronchopneumonia indicating that an infection of the lungs could be a secondary infection (McCollum and Damon, 2014). In others, vomiting or diarrhoea leading to acute dehydration was noticed (McCollum and Damon, 2014). Encephalitis, ocular infections and septicaemia were also recorded, with an average case-fatality rate of 11% in unvaccinated patients with children being highly vulnerable (McCollum and Damon, 2014).

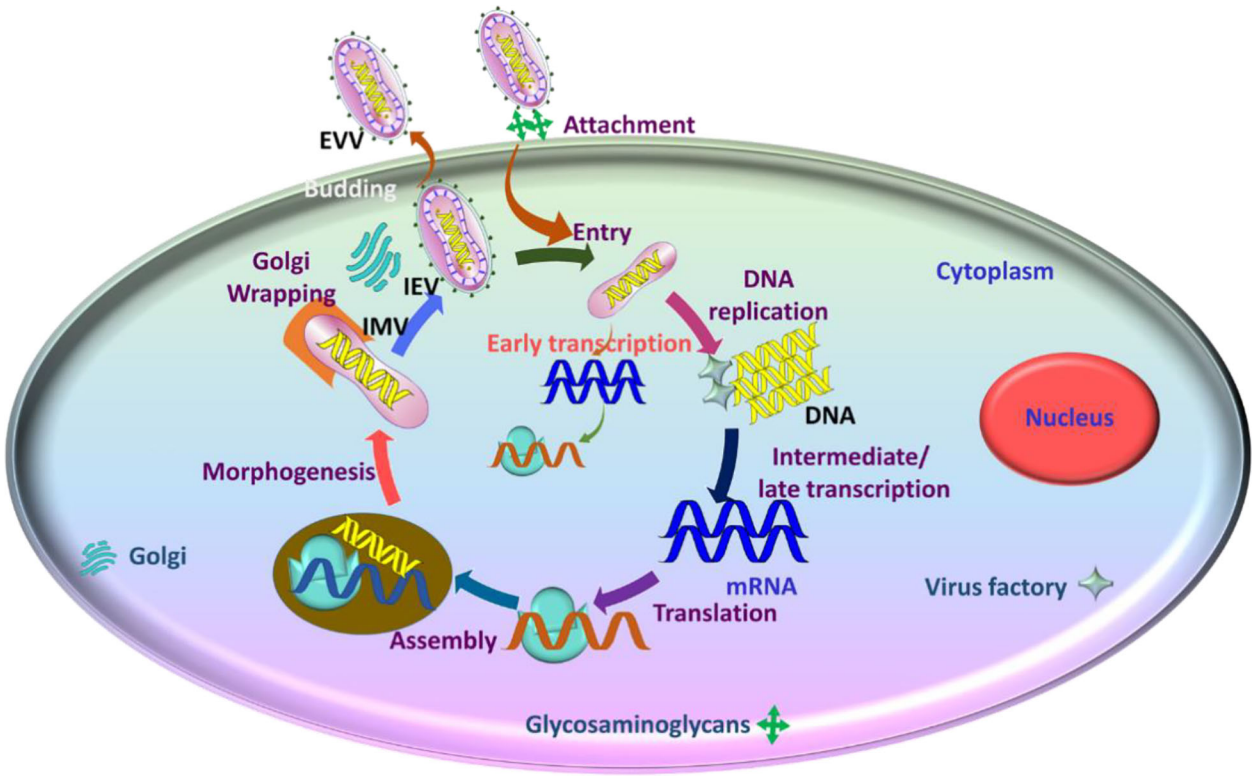


FIGURE 4
The steps involved in the replication of MPV and the generation of new viral progeny.

## 9 Virus-host interaction

It has been reported that the MPV encompasses a substantial number of accessory genes and notably hasa broader host range (Bonilla-Aldana and Rodriguez-Morales, 2022; Xiang and White, 2022). Interestingly, the loss of an accessory gene in approximately 17% of samples from West African strain demonstrated an association with an upsurge in human-to-human transmission (Xiang and White, 2022).

A gene-expression profile study was conducted to understand the viral-host biology (Alkhalil et al., 2010). The experiment was conducted on kidney epithelial cells (MK2) of Macaca mulatta using the GeneChip rhesus macaque genome microarrays (Alkhalil et al., 2010). The findings from this study demonstrate the function of ion channels, cell cycle regulators, histones, and actin play a role in MPV infection (Alkhalil et al., 2010).

A recent bioinformatics study based on transcriptome analysis was conducted to identify biomarkers and signaling pathways in cells infected with monkeypox (Tang et al., 2022). GSE36854 and GSE11234 were retrieved from Gene Expression Omnibus (GEO) (Tang et al., 2022). The results showed that from the GSE36854 dataset, 84 genes were significantly different. The protein-protein interaction (PPI) interactions and the identification of hub genes has revealed that the genes such as ZC3H12A, IER3, EREG, IFIT2, AREG, IL11, IFIT1 IER2, NFKBIE, and FST as the 10 hub genes. (Tang et al., 2022). The genes IFIT1 and IFIT2 (antiviral genes) were noticed to be remarkably repressed. Furthermore, upon searching the drugs that essentially target the hub genes, itraconazole and AP-26113 were found to stimulate the expression levels of IFIT1 and IFIT2 illuminating their ability as MP therapeutics (Tang et al., 2022).

To discover variations in the expression of genes, co-regulated genes, and the pathways that are of concern during MP progression, another research group worked on *in vitro* models that were infected with MPV (Xuan et al., 2022). The two models on which several analysis were performed were, rhesus monkey (*Macaca mulatta*) kidney epithelial (MK2) cells and human immortal epithelial cancer (HeLa) cells (Xuan et al., 2022). The results showed that in the animal cell line model, the prominent regulators were histamine, plasmin and cluster of differentiation 40 (CD40), whereas in the human cell line model, the neutrophil-related signaling pathways and macrophages were noted (Xuan et al., 2022). Additionally, in both models, certain genes that were remarkably expressed during the progression of the infection including, TNFAIP3, IL11, ADORA2A, DUOX1, BIRC3, PTX3, CXCL1, LIF, IER3, IL6, ZC3H12A, EGR1, CSF2, and CCL2. Additionally, epigenetic regulators were also observed, namely, HIST1H3D and HIST1H2BJ (Xuan et al., 2022). Furthermore, a computational study uncovered the over-expression of some histones in both humans and monkeys infected with monkeypox-infected cells (Xuan et al., 2022). The elevated histone members are HIST1H2BB, HIST1H2AK, HIST2H2AB, HIST1H2AC, HIST1H2BM, HIST1H2BJ, HIST1H2BH, HIST1H2AD, HIST1H3D, and HIST1H1B highlighting the possibility of the role epigenetic regulators in monkeypox infection (Xuan et al., 2022).

Another study also has identified the upregulation of histone genes namely, HIST1H2BJ, HIST4H4, HIST1H3I, HIST1H2AD, and, HIST1H1D, while H1F0, the linker histone was downregulated

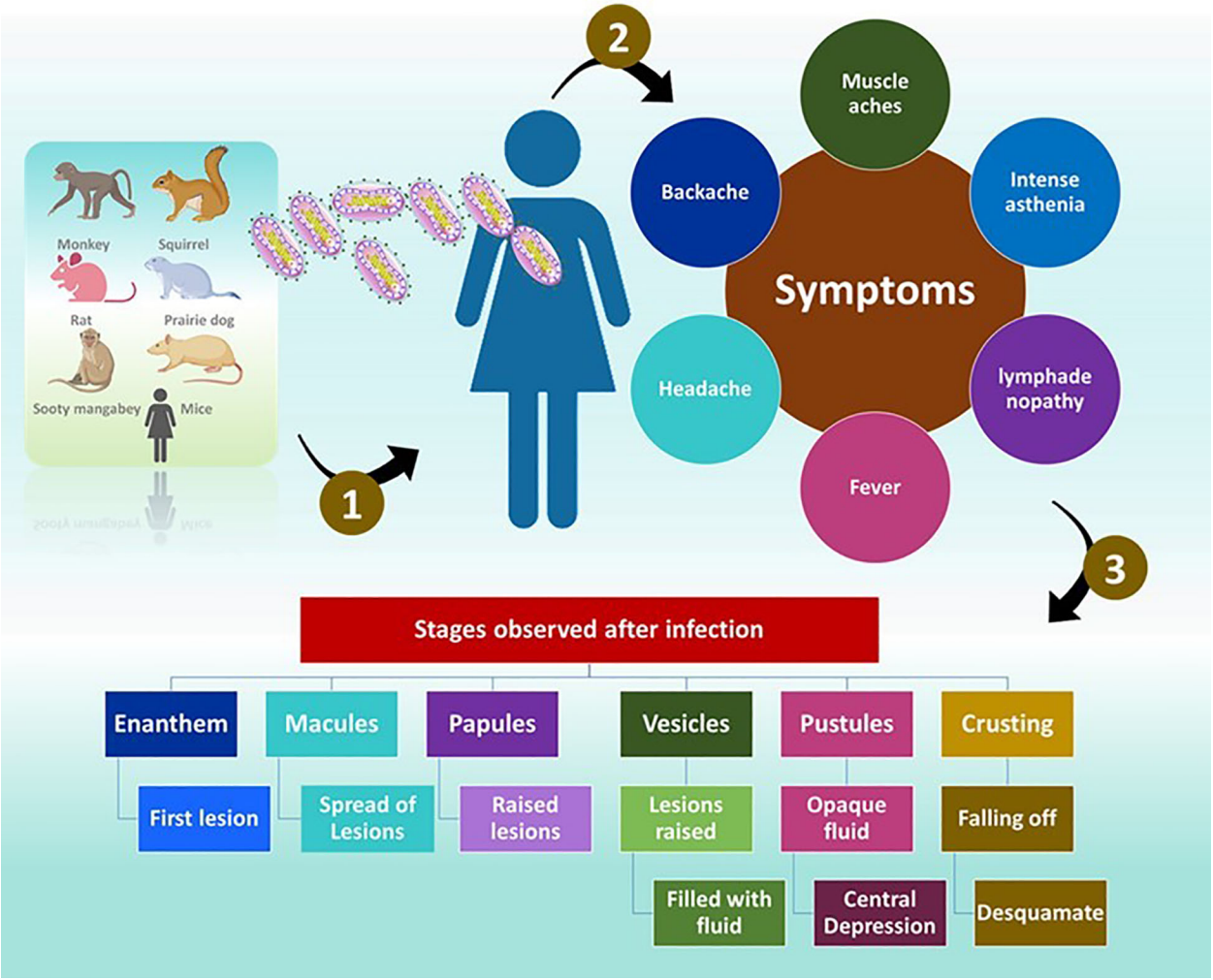


FIGURE 5
Infection with MPV. The symptoms and different stages observed in an infected individual.

(Alkhalil et al., 2010). Certain genes such as MYCBP2, PRMT3, RARS2, FBXO11 and MYST2 were repressed (Alkhalil et al., 2010). During the infection, the repression of ten ion channels and transporters was observed. The cell cycle regulators and actin MPV infection were noticed to participate in MPV infection (Alkhalil et al., 2010). Furthermore, the disease biomarkers can be discovered through proteomics analysis (Wang et al., 2022).

Bourquain et al, conducted an experiment to determine how poxviruses vary their host cell gene expression. HeLa cells were selected, and the changes were recorded using microarrays that are representative of the whole human genome (Bourquain et al., 2013). HeLa cells were treated with CPXV Brighton Red (BR) reference strain, central African MPV strain MSF-6, or the mouse-pathogenic VACV strain IHD-W (Bourquain et al., 2013). In particular to the MPV results, merely 321 (1.1%) transcripts showed 2-fold expression variations with 219 (68.2%) upregulated transcripts and 102 downregulated transcripts (Bourquain et al., 2013). Interestingly, the results also showed common transcripts among infections (Bourquain et al., 2013). The MPV has regulated 321 host transcripts of which 241 (75.1%) were observed with cowpox virus (CPXV) and 148 (46.1%) within VACV infection (Bourquain et al., 2013). Genes such as DUSP5/6, SPRED1/2, and SPRY2/4 are upregulated along with EGR1 and EGR2. After infection, the EGR1 upregulation was observed *via* the MAPK-ERK pathway (Bourquain et al., 2013).

In MPV infection, the genes that take part in the negative regulation of MAPK activity and intracellular protein kinase cascade were enriched, and they induced genes associated with chemotaxis or leukocyte activation (Bourquain et al., 2013). Hammarlund et al, reported that monocytes infected with MPV do not recognize antiviral $CD4^+$ and $CD8^+$ T Cells (Hammarlund et al., 2008). MPV can cause infection in primary human monocytes without eliciting the production of inflammatory cytokines (IFNγ or TNFα) (Hammarlund et al., 2008). It was also reported that MPV impedes the activation of T- cell by VV, implying that MPV bears an immunomodulatory protein which is absent in VV (Hammarlund et al., 2008). This inhibition *in trans* can also obstruct the activation of T - cells by other virus-infected cells that are not precisely MPV-infected, suggesting that MPV is immunosuppressive and immune-evasive (Hammarlund et al., 2008). Cell-associated factor/factors

generated in MPV hinder the activation of T - cells autonomously by MHC class I or class II processing or presentation (Hammarlund et al., 2008).

# 10 Treatments against MPV

## 10.1 Small molecule inhibitors

The replication of MPV can be brought about by the RNA interference using genome-wide expression studies combined with a bioinformatics approach (Alkhalil et al., 2009). Accordingly 12 viral genes were targeted using small interfering RNA (siRNAs). Eventually, siA6-a inhibited replication of the virus for 7 days when administered at 10 nM (Alkhalil et al., 2009). Additionally, this study also demonstrated the significance of the A6R gene in replication (Alkhalil et al., 2009). The use of interferon-β (IFN-β) is another approach to curb MPV spread and multiplication (Johnston et al., 2012). IFN-β has been approved by the FDA for treating multiple sclerosis (Johnston et al., 2012). IFN-β administration after 6-8 hours after infection remarkably inhibited the production and spread of MPV, thereby highlighting the potential of IFN-β as an effective therapeutic option against MPV (Johnston et al., 2012). In previous studies, silver-based nanoparticles have been found to repress plaque formation of MPV with a diameter of 10 nm (Rogers et al., 2008), suggesting the role of silver-based nanoparticles as MPV treatment option (Rogers et al., 2008).

Tecovirimat may offer improved survival when administered for up to 8 days after the lethal aerosol MPV challenge in cynomolgus macaques (Russo et al., 2018). It offers a shield from the clinical effects of the disease earlier than 5 days after the challenge (Russo et al., 2018). This FDA approved drug is used to treat smallpox (Smith et al., 2011; Grosenbach et al., 2018; Laudisoit et al., 2018; Russo et al., 2018). The compound tecovirimat is believed to repress the product of F13L gene which is present across orthopoxviruses (Matias et al., 2022) and is used for orthopoxvirus wrapping (Duraffour et al., 2008; Duraffour et al., 2015). The drug brincidofovir, a nucleotide analogue, has demonstrated promising results in animal models when assessed against MPV (Adler et al., 2022). Improved survival was observed in prairie dog models, administered with brincidofovir soon after monkeypox contact (Hutson et al., 2021; Siegrist and Sassine, 2022).

Computational methods are paramount in the development and design of new drugs. The predominant methods include molecular docking (Morris and Lim-Wilby, 2008; Rampogu and Lee, 2021c; Rampogu and Lee, 2021a), and molecular dynamics simulation (Durrant and McCammon, 2011; Rampogu and Lee, 2021a). Pharmacophore modeling can also be adapted when there are any experimentally known ligands/inhibitors or when the X-ray structure of the target protein is present (Yang, 2010; Rampogu et al., 2020). Homology modeling can be used to build a structure when the X-ray structure is not resolved (Muhammed and Aki-Yalcin, 2019). In one study, five targets were investigated, and compounds, such as NMCT, rutaecarpine, nilotinib, simeprevir, hypericin, naldemedine, fosdagrocorat and lixivaptan (Lam et al., 2022), were found to have potential inhibitory activities. These five targets were A48R, A50R, D13L, F13L and I7L (Lam et al., 2022). An *in silico* study revealed that the compound fludarabine is a potential inhibitor of the MPV target DNA-dependent RNA polymerase subunit (A6R) along with two other targets, protein catalysing the envelopment of intracellular mature virus particles (F13L) and proteins involved in cell entry (D8L) (Altayb, 2022). Sahoo et al., identified four potential inhibitors, Tipranavir, Cefiderocol, Doxorubicin, and Dolutegravir towards the targets thymidylate kinase and D9 (decapping enzyme) (Sahoo et al., 2022) (Table 1).

A study was conducted to evaluate the role of specific genes in viral replication and pathogenicity (Lopera et al., 2015). Correspondingly, a bioinformatics approach has been adapted to discover genomic regions in MPV possessing numerous virulence genes (Lopera et al., 2015). Following this, two regions were selected, and the study was conducted *in vitro* and *in vivo* after single deletion

TABLE 1 Current treatments/small molecules available for MPV therapeutics.

| Inhibitor | Method | Acts on | Dosage | Reference |
|---|---|---|---|---|
| siA6-a | genome-wide expression, bioinformatics | A6R | 10 nM | (Alkhalil et al., 2009) |
| IFN-β | *in vitro* | MPV spread and multiplication | – | (Johnston et al., 2012) |
| NMCT, Rutaecarpine, Nilotinib, Simeprevir, Hypericin, Naldemedine, Fosdagrocorat and Lixivaptan | Computational | A48R, A50R, D13L, F13L, I7L | – | (Lam et al., 2022) |
| Fludarabine | Computational | A6R | – | (Altayb, 2022) |
| Tipranavir, Cefiderocol, Doxorubicin, and Dolutegravir | Computational | Thymidylate kinase and D9 | – | (Sahoo et al., 2022) |
| Tecovirimat | FDA-approved drug for smallpox | F13L | – | (Matias et al., 2022) |
| Brincidofovir | FDA-approved drug for smallpox | The effect shown in prairie dogs | | (Adler et al., 2022) |
| Vaccinia immune globulin (VIG) | FDA-approved | intramuscular | – | (Siegrist et al., 2020) |
| CRISPR/Cas9 | vaccinia virus (VACV) model | A17L, E3L, and I2L | – | (Siegrist et al., 2020) |

and double deletion of the selected genes. The results demonstrated that simultaneous deletion of both genes led to a decrease in replication observed in cell culture (Lopera et al., 2015). When either region was deleted, a remarkable amplified attenuation *in vivo* was observed (Lopera et al., 2015).

### 10.2 Vaccination

In addition to small molecules, vaccines are also widely used as a treatment option (Hooper et al., 2004). Notably, the smallpox vaccine has demonstrated protection in Nonhuman Primates against MPV (Hooper et al., 2004). It is reported that smallpox has provided approximately 85% protection against monkeypox (Bunge et al., 2022). JYNNEOS is a modified vaccinia ankara (MVA) vaccine that has been approved for MPV disease in adults ≥18 years of age (Rao et al., 2022). This was a subcutaneous injection administered at two doses with 28 days apart (Rao et al., 2022). In 2017, healthcare individuals were administered with IMVAMUNE® which is a smallpox vaccine (Petersen et al., 2019). This study aimed to understand the immunogenicity, effectiveness, and safety of IMVAMUNE® in patients at high risk for MPV (Petersen et al., 2019). Another smallpox vaccine, the live VACV Dryvax protects against monkeypox (Heraud et al., 2006; Earl et al., 2008). A multivalent DNA vaccine with eight VACVs virus (Western Reserve strain genes: A4L, A27L, A33R, A56R, B5R, F9L, H3L, and L1R) confers protection against MPV in nonhuman primates *cynomolgus macaques* (Hirao et al., 2011).

It has been observed that, patients vaccinated for smallpox showed immunity (orthopoxvirus [OPXV], IgG and memory B cells) upon exposure to MPV (Nguyen et al., 2021). Interestingly, the smallpox vaccine triggers both humoral and cell-mediated responses towards OPXV which also includes MPV (Nguyen et al., 2021). They target a host of viral elements, thereby hindering viral replication (Nguyen et al., 2021). Vaccinia immune globulin is an approved medication used after contacting the MPV, which is administered intramuscularly (Lederman et al., 2012; Siegrist et al., 2020; Parker et al., 2021). In one study, CRISPR/Cas9 was used to treat orthopoxviruses with VACV, which was used as a model organism (Siegrist et al., 2020). This study focused on the indispensable conserved genes A17L, E3L, and I2L using an adeno-associated virus as a vector (Siegrist et al., 2020). The results have shown a reduction in the viral titre further protecting host cells (Siegrist et al., 2020). An assay based on CRISPR-Cas12a and real-time PCR was used to detect MPV (Li et al., 2006; Sui et al., 2022).

In order to find effective therapeutics, few clinical trials have been conducted. Upon searching for clinical trial information, it was found that nine studies are currently being performed that are either on-going or completed (https://clinicaltrials.gov/)

## 11 Conclusion and future outlook

Viruses are notorious microorganisms that cause serious human infections. Pox viruses are not new as they exist in reptiles, birds, insects, and mammals. Hence, they are also known as ancient viruses (Alakunle et al., 2020). Human MP is a zoonotic disease that is recently spreading globally. In order to prevent the spread of the disease, the contact tracing is an important step (Titanji et al., 2022). If a person is in close proximity with a confirmed case of monkeypox, then that individual should be observed for development of symptoms for 21 days (Titanji et al., 2022).

Since the treatment options for monkeypox are limited (Kaler et al., 2022), the available research methods, such as computational drug discovery, could be an effective method to identify drugs (Ou-Yang et al., 2012; Sliwoski et al., 2014). This approach is useful for discovering new drugs over a short period (Leelananda and Lindert, 2016). Drug repurposing is another approach that can be used to identify mmediate candidate compounds (Pushpakom et al., 2018; Begley et al., 2021). This approach has been proven to be promising for the treatment of SARS-CoV-2. Remdesivir is one such candidate (Li and Peng, 2021). To prevent the spread of virus in the developing countries the awareness on health hygiene is essentially important as the samples from the excreta are reported to have monkeypox virus DNA (Singla and Shen, 2022). Additionally, the knowledge and treat from the pandemics and similar kind of diseases should be widely spread among all the sections of the people (Poland et al., 2022). Countries need to review their approach and preventive measure are to be taken during the outbreaks and be prepared for any such kind in the future (Poland et al., 2022). To conclude, since the spread of MPV is swift, it demands extra awareness and thoughtfulness to control the transmission and thereby to mitigate the disease and further its future reappearance.

## Author contributions

SR and KW conceived the idea. SR wrote the manuscript. KW, S-WK, and YK reviewed the manuscript and revised. All authors contributed to the article and approved the submitted version.

## Funding

This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (MSIT) (No. 2021R1A5A8029490).

## Acknowledgments

The icons are taken from BioRender.com and the figures are created accordingly.

## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

## Publisher's note



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frontiers | Frontiers in Public Health

TYPE Systematic Review
PUBLISHED 09 March 2023
DOI 10.3389/fpubh.2023.1144325



OPEN ACCESS

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SPECIALTY SECTION
This article was submitted to Infectious Diseases: Epidemiology and Prevention, a section of the journal Frontiers in Public Health

RECEIVED 14 January 2023
ACCEPTED 22 February 2023
PUBLISHED 09 March 2023

CITATION
Alorfi NM, Alshehri FS and Haseeb A (2023) Characterization of interventional clinical trials for monkeypox; systematic review of ClinicalTrials.gov database. *Front. Public Health* 11:1144325. doi: 10.3389/fpubh.2023.1144325



# Characterization of interventional clinical trials for monkeypox; systematic review of ClinicalTrials.gov database

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**Background:** Monkeypox (mpox), a zoonotic viral infection, poses a global threat that is being acknowledged at the national and international levels. This systematic review aims to identify and characterize interventional clinical trials for mpox.

**Method:** All interventional clinical trials registered at ClinicalTrials.gov for mpox were searched up to January 6, 2023. We described the characteristics of interventional clinical trials, and drug interventions (including drugs and vaccines).

**Results:** As of January 6, 2023, there were 10 clinical trials in the ClinicalTrials.gov registry that met our criteria. Most of the interventional clinical trials were focused on the treatment ($N$ = 4, 40%) and prevention ($N$ = 4, 40%) of mpox. From the 10 trials, 50% used random treatment allocation, and six (60%) chose the parallel assignment intervention model. All 10 studies were blinded, and six were open-label blinded. The largest proportion of the clinical trials ($N$ = 4, 40%) were registered in Europe, followed by America ($N$ = 3, 30%) and Africa and others ($N$ = 3, 30%). The JYNNEOS vaccine (40%), followed by Tecovirimat (30%) were the most frequently studied drugs used against mpox.

**Conclusion:** A limited number of clinical trials have been registered on ClinicalTrials.gov since the first case of mpox was reported. Therefore, there is an urgent need to conduct large-scale randomized clinical trials to assess the safety and efficacy of the drugs and vaccines being used against the mpox virus.

KEYWORDS

**monkeypox, viral, clinical trials, vaccination, immunity**

## Introduction

In addition to the global scourge of Coronavirus disease 2019 (COVID-19), the monkeypox virus (MPXV) has raised health authorities' concerns (1). Monkeypox (mpox) is a zoonotic viral infection caused by MPXV, a double-stranded DNA virus of the genus orthopox. The smallpox virus, camelpox virus (CMLV), cowpox virus (CPXV), and vaccinia also belong to this genus (2). As early as 1958, mpox was confirmed to contain MPXV, but it was 1970 in Congo until the first case in humans was identified (3, 4). Mpox shares many of the clinical features of smallpox, typically headache, tiredness, rashes, fever and lesions. These lesions evolve sequentially from macules to papules, vesicles and pastules and then crusts, which later on dry up and fall off (5).

The World Health Organization (WHO) has received reports of mpox cases from all around the world. According to preliminary data from the WHO, a total of 84,330 laboratory-confirmed cases and 1,343 suspected cases of mpox had been reported from over

100 countries by January 6, 2023 (6). According to geographic distribution, the regions of America, Africa, Europe, South East Asia, and the Eastern Mediterranean account for most of the confirmed cases of mpox (7, 8). The WHO has reported 74 deaths, emphasizing the importance of conducting further public health investigations in countries where mpox is not endemic. This includes identifying cases, careful contact tracing, maintaining effective surveillance, conducting laboratory tests, and managing clinical care (9).

Global health concerns have been raised by current mpox and COVID-19 outbreaks (10). The WHO authorities have issued interim guidelines for public health officials and healthcare professionals regarding the use of mpox vaccines for the prevention of viral infection (11). Previous foreign studies reported that children are particularly susceptible, as those aged 15 years and under account for 90% of mpox cases (12). Insufficient laboratory diagnostics, vaccines, and antivirals may hinder the effective clinical management of patients with confirmed mpox cases (13). Most researchers are conducting clinical trials to find effective treatments for mpox, evaluate the safety and efficacy of existing antivirals, reduce mortality and morbidity rates, and assess the pharmacokinetic parameters of certain drugs.

ClinicalTrials.gov is the biggest clinical trial database currently available. A previous publication described the organization's process for registration and its use for analyzing clinical trials (14). In this review, we focused on the ongoing clinical trials for mpox registered on ClinicalTrials.gov in order to identify and characterize interventional clinical trials for mpox. We believe this to be important because the incidence rate of mpox is expected to escalate and we need to take necessary precautions, including effective surveillance, isolation, and contact tracing.

# Methods

## Clinical trials search

On January 6, 2023, relevant studies were searched for on ClinicalTrials.gov using the single search term "Monkeypox." The dataset for the monkeypox clinical trials was limited to only include interventional studies that were registered within the larger data system, up to that date. Any registered clinical trials that had been terminated, withdrawn or suspended were also excluded from the final analysis.

## Clinical trials collection

Two of the reviewers independently collected data from the downloaded registration information files, after which the data were reviewed by a third reviewer. The data collected included the aim of the study, the type of study, the study design, the inclusion criteria, sponsors, estimated enrolment, where the trial was conducted, and other protocol information.

We conducted the analysis, describing the results of interventional clinical trials, and trials on drug interventions, including vaccines and antivirals. Drugs administered, imaging technologies and surgical procedures were categorized as either standard treatment or experimental intervention based on their role in the trial. If a procedure was specifically targeted for a particular trial, it was categorized as an experimental intervention.

We grouped the study locations into continents based on the website allocation. Also, we categorized the funding sources under "sponsors" in the ClinicalTrials.gov database into government funding agency, medical institute or research institute. If a study was not sponsored by any of the aforementioned funding bodies, it was categorized as "others".

# Results

## Number of studies

As of January 6, 2023, a total of 22 registered clinical trials related to mpox were identified in the ClinicalTrials.gov database. Two studies were excluded because they did not provide any information about mpox, and 10 studies were excluded because they were observational studies. The remaining 10 studies were then available to be analyzed (Figure 1).

## Characteristics of interventional trials

Table 1 depicts the characteristics of the 10 interventional trials. Five (50%) had not started recruiting and four (40%) were still recruiting. Most of the interventional clinical trials were focused on the treatment ($N = 4$, 40%) and prevention ($N = 4$, 40%) of mpox. Treatment allocation was randomized in five clinical trials (50%) and six clinical trials (60%) chose the parallel assignment intervention model. All of the studies were blinded, with six employing open-label blinding. Half had 250 or fewer participants. The majority of the clinical trials were performed with adults aged above 18 years old. Government agencies and medical institutes sponsored 40 and 30% of the trials, respectively. Of these, two projects were funded by a Federal US agency and one project was funded by CDC. Figure 2 illustrates that most of the clinical trials ($N = 8$, 80%) were conducted in 2022. The most common countries for the clinical trials were Europe ($N = 4$, 40%), followed by America ($N = 3$, 30.00%) and Africa ($N = 2$, 20%) as shown in Figure 3.

## Analysis of drugs studied in clinical trials

As shown in Table 2, tecovirimat was used for the treatment of patients with confirmed mpox while the vaccine Imvanex (also known as JYNNEOS and Imvamune) was used for the prevention of mpox.

### Tecovirimat

Patients were treated with tecovirimat (also named ST-246) or a placebo for 14 days, each being administered in a hospital with standard-of-care (SOC) treatment. Afterwards, the patients were examined weekly for 28 days to further evaluate their mpox infection, and to carry out a safety assessment. Participants were

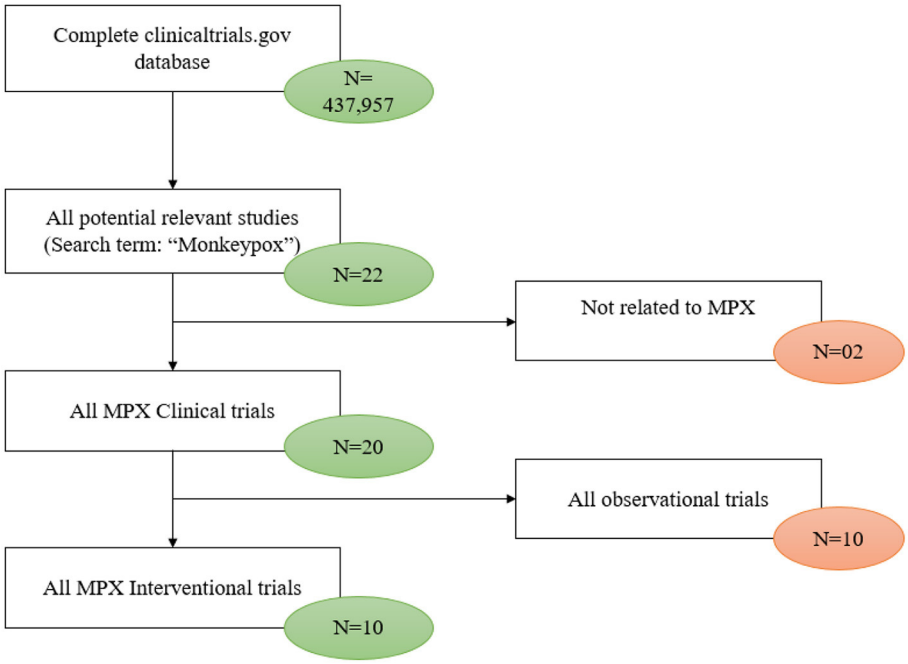


FIGURE 1
Flow chart of clinical trial selection process.

then followed-up with an optional visit between days 57 and 59 for possible recrudescence of the infection.

In a double-blind, randomized, cross-over study of the pharmacokinetics of an oral dose of anti-orthopoxvirus compound, a single dose of ST-246 was administered in healthy patients with mpox. The primary outcome measures were the assessment of the pharmacokinetic parameters of a single dose of ST-246. Six patients received ST-246 Form V (hemihydrate) followed 10 days later after a wash-out period by Form I (monohydrate), and the remaining received ST-246 Form I (monohydrate) followed by Form V (hemihydrate).

### JYNNEOS/Imvamune/Imvanex

Healthy volunteers aged 18 years or older participated in the open-label clinical trial. Participants receive the vaccine Imvamune on days 0 and 28. Blood samples were collected on days 0, 14, 28, 42, 280, 365, 545, and 730 for immunogenicity analysis. The participants were observed for at least 30 min to assess if any adverse events occurred. The recorded exposure to mpox was also maintained at each follow-up visit.

## Discussion

This systematic review provides an initial overview of the mpox clinical trials that have been registered on ClinicalTrials.gov, with a specific emphasis on treating and managing mpox. Multiple noteworthy observations emerged from this review of interventional clinical trials. We found a limited number of mpox clinical trials. Most of the interventional trials enrolled <250 participants.

Small-scale studies are prone to type II errors, otherwise known as a false negative, which occur when the null hypothesis is not rejected. This leads to the inaccurate conclusion that an intervention or treatment is ineffective, because the sample is of an inadequate size, meaning that it is difficult to detect a significant effect (15). Although many interventional clinical trials share favorable features in their design, such as similar rates of randomized and non-randomized trials with two treatment arms across different time periods, some clinical trials also exhibit unfavorable study design characteristics, such as those with active comparator data monitoring committees (as observed in clinical trials NCT05597735 and NCT05534165). These findings underscore the need for better-designed trials and monitoring practices.

Mpox management varies from patient to patient and should involve decision making centered on patients, and multidisciplinary discussions that aim to augment disease control, reduce and improving quality of life among patients (16). Current treatment options for patients with mpox infection include vaccines such as JYNNEOS and administration of an antiviral drug, i.e., tecovirimat (17). Tecovirimat is a FDA-approved drug that is used for the treatment of smallpox. Prior to the onset of mpox, a randomized clinical trial (RCT) was being planned by the National Institute of Health (NIH) in Congo with the aim of evaluating the effectiveness and safety of tecovirimat (18). Based on a study with healthy volunteers, tecovirimat appears to have a favorable clinical profile (19). A double-blind, randomized, cross-over clinical trial for the assessment of the anti-orthopoxvirus compound, ST-246, is in process to assess the pharmacokinetic parameters of an oral

10.3389/fpubh.2023.1144325

TABLE 1 Characteristics of interventional clinical trials registered on ClinicalTrials.gov.

| | Number (*N*) | Percentage (%) |
|---|---|---|
| **Interventional model** | | |
| Single group assignment | 4 | 40.00 |
| Parallel assignment | 6 | 60.00 |
| **Recruitment status** | | |
| Recruiting | 4 | 40.00 |
| Active, not recruiting | 1 | 10.00 |
| Not yet recruiting | 5 | 50.00 |
| **Study purpose** | | |
| Prevention | 4 | 40.00 |
| Treatment | 4 | 40.00 |
| Supportive care | 1 | 10.00 |
| Others | 1 | 10.00 |
| **Sample size** | | |
| 0–250 | 5 | 50.00 |
| 251–500 | 2 | 20.00 |
| >500 | 2 | 20.00 |
| Not available | 1 | 10.00 |
| **Trial phase** | | |
| Phase 2 | 2 | 20.00 |
| Phase 3 | 4 | 40.00 |
| Not available | 4 | 40.00 |
| **Treatment allocation** | | |
| Randomized | 5 | 50.00 |
| Non-randomized | 1 | 10.00 |
| NR | 4 | 40.00 |
| **Masking (blinding)** | | |
| Double | 2 | 20.00 |
| Open | 6 | 60.00 |
| Others | 2 | 20.00 |
| **Funding source** | | |
| Government | 4 | 40.00 |
| Medical institution | 3 | 40.00 |
| Others | 3 | 30.00 |

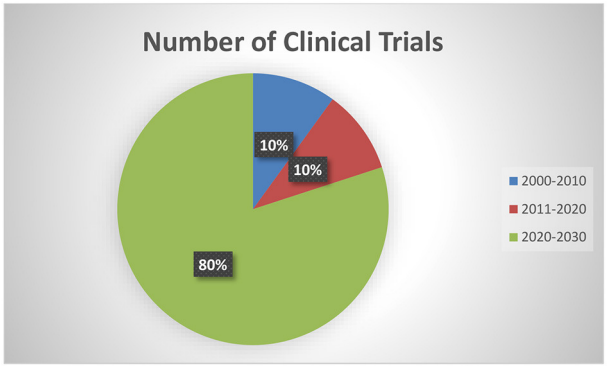


FIGURE 2
Number of clinical trials conducted in years.

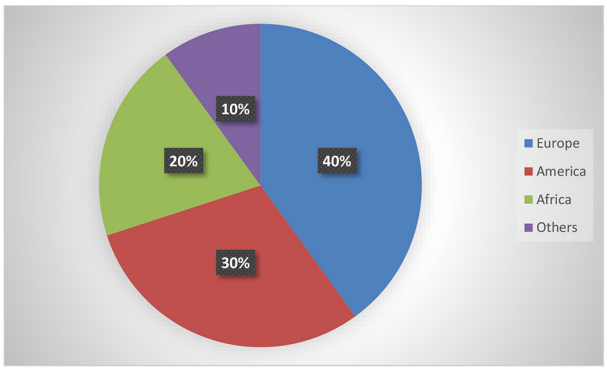


FIGURE 3
Geographical distribution of clinical trials.

TABLE 2 Most studied drugs in clinical trials.

| Drugs/vaccines | Number of trials |
|---|---|
| Tecovirimat/ST-246 | 4 |
| Imvanex/JYNNEOS/Imvamune | 3 |
| Others | 3 |

dose of that drug in patients with mpox. Pharmacometrics utilized pharmacokinetic and pharmacodynamic data to establish models that describe drug efficacy factors such as the progression of disease, compliance with treatment, and viral growth. These models provide guidance for the design of trials, comparisons of effectiveness, changes in drug dosage, and decision making for patient care in particular populations (20).

Our analysis shows that the four clinical trials focused on the prevention and management of mpox. Previous studies have reported that smallpox vaccines could provide some protection against mpox and lessen its clinical manifestations (5, 21, 22). Currently, JYNNEOS (also named as Imvanex, MVA-BN, or Imvamune) is approved for smallpox and it is being studies as a potential drug that can prevent infections with MPXV (23). JYNNEOS is a viral vaccine made from the MVA-BN strain, which is a modified vaccinia Ankara-Bavarian Nordic strain of orthopoxvirus that has been weakened and is no longer able to reproduce itself (24). This vaccine was licensed by US-FDA on September 24, 2019 and is currently recommended as a vaccine for mpox and small pox, for adults (>18 years old) who are classified as being at high risk of exposure to these viral diseases (25). In most of the interventional clinical trials, the Immavune vaccine was given to healthy volunteers on days 0 and 28 for the prevention of MPXV.

The current mpox outbreak requires a multi-faceted approach that includes more than just treatment (26). Public awareness, robust testing, containment measures and vaccination of high-risk

groups all have significant roles to play in minimizing the spread of MPXV (27, 28). Nonetheless, significant challenges persist. While vaccines are believed to be safe and effective for individuals with smallpox infection, there is a shortage of data on their effectiveness in terms of managing mpox. The re-emergence of this zoonotic viral disease raises concerns, and additional research is merited into measures and treatments that could be employed to help prevent and combat the disease, which is now present in multiple nations through possible new routes of transmission. To the best of our understanding, key interventions to prevent mpox outbreaks include early identification, barrier nursing, raising awareness, and strict infection prevention control practices that include isolating individuals diagnosed with mpox. This requires efforts by public health officials and healthcare professionals. Also, epidemiological studies should focus on how the virus can be transmitted from animals to people and identify possible sources of infections, such as people who work closely with animals (29, 30). Furthermore, more clinical trials should be carried out to ensure that patients receive optimal care.

## Conclusion

The periodic occurrence and frequency of mpox outbreaks bring to light the need for outbreak awareness, research and preparedness. There is only a small number of interventional clinical trials registered on ClinicalTrials.gov that are focusing on mpox vaccines (JYNNEOS) and antiviral drugs (tecovirimat), and assessing how effective and safe they are. Therefore, we call for more large-scale randomized clinical trials to be conducted in order to assess mpox drugs and vaccines, particularly in Africa.

## Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

## Author contributions

All authors wrote sections of the manuscript, contributed to manuscript revision, read, and approved the submitted version.

## Funding

This project was funded by the Deputyship for Research and Innovation and Ministry of Education in Saudi Arabia through project number: IFP22UQU4310453DSR244.

## Acknowledgments

The authors extend their appreciation to the Deanship for Research and Innovation and Ministry of Education in Saudi Arabia for funding this research work through project number: IFP22UQU4310453DSR244.

## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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frontiers | Frontiers in Cellular and Infection Microbiology

TYPE Original Research
PUBLISHED 22 March 2023
DOI 10.3389/fcimb.2023.1157627



OPEN ACCESS

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SPECIALTY SECTION
This article was submitted to Virus and Host, a section of the journal Frontiers in Cellular and Infection Microbiology

RECEIVED 02 February 2023
ACCEPTED 02 March 2023
PUBLISHED 22 March 2023

CITATION
Akash S, Hossain A, Hossain MS, Rahman MM, Ahmed MZ, Ali N, Valis M, Kuca K and Sharma R (2023) Anti-viral drug discovery against monkeypox and smallpox infection by natural curcumin derivatives: A Computational drug design approach. *Front. Cell. Infect. Microbiol.* 13:1157627. doi: 10.3389/fcimb.2023.1157627



# Anti-viral drug discovery against monkeypox and smallpox infection by natural curcumin derivatives: A Computational drug design approach

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**Background:** In the last couple of years, viral infections have been leading the globe, considered one of the most widespread and extremely damaging health problems and one of the leading causes of mortality in the modern period. Although several viral infections are discovered, such as SARS CoV-2, Langya Henipavirus, there have only been a limited number of discoveries of possible antiviral drug, and vaccine that have even received authorization for the protection of human health. Recently, another virial infection is infecting worldwide (Monkeypox, and Smallpox), which concerns pharmacists, biochemists, doctors, and healthcare providers about another epidemic. Also, currently no specific treatment is available against Monkeypox. This research gap encouraged us to develop a new molecule to fight against monkeypox and smallpox disease. So, firstly, fifty different curcumin derivatives were collected from natural sources, which are available in the PubChem database, to determine antiviral capabilities against Monkeypox and Smallpox.

**Material and method:** Preliminarily, the molecular docking experiment of fifty different curcumin derivatives were conducted, and the majority of the substances produced the expected binding affinities. Then, twelve curcumin derivatives were picked up for further analysis based on the maximum docking score. After that, the density functional theory (DFT) was used to determine chemical characterizations such as the highest occupied molecular orbital (HOMO), lowest unoccupied molecular orbital (LUMO), softness, and hardness, etc.

**Results:** The mentioned derivatives demonstrated docking scores greater than -6.80 kcal/mol, and the most significant binding affinity was at -8.90 kcal/mol, even though 12 molecules had higher binding scores (-8.00 kcal/mol to

-8.9 kcal/mol), and better than the standard medications. The molecular dynamic simulation is described by root mean square deviation (RMSD) and root-mean-square fluctuation (RMSF), demonstrating that all the compounds might be stable in the physiological system.

**Conclusion:** In conclusion, each derivative of curcumin has outstanding absorption, distribution, metabolism, excretion, and toxicity (ADMET) characteristics. Hence, we recommended the aforementioned curcumin derivatives as potential antiviral agents for the treatment of Monkeypox and Smallpox virus, and more in vivo investigations are warranted to substantiate our findings.

KEYWORDS

**curcumin, monkeypox, smallpox virus, molecular docking, DFT, admet, molecular dynamic simulation**

## 1 Introduction

Viruses have been considered obligatory microscopic biological infections that are incredibly tiny and pathogenic (Greenwood et al., 2012). They are very complex nonliving substances or basic biological microorganisms that may act as live-in host cells and act as a particle outside the host cell (Villarreal, 2004). They are obligatory intracellular parasitic since they do not possess the metabolic enzymes or the infrastructure necessary to produce proteins (Vlachakis et al., 2013). The viral genome is formed of a single form of nucleic acid, either deoxyribonucleic acid (DNA) or ribonucleic acid (RNA), as well as a capsid (Lucas and Knipe, 2010). The protein coat is occasionally surrounded by an enclosure that is made up of lipids, proteins, and carbohydrates (Parvez, 2020). Viruses can infect every living thing, such as animals, plants, bacteria, and archaea6, and can only multiply and execute through their physiological activities in a host cell.

In the last couple of years, viral disease has developed very rapidly and infected millions of people around the globe, such as SARS CoV-2 (Khattak et al., 2022; Miller et al., 2023), Monkeypox virus (Mahase, 2022), Ebola virus (Malvy et al., 2019), HIV (Zhou and Saksena, 2013), Smallpox (Smith and McFadden, 2002), Hantavirus (Vaheri et al., 2013), Influenza (Dunn and Miller, 2014), Langya Henipavirus (Akash et al., 2022b), and Dengue (Pawitan, 2011). This viral infection is sometimes converted into global pandemics such as the SARS-CoV-2 pandemic and the Ebola pandemic (Islam et al., 2022), which directly impact world economics and the health sector (Kumer et al., 2022b). Several studies and findings have reported that millions of people die annually due to viral infection.

The *Orthopoxvirus* genus of the *Poxviridae* family includes the enveloped double-stranded DNA virus known as the monkeypox virus. Monkeypox is a viral zoonosis that is clinically less severe than Smallpox. It has symptoms comparable to those of Smallpox (Di Giulio and Eckburg, 2004; Rizk et al., 2022). The Monkeypox virus is susceptible to several animal species. A 9-month-old boy in the Democratic Republic of the Congo, where Smallpox had been eradicated in 1968, was the first person to be diagnosed with human Monkeypox in 1970 (Breman et al., 1980). Contacting infected animals' blood, body fluids, skin, or mucosal lesions can result in animal-to-human (zoonotic) transmission. Since it affects the rest of the world, in addition to nations in west and central Africa, Monkeypox is a disease of worldwide public health concern now (Di Giulio and Eckburg, 2004; McCollum and Damon, 2014).

The World Health Organization declared Monkeypox an "evolving threat of moderate public health concern" on June 23, 2022, after more than 3000 Monkeypox virus infections were detected in more than 50 nations (Thornhill et al., 2022). There isn't a specific medication for the Monkeypox virus infection right now. However, a number of antiviral drugs used to treat Smallpox and other ailments could be beneficial for those with Monkeypox infection. According to the Centers for Disease Control and Prevention (CDC), supportive care is often sufficient for people with a Monkeypox virus infection because no particular medicines are available (Rizk et al., 2022). The computational technique is used to develop a safe drug against Monkeypox and Smallpox virus infection. Because developing any medication takes more than 10-12 years, huge cost, time, and resources, but this new computational technology creates a new era in which effective drugs and new biological substances can be developed within a concise period of time, reducing both time and costs (Rahman et al., 2012; Tropsha and Bajorath, 2016; Stanzione et al., 2021). For more than three decades, the discovery of clinically significant compounds has been greatly helped by computer-aided drug discovery and design techniques (Marhöfer et al., 2011; Hung and Chen, 2014). Although, computer-aided drug discovery and design has a lot of advantages and reduce the time, cost and resources to develop a potential drug, but it has some its own limitations.

One of the most complicated issues has to be solved in drug development is considered about target flexibility. The use of a toxicity prediction model is helpful in determining whether or not a medication candidate is harmful to organs such as the liver, kidneys,

heart, and lungs. Besides, the accuracy of prediction models is hindered by a lack of trustworthy experimental data and factors relevant to ADME and toxicity. It is also noteworthy to know that only forty percent of medicine candidates are being tested in clinical trials by the forecast computer model (Singh, 2014; Hassan Baig et al., 2016).

Curcumin and its derivatives are thought to have carried out a study against Monkeypox and Smallpox infection. According to research, curcumin, the primary biologically active component of turmeric (*Curcuma longa L.*), functions as a potent anti-inflammatory, antioxidant, antibacterial, antifungal, and antiviral activity (Rathore et al., 2020). The chemical formula $C_{21}H_{20}O_6$ may represent it, and its molecular mass is determined to be 368.38 g/mol. This lipophilic polyphenol is principally abundant in the rhizomes of turmeric (*Curcuma longa L)*, which is a member of the ginger family (Zingiberaceae) and is indigenous to the tropical regions of South Asia. It contains a yellow-to-orange hue. Turmeric powder is an eastern flavor that is typically derived from this plant. Curcumin, along with natural ingredients and other coumarins, is one of the most prominent bioactive substances recognized in turmeric powder (Basnet and Skalko-Basnet, 2011; Kotha and Luthria, 2019; Adamczak et al., 2020). Due to its antimicrobials, antioxidative, anti-inflammatory, and anti-cancerous properties, curcumin has long been anticipated to be a therapeutic or preventive agent for many human diseases (Tønnesen and Karlsen, 1985; Hsu and Cheng, 2007; Hatcher et al., 2008; Lestari and Indrayanto, 2014).

# 2 Literature review of curcumin

## 2.1 MPXV: Genome and physiological characteristics

Since its first appearance, the Monkeypox virus (MPXV) has been found throughout West and Central Africa. Recently, occasional cases of MPXV infections have been reported in a number of nations. According to recent findings, the MPXV-2022 strains are members of the same family as the MPXV strain discovered in 2018. In comparison to the MPXV strain that was found in 2018, the MPXV-2022 strains have been shown to include a total of 46 additional consensus substitutions, comprising 24 variants that are nonsynonymous (Wang et al., 2022). Besides, the MPXV genome consists of about 197,000 base pairs and has hairpin termini in addition to more than 190 open reading frames that do not overlap (ORFs) (Shchelkunov et al., 2001). The highly conserved segment in the middle of the genome that codes for proteins is surrounded on both sides by flexible endpoints that include inverted terminal repeats. At least ninety open reading frames (ORFs) are documented to be necessary for the proliferation and development of poxviruses. Many other ORFs considered non-essential have a role in the abnormalities in host tropism, immunomodulation, and pathogenicity caused by poxviruses (Seet et al., 2003). The form of MPXV virions may be described as either barrel or oval, and their sizes range from around 280 to 220 nanometers in general (Erez et al., 2019; Kmiec and Kirchhoff, 2022). The physiological features and structures of MPXV are displayed in Figure 1 (Miller, 2013).

## 2.2 Effect of curcumin on viral infections

Curcumin has been shown to interfere with the progress of viral infection *via* various pathways, explicitly targeting the viral genome, reducing protein assembly and cell proliferation, and preventing the virus from entering the host cells and replicating (Basu et al., 2013; Wang et al., 2020). An updated research investigation reported that curcumin might be suppressed the respiratory syndrome virus (RSV) by preventing the virus' interaction with hosting cells (Yang et al., 2017). According to new findings, curcumin seems to hinder the attachment of the porcine reproductive and respiratory syndrome virus (PRRSV). This may be accomplished by affecting the flexibility of viral envelopes. In addition, curcumin can prevent viral infection by blocking PRRSV-mediated cell fusion (Du et al., 2017). Another study reported that curcumin and its derivatives had been shown to have a high binding affinity to hemagglutinin (HA), the primary capsid glycoprotein of the influenza virus that is responsible for viral attachment, and disrupts the integrity of the membrane structure to inhibit IAV entrance. This stops the virus from adhering to host cells and prevents the virus from entering the cell (Liu and Ying, 2020).

## 2.3 Effects of curcumin on the SARS CoV-2

The primary antiviral function of curcumin against SARS-CoV-2 is its capacity to block the folding of viral spike protein to the ACE2 receptors, which is the first stage in the development of infecting the host organism. The inflammatory response brought on by COVID-19 is a complicated and multi-step phenomenon. Patients with a severe illness are more likely to be influenced by a hyperinflammatory phenomenon known as a cytokine storm. This fact highlights the necessity for anti-inflammatory therapies to

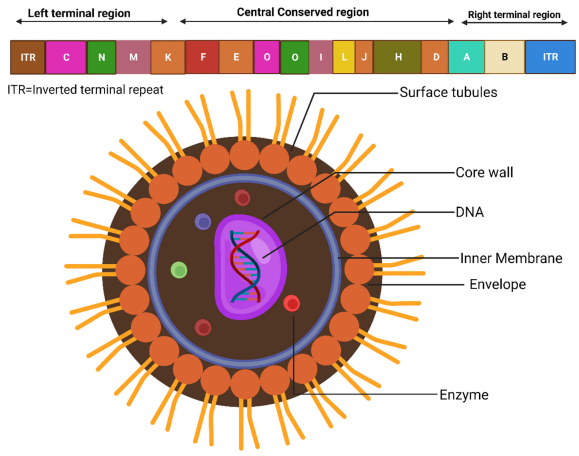


FIGURE 1
Physiological structures of MPXV.

minimize the hyperactivation of the immune reaction that occurs when it causes the cytokine storm (Rattis et al., 2021). Two investigations were performed with COVID-19-infected patients, one of which focused on the anti-inflammatory effects of curcumin. The first investigation that the research team undertook looked at how nano curcumin affected the production of cytokines that promote inflammation. Patients diagnosed with COVID-19 had an extreme amount of mRNA generation and production of the cytokines IL-1β, IL-6, TNF-α, and IL-18; however, patients treated with nano curcumin exhibited a substantial decline in IL-6 and IL-1β levels (Valizadeh et al., 2020; Tahmasebi et al., 2021).

## 2.4 Curcumin function on dengue protease

Research and investigation reported that curcumin might inhibit plaque formation caused by dengue virus strains (DENV-1-4, IC50 of 9.37, 3.07, 2.09, and 4.83 M, correspondingly) that were tested in LLC-MK2 cells and exhibited only a moderate level of toxicity (CC50 of 59.42 M) (Gao et al., 2019). Even though the method of suppression was not investigated, previous research indicated that curcumin probably suppresses DENV-2 implicitly *via* its influence on cellular systems rather than immediately on viral activities. Besides, curcumin and its four analogs could suppress viral protease activity (IC50 values ranged from 36–66 M) when tested in an *in vitro* experiment. The multiplication of a DENV2 reporter plasmid mutant was only moderately suppressed by these substances, with the acyclic and cyclohexanone analogs of curcumin functioning substantially better than the natural curcuminoids (50% effective concentration (EC50) of 8.61 and 8.07 μM vs. 13.91 μM) (Balasubramanian et al., 2019). It appears that the actions of curcumin on cellular lipid metabolism were responsible for the virus-inhibiting properties of curcumin against DENV. Curcumin and its analogs could inhibit the enzymes acetyl-CoA carboxylase and fatty acid synthase and reduce the development of lipid droplets (LD). These mechanisms would ordinarily serve to make DENV acquisition more prevalent. In addition, therapy with curcumin culminated in the disarray of actin filaments and abnormalities in polymerization, which is another function that is inherently essential for DENV entrance and reproduction (Balasubramanian et al., 2019; Jennings and Parks, 2020).

## 2.5 Effects of curcumin on the influenza A virus

Curcumin is another potent inhibitor of the Influenza A virus (IAV), and it probably impacts the virus at various phases during its lifespan. When IAV is incubated with curcumin, the pathogenicity of the virus is diminished; this is presumably owing to the capacity of curcumin to compromise with the viral haemagglutinin function (Chen et al., 2010; Han et al., 2018). Curcumin significantly blocks NF-kB signaling, which is essential for the reproduction of the influenza A virus (Nimmerjahn et al., 2004). For instance, curcumin blocked various IAV-induced toll-like receptor (TLR) signaling pathways and enzymes, such as TLR2/4/7, MyD88, TRIF, and TRAF6, which are generally essential for effective virus assembly. Stimulating cells with agonists for TLR2/4, p38/JNK MAPK (Dai et al., 2018).

## 2.6 Effects of curcumin on Zika, and chikungunya virus

Several curcumin analogs that are effective against the enveloped viruses Zika (ZIKV), Chikungunya (CHIKV), and Vesicular Stomatitis (VSV), as well as the non-enveloped virus Coxsackie B3 (CVB3) (Adams et al., 2004). Curcumin could be effective in inhibiting when applied to cells both before and after being infected with Zika or chikungunya; however, it works against Zika solely during cell attachment or entrance and does not affect the final stages of the disease (Ardebili et al., 2021). Curcumin at concentrations of 5 M was demonstrated to be significantly efficient. This resulted in a drop in virus intensity of more than 0.5 log10 despite causing negative impacts. Additionally, the IC50 values for curcumin against Zika and Chikungunya were significantly 1.9 M and 3.89 M (Mounce et al., 2017). In addition, they encountered that curcumin stopped the chikungunya and vesicular stomatitis virus from entering cells or attaching to them (von Rhein et al., 2016).

## 2.7 Effects of curcumin on hepatitis C virus instead of herpes simplex virus

Components of curcumin that include -unsaturated ketone groups make the HCV membranes less flexible. This, in turn, prevents the virus from attaching to cells and fusing with them. Therefore, curcumin prevents the entry of all HCV genotypes into cells that have been evaluated in a dose-dependent manner, with a half-maximal inhibition zone (IC50) of about 8.46 1.27 M (Colpitts et al., 2014; Qin et al., 2014). Curcumin's ability to block the PI3K-AKT and Akt-SREBP-1 pathways and induce heme oxygenase is responsible for its antiviral effects (Calland et al., 2015; Wahyuni et al., 2018).

## 2.8 Curcumin as HIV protease inhibitor

Curcumin has been the subject of a significant number of research, all of which have shown to be an effective inhibitor of HIV protease. Curcumin meanly inhibit HIV-1 proteases (IC50 = 100 M) and HIV-2 proteases (IC50 = 250 M), which may be responsible for its anti-HIV activities (Prasad and Tyagi, 2015). By engaging with the catalytic center of isolated HIV-1 integrase, curcumin was capable of inhibiting HIV-1 integrase with an IC50 value of 40 microM. Additional research demonstrated that the anti-integrase action of curcumin was connected to the intramolecular arrangement of two phenyl rings, which brought the hydroxyl groups into direct connection with one another (Prasad and Tyagi, 2015). Not only does curcumin control the

infectious potential of HIV, but it also boosts the potency of medications used to treat HIV and AIDS. In another research, curcumin was demonstrated to increase the systemic exposure of saquinavir in rats, but it did not affect the intravenous pharmacokinetics of saquinavir (Kim et al., 2013). In the appearance of the curcumin-loaded microemulsion, the oral administration of saquinavir led to a rise in both the AUC and Cmax by a factor of 3.8 and 2.7, significantly (Prasad and Tyagi, 2015).

As curcumin is already established as potential effectiveness against the different viruses in clinical and laboratory trials (Table 1), so, we believe that the curcumin derivatives might have capabilities to inhibit viral entry/gene expression. Thus, we have selected more than 50 curcumin derivatives available in natural sources to find an effective medication against smallpox and Monkeypox virus treatment. At the beginning of the studies, these 50 derivatives were conducted molecular docking against the Monkeypox virus and selected best 12 compounds according to maximum binding energy for further investigation (Figures 2, 3 displayed selected the most potent 12 curcumin derivatives from 50 natural curcumin analogs, Figure 4 is illustration for antiviral mechanism of curcumin).

The phases of the viral life cycle are complex and occur through different processes. These processes consist of the attachment of the virion to its cell surface receptor, the subsequent entrance of the virion, the phase of viral genome replication and transcription, the process of translation of the viral genome, the congregation of the virion, and ultimately the release of the virion. As a result of curcumin's ability to suppress the function of viral envelope proteins, viral attachments and entrance are both blocked.

Furthermore, curcumin has been shown to affect specific signaling pathways, inflammatory processes, and translation and transcription, which ultimately results in a blockage of viral replication. Then, curcumin competitively inhibits the synthesis of viral DNA within the host cell. As a result, the virus cannot multiply and is unable to survive within the host cell (Hussain et al., 2022).

## 2.9 Transmission and clinical manifestation

The natural reservoir of MPX has not been identified; however, rats are a leading possibility. Consumption of uncooked or improperly prepared meat or other animal products from infected animals is a potential risk factor. People who reside in or near forested areas may also be at risk of low-level or indirect exposure to diseased animals. Though transmission of MPX is infrequent, it may occur *via* direct or indirect contact with infected bodily fluids (Guarner et al., 2022). The MPX may be transmitted *via* the placenta and spread through sexual intercourse before, during, and after childbirth (Di Gennaro et al., 2022). Rash, fever, chills, headache, adenopathy, and myalgia have been the ailments and indicators of MPX disease (Sale et al., 2006). Occasionally, in the first stages of MPX disease, the rash has present primarily in the genitalia and perineal regions. The incubation period for Monkeypox may be from around 5 to 21 days, although it is most often between 6 and 13 days. After 14–21 days, in most cases, patients recover completely from the disease on their immunity (Kumar et al., 2022; Pal et al., 2017). In Figure 5, the transmission pathway of MPX is graphically displayed.

# 3 Computational method

## 3.1 Preparation of Ligand and geometry optimization

Curcumin is a natural flavonoid polyphenol class compound, which is the primary and bioactive component of turmeric. Around 8000 different flavonoid compounds have been discovered, making flavonoids the most abundant family of phenolic chemicals. In addition to this, they are considered nutritional supplements that improve health and guard against infection (Zeghbib et al., 2022). Numerous studies demonstrate that curcumin reflects a variety of biological actions, suggesting that they may have preventive benefits against a broad range of diseases (Table 1). In this investigation, more than 50 compounds were taken from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/) (50 derivatives displayed in Supplementary Table 1), and the canonical SMILES of the exploration molecules were also acquired from the PubChem database. The 2D structures of the molecules were obtained by importing the canonical SMILES into the ChemBioOffice program. The Molecular optimization of 3D structure of molecules were carried out with the assistance of a method known as density functional theory (DFT) then optimized geometrically, and energy was minimized by employing B3LYP and the functional

TABLE 1 Experimental and clinical data of antiviral activities against viral strains reported in different research investigations (throughout 2018-2022).

| No | Virus species | Antiviral Activity | References |
|---|---|---|---|
| 01 | SARS CoV-2 | Inhibiting the Endosomal acidification | (Rattis et al., 2021) |
| 02 | Dengue virus | Entry inhibitor | (Balasubramanian et al., 2019) |
| 03 | Influenza A virus | Replication inhibitor | (Dai et al., 2018) |
| 04 | Herpes simplex virus | Gene expression inhibition | (van de Sand et al., 2021) |
| 05 | Human immunodeficiency virus | Viral protein degradation/Protease inhibitor | (Butnariu et al., 2022) |
| 06 | Zika virus | Entry inhibitor | (Pacho et al., 2021) |

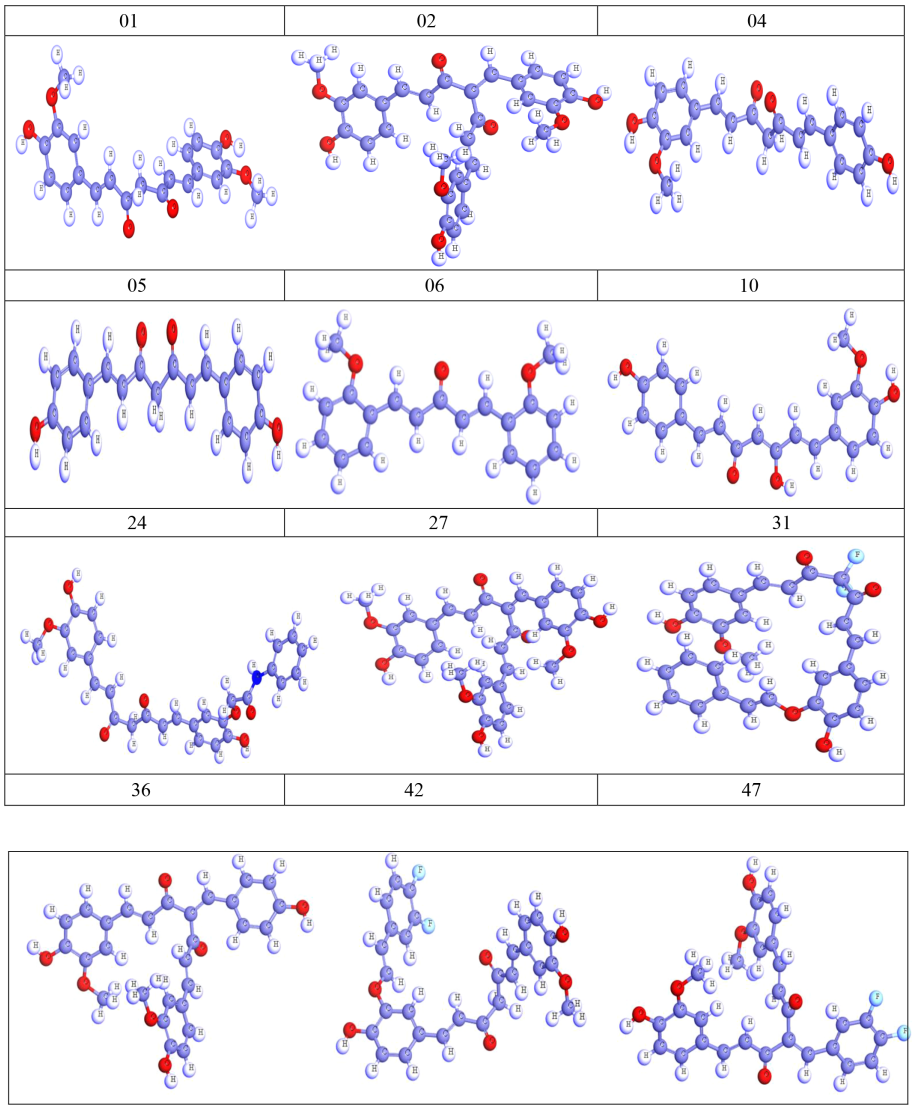


FIGURE 2
Optimized structure of curcumin derivatives.

unit DFT procedure of DMoL3 code from material studio 08 (Delley, 1995; Delley, 2010; Ramos, 2020). The HOMO-LUMO expression was then calculated using the optimized structures, and the optimized molecules were saved in PDB format for further work. The optimized chemical structures of curcumin derivatives are given in Figure 2.

## 3.2 ADMET profile prediction, Lipinski rule, and drug-likeness

The advancement of computer technology has enabled scientists to create innovative drug targets, which has cut down on the number of experiments that need to be conducted while simultaneously raising the success rate; in order to facilitate preliminary evaluation during drug discovery and development, ADMET pharmacokinetic features and drug-likeness play a major role. The ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) characteristics might be obtained using the in silico investigation (Kumer et al., 2021; Hassan et al., 2022). In this current study, the pkCSM(https://biosig.lab.uq.edu.au/pkcsm/) and SwissADME (http://www.swissadme.ch/index.php) online tools are used to investigate the ADMET features, Lipinski Rule and drug likeness (Mvondo et al., 2021).

## 3.3 Target structure selection and preparation

The 3D crystal protein-structures Monkeypox virus (PDB ID 4QWO) and smallpox virus (PDB 3IGC) were downloaded from the RCSB PDB database (https://www.rcsb.org/). To design any drug, must be needed a receptor. So, the these mentioned target receptor were taken since they are responsible for pathogenic effects. So, if this

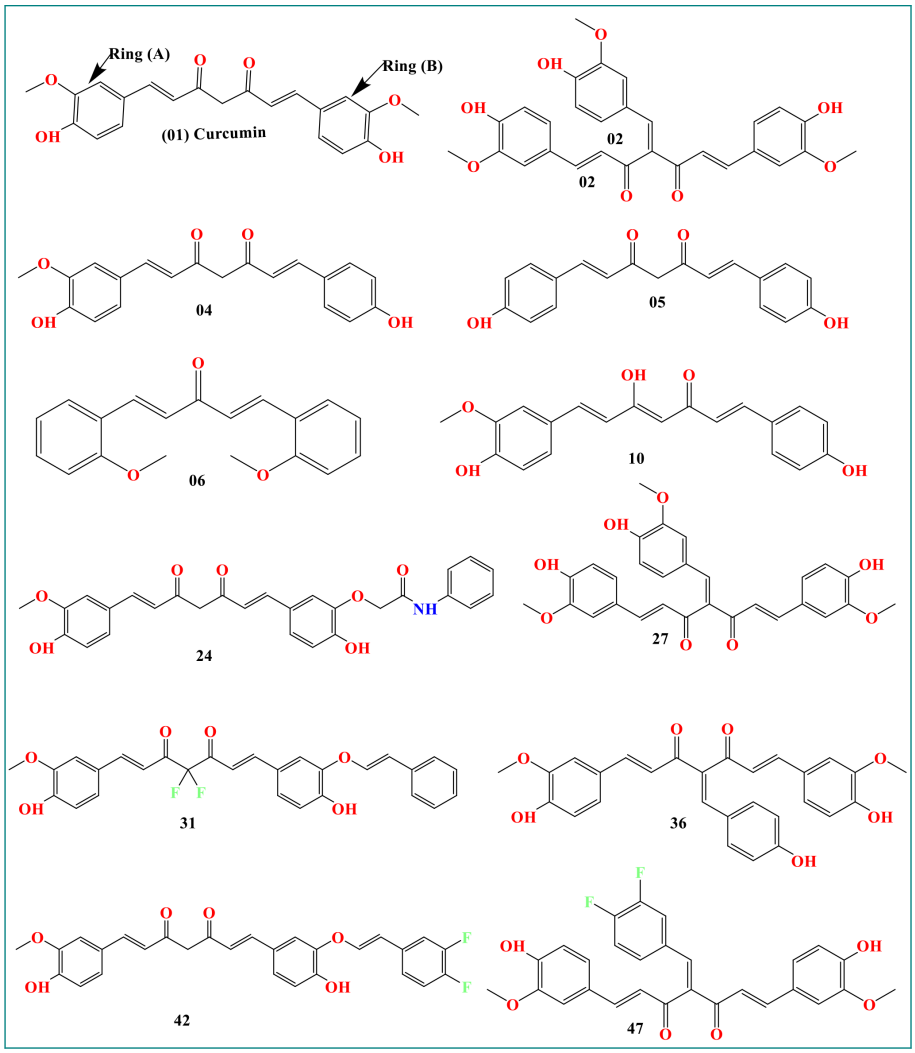


FIGURE 3
Chemical structure of curcumin and its derivatives.

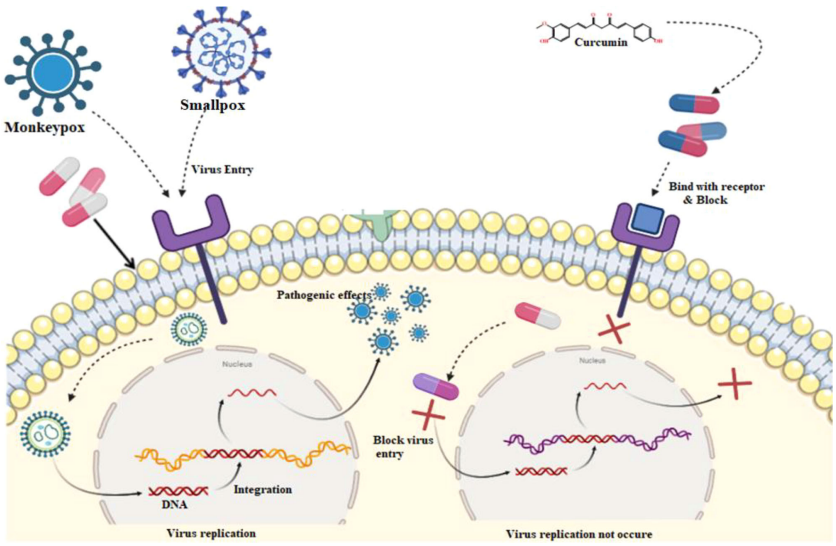


FIGURE 4
Probable mechanism action of curcumin against the virus.

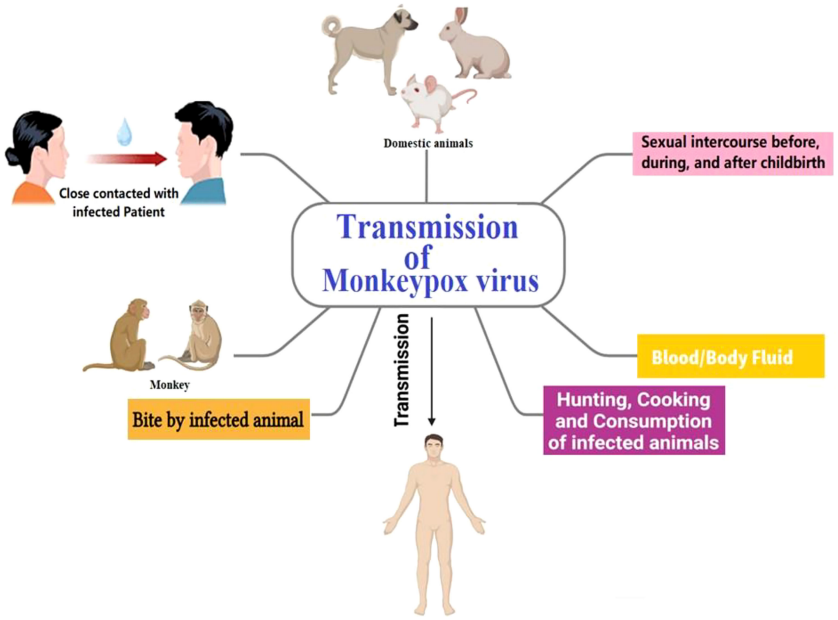


FIGURE 5
Monkeypox transmission pathway.

target protein is possible to inhibited, virus cannot replicate within the host cell and as a result, they cannot produce pathogenicity. Besides, to bind a drug in a specific side, like as lock and key model, must be cleaned or fresh target receptor, excess molecules such as water and other unwanted substances may interfere to bind specific site. So, the water and other unwanted substances are cleaned before docking. By utilizing the Discovery Studio v16.1.0.15350, and Pymol version 2021 program, the 3D crystal structures of Monkeypox and the small virus were cleaned up in preparation for molecular docking. This included the removal of all ligands, non-protein components, and molecules of water (Mahmud et al., 2021). Before molecular docking, the energy of the targeted protein was minimize using swisspdbviwer (Rangisetty et al., 2023).

The details of Monkeypox and the small virus are listed in Figure 6.

## 3.4 Molecular docking analysis

The docking studies modeling was executed to investigate the molecular interaction strategy and acquire documentation on the binding capacity and ligand effectiveness to inhibit the targeted protein. In the current study, the molecular docking analysis was evaluated with the cooperation of the PyRx AutoDock tools application, version 4.2 (Akash et al., 2022a; Rizvi et al., 2013). The automatic maximized function was used to compose the 3D grid for ligand-receptor interaction during molecular docking. The grid box parameters were generated as center (x = 12.0443, y = 18.445, z = 16.0634), dimension (x= 35.14496, y= 37.645, z=36.966) for monkeypox virus, and center (x = 23.2575, y = -9.6147, z = 31.6074), dimension (x= 93.9023, y= 79.8679, z= 63.0821). When the docking analysis was done, The BIOVIA Discovery Studio

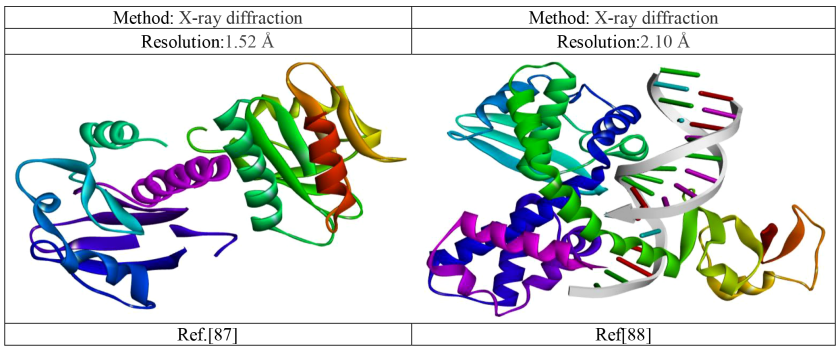


FIGURE 6
Experimental three-dimensional protein structures information (Minasov et al., 2022; Perry et al., 2010).

Visualizer v16.1.0.15350 was applied to generate docked residues, ligand-protein complex structures, and 2D and 3D representations.

## 3.5 Molecular dynamics simulations

YASARA v21.6.17 software was used to run an MD simulation to investigate the relationship between curcumin and 4QWO viral protein. The simulation used the aided model construction with an AMBER 14 force field (Zhang et al., 2020). By combining Cl- and $Na^+$ ions, the exchangeable Inter - molecular Potential3 (TIP3P) water model was utilized. Each system operates with the most effective gradient strategy for energy minimization (5000 cycles). The simulations used a periodic boundary condition in which the cell size was always ten times larger than the protein size. Using particle-mesh Ewald (PME) methods, MD simulations and electrostatic interactions were carried out, and also some physiological settings were set at 298 K, 0.9% NaCl, and pH 7.4 (Krieger et al., 2006). A Berendsen thermostat was used to control the simulation temperature while keeping the pressure fixed. Finally, 100 ns of MD simulations were run under constant pressure, and subsequent analysis was handled *via* the built-in YASARA MACRO script (Krieger et al., 2002).

# 4 Results and discussion

## 4.1 Chemistry

The molecular structure of curcumin exposes the myriad of functional groups (1, 7-bis [4-hydroxy-3- methoxyphenyl]-1,6-heptadiene-3,5-dione, Figure 3). Rings A and B, both of which are aromatic phenol rings, are joined to one another by different pairs of -unsaturated carbonyl groups (Pullakhandam et al., 2009). These carbonyl groups are excellent Michael receivers and can react with glutathione and other nucleophiles. Other essential structural properties of curcumin are the two aryl methoxy groups located in the ortho position, the hydroxyl component, and the connected -diketone subunits. So, when different substitutes are added, generating various analogs, they act as potential drug candidates and provide better pharmacological efficacy (Nabavi et al., 2014). The studied analogs of curcumin are given in Figure 3.

## 4.2 Lipinski rule, pharmacokinetics

The Lipinski rule, commonly referred to as the rule of five, states that one of the most crucial aspects of a drug is how similar it is to other substances. Molecular weight, hydrogen bond donors and acceptors, Topological polar surface area, and Consensus (Log $P_{o/w}$) criteria for determining drug similarity characteristics must be followed (Akash, 2022; Kumer et al., 2022a). The molecular weight should be between 150 and 500 g/mol, the number of hydrogen bond donors should be five or fewer, and the number of hydrogen bond acceptors ought to be ten or fewer. The value of TPSA must fall within the limit of 20 and 130, whereas the acceptable range for Log $P_{o/w}$ must not be higher than 6.00 (Chen et al., 2020).

In our studied compounds, the molecular weight 294.34 – 492.49 Dalton, but two compounds (02 and 27) showed 502.51 Dalton as molecular weight, hydrogen bond donors (03-03) and acceptors (03-08), Topological polar surface area (35.53 $Å^2$-122.16 $Å^2$), and Log $P_{o/w}$ (2.83 – 5.19), which all falls into standard ranges. Lipinski was used to predict the drug likeness accurately, and all the chosen compounds accept the Lipinski rule. So, it is noticed that they might be used as drugs. The *in-silico* prediction of druglike qualities has been compiled in Table 2, and it was accomplished with the help of the web program SwissADME.

TABLE 2 Predicted data of Lipinski rule, pharmacokinetics.

| Ligand No | Molecular Weight(g/mol) | Hydrogen bond acceptor | Hydrogen bond donor | Topological polar surface area ($Å^2$) | Consensus Log $P_{o/w}$ | Lipinski rule | |
|---|---|---|---|---|---|---|---|
| | | | | | | Result | violation |
| 01 | 368.38 | 06 | 02 | 93.06 | 3.03 | Yes | 00 |
| 02 | 502.51 | 08 | 03 | 122.52 | 4.16 | Yes | 01 |
| 04 | 338.35 | 05 | 02 | 83.83 | 3.00 | Yes | 00 |
| 05 | 308.33 | 04 | 02 | 74.60 | 2.83 | Yes | 00 |
| 06 | 294.34 | 03 | 00 | 35.53 | 3.86 | Yes | 00 |
| 10 | 338.35 | 05 | 03 | 86.99 | 3.18 | Yes | 00 |
| 24 | 487.50 | 07 | 03 | 122.16 | 3.61 | Yes | 00 |
| 27 | 502.51 | 08 | 03 | 122.52 | 4.16 | Yes | 01 |
| 31 | 492.47 | 08 | 02 | 93.06 | 5.05 | Yes | 00 |
| 36 | 472.49 | 07 | 03 | 113.29 | 4.05 | Yes | 00 |
| 42 | 492.47 | 08 | 02 | 93.06 | 5.19 | Yes | 00 |
| 47 | 492.47 | 08 | 02 | 93.06 | 4.99 | Yes | 00 |

## 4.3 Molecular docking analysis against monkeypox and smallpox virus

Molecular docking investigation is the key, innovative drug development strategy in the computational chemistry (structure-based drug design), which sorts residual interactions between targeting ligands and a protein's receptor active site (Ferreira et al., 2015). The docking value is calculated to determine the degree to which ligand molecules bind with the active region of the specific receptor. The greater the negative value of the binding energy, the more strongly preferred the orientation will be, and the more persistent the structure of the ligand-receptor complex is formed (Cerqueira et al., 2015). To become a potential drug candidate, the docking score might be greater than -6.0 kcal/mol (Kumer et al., 2022a; Kumer et al., 2022c). So, the molecular docking was performed against Monkeypox Virus (PDB ID 4QWO) targeted protein initially. When, the binding affinity was achieved outstanding result against Monkeypox Virus (PDB ID 4QWO), then the Smallpox virus (PDB 3IGC) were also taken to determine how much affinity present. In these current studies, the binding affinities are generated from -7.7 kcal/mol to -8.9 kcal/mol against Monkeypox virus (PDB ID 4QWO) and -7.3 kcal/mol to -8.8 kcal/mol against smallpox virus (PDB ID 3IGC). The standard Acyclovir displayed -6.4 kcal/mol and -6.5 kcal/mol. In both cases, the binding energy is more remarkable than -6.00 kcal/mol, much better than standard Acyclovir. Among all derivatives, the best bonding affinity was reported -8.9 kcal/mol against Monkeypox Virus (PDB ID 4QWO) in ligand 24, and -8.8 kcal/mol against Smallpox virus (PDB 3IGC) in ligand 42. So, these reported natural curcumin derivatives should be effective against monkeypox and smallpox viruses.

Noted that fifteen curcumin was taken from PubChem database (Shown in Supplementary Table 1), and selected best twelve compounds based on their binding affinities (Table 3).

## 4.4 Protein-ligand interaction analysis

The application known as BIOVIA discovery studio visualizer v16.1.0.15350 was used to visualize the interaction and binding between the ligands and the target proteins (Biovia, 2015). When generating protein-ligand complexes, the discovery studio visualizer was used to visualize various non-bonded interactions, such as hydrogen bond interactions and hydrophobic bond interactions with specific distances, as well as active amino acid residues, which are shown in Figure 7. The visualization of intermolecular interaction modes to estimate antiviral functions was further investigated so that the mechanism of interactions between bioactive substances and the enzyme substrates specific to each of those medicines could be evaluated.

It has been determined that the significant sites in the instance of monkeypox and smallpox virus constituted the active site pocket are situated at ASN A-14, PHE A-17, ALA A – 34, ILE A – 35, VAL A – 31, ARG A -38, PRO A – 36, LYS A-13, GLU A – 18, HIS A-15, TYR A-136. Besides, the two-dimensional visualizations of the most effective active molecules, as determined by the number of hydrogen bonds formed with the significant amino acids of the Monkeypox and smallpox active sites, are shown in Figure 7A–C, correspondingly.

These findings imply that all these ligands might be explored as a viable therapeutic strategy against Monkeypox and smallpox viruses by decreasing viral multiplication and expression. Therefore, the binding of ligands in the active site and their

TABLE 3 Summary of binding affinities against Monkeypox, and Smallpox virus.

| Drug No | PubChem CID | Monkeypox Virus (PDB ID 4QWO) | Smallpox virus (PDB 3IGC) |
|---|---|---|---|
| | | Binding Affinity(kcal/mol) | Binding Affinity(kcal/mol) |
| 01 | 969516 | -7.7 | -7.3 |
| 02 | 44195235 | -8.2 | -8.1 |
| 04 | 146723 | -8.1 | -6.8 |
| 05 | 147439 | -8.2 | -7.7 |
| 06 | 830608 | -8.1 | -7.8 |
| 10 | 5324476 | -8.1 | -7.3 |
| 24 | 122515213 | -8.9 | -8.7 |
| 27 | 44195235 | -8.2 | -8.0 |
| 31 | 162394524 | -8.5 | -8.2 |
| 36 | 44452370 | -8.2 | -7.6 |
| 42 | 132993165 | -8.8 | -8.8 |
| 47 | 54597187 | -8.4 | -8.7 |
| **Standard (Acyclovir)** | 135398513 | -6.4 | -5.5 |

10.3389/fcimb.2023.1157627

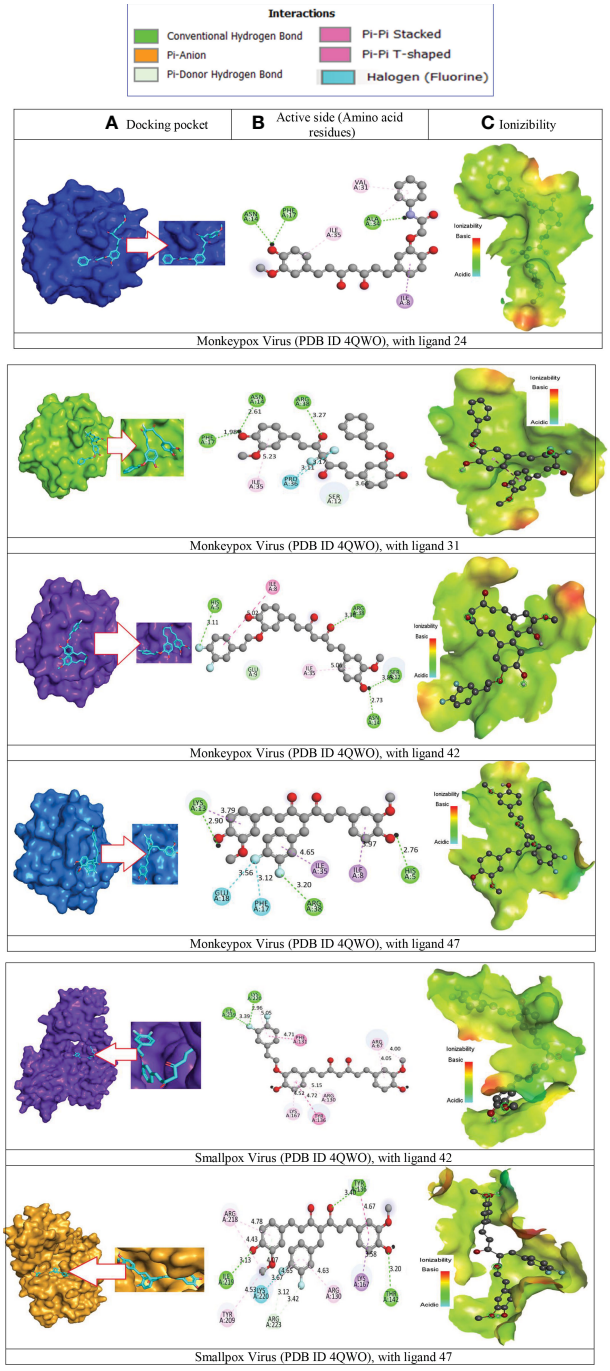


FIGURE 7
Molecular docking pocket, hydrogen bonding, and binding interaction. **(A)** Docking pocket, **(B)** Active side (Amino acid residues), **(C)** Ionizibility.

durability are essential factors in determining the possible treatment of Monkeypox and Smallpox viruses. Consequently, they have the capability of monkeypox and smallpox viral agonists since they may potentially attach to the pocket generated by the protein residues of the virus and disrupt the virus's functionality. The bonding residue of active amino acid for best two complexes are given in Supplementary Table 1.

## 4.5 Chemical descriptors calculation

The highest occupied molecular orbitals (HOMOs) and the lowest unoccupied molecular orbitals (LUMO) have a significant role in biochemical reactions. The kinetic resilience, electrochemical stability, chemical hardness, and softness of a drug molecule are all correlated to the HOMO-LUMO energy gap exists between them. The density functional theory was adopted to evaluate the chemical potential, electronegativity, hardness, and softness of our biomolecules (Table 4). The HOMO and LUMO frequencies are used to derive these reactivity parameters. The HOMO and LUMO frequencies are used to derive these reactivity parameters. Table 4 expresses the chemical reactivities of the 12 different curcumin derivatives available in nature.

A larger energy gap suggests significant kinetic stability but low electrochemical stability, while a relatively low energy difference and enhanced softness imply that the molecules have a greater level of polarity and chemical conductivity. Besides, the chemical reactivity of molecules is reduced when the HOMO-LUMO energy gap is large, indicating a lower electronic transition of the electron, and is increased when the gap is small, representing a higher atomic system and higher electrochemical stability, both of which are closely relevant to bind with a targeted protein receptor. The energy gap for organic or aromatic chemicals are typically between 7.00 eV and 9.00 eV, which allows them to attach to any protein efficiency (Kobir et al., 2022). Based on Table 4, it is reported that all chemicals maintain the energy gap within the acceptable number (7.00 eV to 9.00 eV). Furthermore, the chemical potential (l), hardness (g), and softness (r), as well as the electronegativity coefficients, might have been utilized to assess therapeutic efficacy (Hoque et al., 2020; Kobir et al., 2022). Usually, the hardness of the material is higher than its softness, and the two properties are inversely proportional to one another. A lower softness score indicates that the components have an outstanding level of dissolving capacity (Kawsar et al., 2022). It is anticipated that the hardness is approximately 3.5 to 4.5, whereas the softness is within 0.24 for all curcumin derivatives. All of the chemical potentials were discovered to be negative, evidence that any form of chemical species or compounds may maintain a satisfactory level of chemical stability and durability. It is clear from looking at Table 4 that the potential chemical levels of all agonists range from -4.93105 to -5.5725 (Kobir et al., 2022).

## 4.6 Frontier molecular orbitals (HOMO and LUMO)

The HOMO and LUMO are remarkable promoters that can manage the physiochemical properties of any chemical molecule, hence deciding and regulating all of the chemical properties of that substance. From this perspective, it predicts the chemical characteristics of the subsequent curcumin analogs. As before, oxygen atoms in these compounds make it difficult to determine whether the aromatic chain or the heterocyclic ring dominates the chemical characteristics without resorting to the HOMO and

TABLE 4 Data of chemical descriptors calculation.

| S/N | I=- HOMO | A=-LUMO | Energy Gap E(gap) =I-A (eV) | Chemical potential $(\mu) = \frac{I + A}{2}$ | Hardness $(\eta) = \frac{I - A}{2}$ | Softness $(\sigma) = \frac{1}{\eta}$ |
|---|---|---|---|---|---|---|
| 01 | -9.8934 | -0.9844 | 8.909 | -5.4389 | 4.4545 | 0.2245 |
| 02 | -9.886 | -1.0205 | 8.8655 | -5.45325 | 4.43275 | 0.2256 |
| 04 | -9.7678 | -0.9827 | 8.7851 | -5.37525 | 4.39255 | 0.2277 |
| 05 | -9.7719 | -0.9936 | 8.7783 | -5.38275 | 4.38915 | 0.2278 |
| 06 | -8.8939 | -0.9682 | 7.9257 | -4.93105 | 3.96285 | 0.2523 |
| 10 | -9.8924 | -0.9944 | 8.898 | -5.4434 | 4.449 | 0.2248 |
| 24 | -8.8996 | -0.9791 | 7.9205 | -4.93935 | 3.96025 | 0.2525 |
| 27 | -9.896 | -0.9952 | 8.9008 | -5.4456 | 4.4504 | 0.2247 |
| 31 | -9.9115 | -1.1298 | 8.7817 | -5.52065 | 4.39085 | 0.2277 |
| 36 | -9.0900 | -0.9885 | 8.1015 | -5.03925 | 4.05075 | 0.2469 |
| 42 | -9.9041 | -1.1225 | 8.7816 | -5.5133 | 4.3908 | 0.2277 |
| 47 | -9.9055 | -1.2395 | 8.666 | -5.5725 | 4.333 | 0.2308 |

LUMO diagrams (Kobir et al., 2022). The discrepancy between HOMO and LUMO is negligible, as seen in Figure 8. (The deep blue and yellow of LUMO are the positive and negative ends of the orbital node, and the green and red color of HOMO are the positive and negative ends of the orbital node, similarly). It is remembered that protein or targeted biomolecules are attached in the LUMO region (Kumer et al., 2019; Kumer et al., 2022c).

## 4.7 Molecular dynamics simulations

The hit's best pose from the virtual screening was chosen compounds (42 and standard Acyclovir), and the YASARA structure's scenario mode was then set up using the default option (Prasasty and Istyastono, 2020). Here, we ran 100ns of MD simulations using the reference derivatives (Acyclovir). Protein and ligand RMSDs of the Cα atoms were determined and shown in (Figure 9) in a time-dependent manner.

The result is shown how proteins behave during an MD simulation; as seen in the plot, compound 42 has shown high fluctuation in the primary stage, but after near 74.3ns and 75.5ns, Acyclovir is given the highest range of pick in RMSD, so ligand 42 is a perfectly stable compound. For better results on how ligands 42 and Acyclovir influence the binding mode with Monkeypox protein (PDB ID 4QWO). The two complexes' structural changes were evaluated by means of root mean square fluctuation (RMSF), the radius of gyration, and the solvent-accessible surface area of the protein-ligand complex (Figure 10).

Solvent can ruin a pocket if it penetrates the binding site. Protein-ligand interactions must be tightly regulated. Figure 10A represents compound 42 showed a high SASA value after close 7ns of simulation, it may not reduce the protein expansion. Figure 10B demonstrates the radius of gyration value, compound 42 showed more excellent value than Acyclovir, denoting loose packing of protein structure, RMSF value, which depicts the flexibility of the entire residue in the protein, is shown in Figure 10C. High fluctuations were reached in some positions, including (13, 55, 59, 66, and 115 residues), which produced positive results. In Figure 11, the hydrogen bond interaction between the protein and the ligand is finally depicted.

Intermolecular hydrogen bonding shows that compound 42 and Acyclovir yield hydrogen bonds with the catalytic domain residue in proteins. Protein folding and de-folding nature depend on intermolecular hydrogen bonds. Acyclovir showed maximum hydrogen bond contact rather than 42 compounds. Eventually, all analyses from the MD simulations suggest that compound 42 is stable and performed a little conformational change of the proteins.

## 4.8 ADME, and aquatic and non-aquatic toxicity

The absorption, distribution, metabolism, excretion, and toxicity of chemicals, abbreviated as ADMET, all contribute a major function significantly to the discovery and development of new drugs. Compounds are absorbed in the human small intestine, move from one tissue to another, undergo chemical biotransformation in the body, and are eliminated from the body *via* excretion; and the level of toxicity of a compound is determined by its toxicity. By using ADMET metrics, we were capable of confirming that the most promising active components against

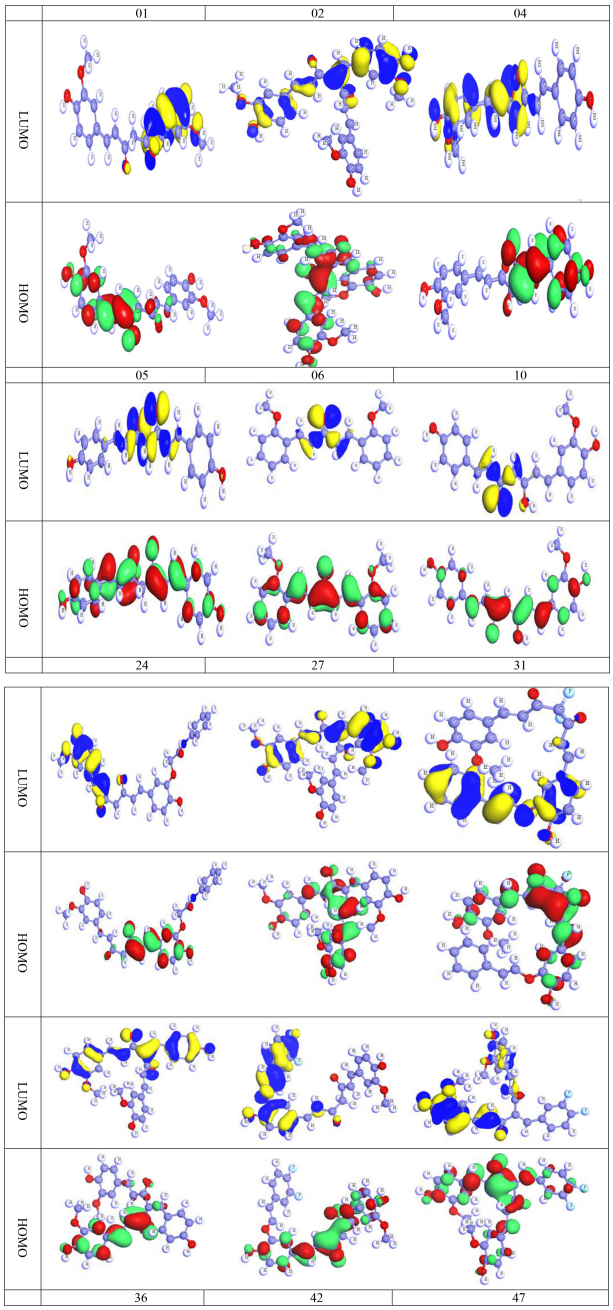


FIGURE 8
HOMO and LUMO diagram of curcumin and its derivatives.

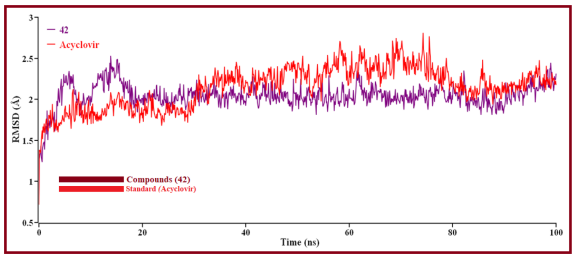


FIGURE 9
The RMSD of Cα atoms over time for proteins and ligands. Here, red and violet lines denote compound 42 and Acyclovir complexes, respectively.

monkeypox and smallpox virus had the potential to become marketable medications. The *in silico* prediction of ADMET characteristics has been compiled in Table 5, and it was accomplished with the help of the online program pkCSM.

If the absorption score is less than 30 percent, it indicates an inadequate, poor absorption rate. The findings above suggest that the human intestine can absorb all the chemicals effectively, most of which absorb up to 90%. Water solubility (Log S) recommendations for slightly and high solubility compounds vary from -4 to -6 and -2 to -4, correspondingly (Rout et al., 2022). Our invented molecules reported that ligands (05, 06, and 31) are in -4 to - 6 ranges, and the rest ligands ranges fall with -4.0, which means ligands (05, 06, and 31) are slightly soluble, and the others ligands (01,02,04,10,24,30,36,42, and 47) are highly soluble in the water system. Moreover, it is believed that the volume of distribution (VDss) is substantial better if the value is more than 0.45, and our studies reported that most of the curcumin derivatives have a lower volume of distribution (VDss) than the standard. It is also noticed that none of the ligands can cross to the BBB. The biotransformation of pharmaceutical drugs inside the body is referred to as drug metabolism, a phrase often used. The metabolism of drugs results in the formation of a number of distinct enzymatic substrates, each of which contains its unique set of physicochemical, pharmacokinetic, and pharmacological characteristics. As a result, it is essential to consider how the medicine will be metabolized and how it will react with other medications. Drug interactions occur due to cytochrome P450 (CYP) inhibition, which is essential in drug metabolism. The substances 04, 05, 06, and 10 were shown to be inhibitory of the enzymes CYP1A2, while all of the agonists were confirmed to effectively substrate with CYP450 2C9. The clearance parameter characterizes the linear connection between the clearance rate and the plasma concentration of medication. A low clearance constant thus suggests enhanced retention of medicines inside the body long time. The cumulative clearance rate of all chemicals demonstrates that the medicine can excrete from the body after an extended period. At the same time, only ligands 42 and 47 have negative scores, which means these two have poor excretion rate constants. Finally, carcinogenicity is applied to characterize the toxicity of AMES; hence, it is essential to ensure that the expected chemicals are not carcinogenic, skin sensitization, or hepatotoxicity. Our projection is predicted that only ligands 04 and 05 may be carcinogenic, and ligands 04 and 10 may be the hepatotoxic effect, while the rest ligands are free from skin sensitization effects. So, the overall finding of ADMET is favorable and suggests them as new drug candidates.

## 5 Conclusions

Fifty different natural curcumin derivatives were used in these computational investigations. In the meantime, twelve compounds

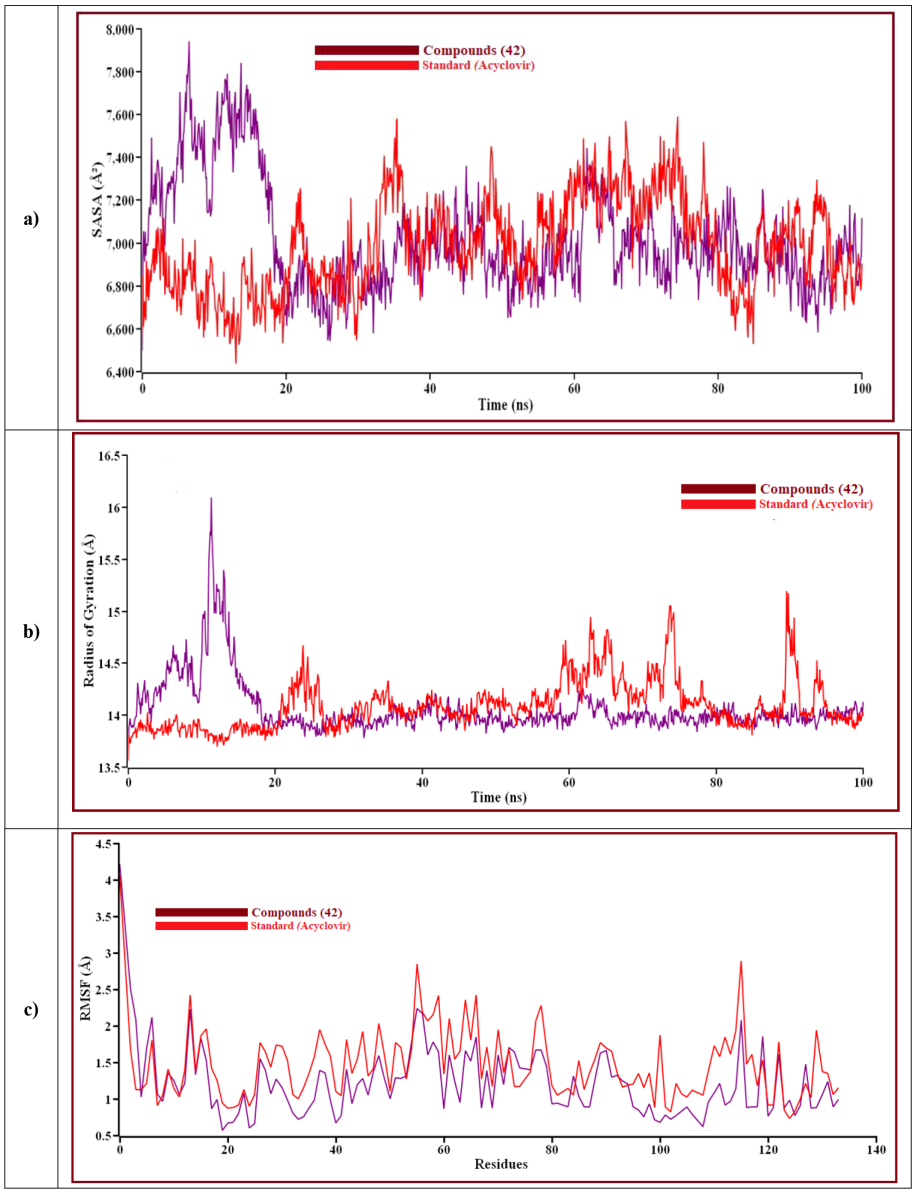


FIGURE 10
The structural behavior change of protein by means of **(A)** solvent accessible surface area (SASA), **(B)** radius of gyration, and **(C)** root means square fluctuations (RMSF) analysis. Here, the red line indicates Acyclovir, and the violet line indicates compound 42 complexes, respectively.

were chosen based on having the highest possible docking score. After that, a multitude of computational studies were carried out, including molecular docking, dynamic modeling, ADMET, and DFT.The molecular docking investigation corroborated these findings, showing promise antiviral effectiveness against monkeypox and smallpox virus. The finding docking score is about -7.7 kcal/mol to -8.9 kcal/mol against monkeypox virus (PDB ID 4QWO) and -7.3 kcal/mol to -8.8 kcal/mol against smallpox virus (PDB ID 3IGC). During molecular docking, different types of bond and active amino acid residues were formed like as ASN A-14, PHE A-17, ALA A – 34, ILE A – 35, VAL A – 31, ARG A -38, PRO A – 36, LYS A-13, GLU A – 18, HIS A-15, TYR A-136. After that, DFT calculation, the curcumin derivatives have shown the hardness is approximately 3.5 to 4.5, whereas the softness is within 0.24 for all

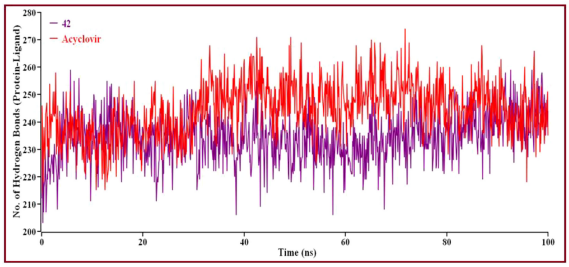


FIGURE 11
The number of hydrogen bonds created overall between protein-ligand complexes *via* MD simulations. Here, the red line indicates Acyclovir complexes, and violet lines indicate compound 42 complexes, respectively.

TABLE 5 Summary of in silico ADMET prediction.

| S/N | Absorption | | | Distribution | | Metabolism | | Excretion | | Toxicity | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Water solubility Log S | Caco-2 Permeability x $10^{-6}$ | Human Intestinal Absorption (%) | VDss (human) | BBB Permeability | CYP450 1A2 Inhibitor | CYP450 2C9 Substrate | Total Clearance (ml/min/kg) | Renal OCT2 substrate | AMES toxicity | Skin Sensitization | Hepatotoxicity |
| 01 | -3.952 | 0.617 | 88.035 | -0.152 | No | No | Yes | 0.117 | No | No | No | No |
| 02 | -3.22 | 0.66 | 97.702 | -0.445 | No | No | Yes | 0.112 | No | No | No | No |
| 04 | -3.967 | 0.955 | 92.354 | -0.112 | No | Yes | Yes | 0.099 | No | Yes | No | Yes |
| 05 | -4.48 | 1.02 | 90.805 | -0.247 | No | Yes | Yes | 0.041 | No | Yes | No | No |
| 06 | -5.346 | 1.64 | 95.334 | 0.015 | No | Yes | Yes | 0.329 | No | No | No | No |
| 10 | -3.895 | 0.894 | 89.952 | 0.057 | No | Yes | Yes | 0.179 | No | No | No | Yes |
| 24 | -3.8 | 0.5 | 82.557 | -0.355 | No | No | Yes | 0.069 | No | No | No | No |
| 27 | -3.22 | 0.66 | 97.702 | -0.445 | No | No | Yes | 0.112 | No | No | No | No |
| 31 | -4.598 | 0.497 | 96.984 | -0.639 | No | No | Yes | 0.012 | No | No | No | No |
| 36 | -3.543 | 0.535 | 95.212 | -0.587 | No | No | Yes | 0.08 | No | No | No | No |
| 42 | -3.428 | 0.72 | 98.664 | -0.608 | No | No | Yes | -0.246 | No | No | No | No |
| 47 | -3.301 | 0.754 | 100 | -0.652 | No | No | Yes | -0.243 | No | No | No | No |

curcumin derivatives. So, it is suggested that these derivatives may easily decompose or breakdown within physiological system. Then, the molecular dynamic simulations were performed. The MDs significantly and emphatically corroborates this observation over 100 ns, which confirms the binding stability of the docked complexes in the trajectory analysis. It suggests that the protein-ligand complexes maintain the strongest stability inside the biological system. In accordance with this, the pharmacological features of these chemicals suggested that the majority of the developed molecules demonstrated enhanced pharmacokinetic parameters, conserved all drug-likeness rules, and increased pharmacological activities. Based on their pharmacokinetic and biological profiles, reported curcumin derivatives were shown to have the most promising use in treating Monkeypox and smallpox viral infections. So, these mentioned derivatives might be further suggested for experimental animal model.

## Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/Supplementary Material.

## Author contributions

*Conceptualization*, SA, AH, and MR. *methodology*, SA, MH. *validation*, SA, MH. *formal analysis*, SA, MH, and MR. *data curation*, SA, and MR. *writing—original draft preparation*, SA. *writing—review and editing*, MA, NA, MV, KK, RS. *supervision and project administration*, RS. All authors contributed to the article and approved the submitted version.

## Acknowledgments

This study was partially supported by grant projects of the Ministry of Health of the Czech Republic (FN HK 00179906) and of the Charles University in Prague, by the Cooperatio Program, research area NEUR. Also supported by Excelence project UHK 2216/2023-2024. Authors are thankful to the Researchers Supporting Project number (RSPD2023R728), King Saud University, Riyadh, Saudi Arabia.

## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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## Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcimb.2023.1157627/full#supplementary-material

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frontiers | Frontiers in Cellular and Infection Microbiology

TYPE Correction
PUBLISHED 09 June 2023
DOI 10.3389/fcimb.2023.1217334



OPEN ACCESS

APPROVED BY
Frontiers Editorial Office,
Frontiers Media SA, Switzerland

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RECEIVED 05 May 2023
ACCEPTED 09 May 2023
PUBLISHED 09 June 2023

CITATION
Akash S, Hossain A, Hossain MS, Rahman MM, Ahmed MZ, Ali N, Valis M, Kuca K and Sharma R (2023) Corrigendum: Anti-viral drug discovery against monkeypox and smallpox infection by natural curcumin derivatives: a computational drug design approach. *Front. Cell. Infect. Microbiol.* 13:1217334. doi: 10.3389/fcimb.2023.1217334



# Corrigendum: Anti-viral drug discovery against monkeypox and smallpox infection by natural curcumin derivatives: a computational drug design approach

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KEYWORDS

**curcumin, monkeypox, smallpox virus, molecular docking, DFT, admet, molecular dynamic simulation**

A Corrigendum on

Anti-viral drug discovery against monkeypox and smallpox infection by natural curcumin derivatives: a computational drug design approach

By Akash S, Hossain A, Hossain MS, Rahman MM, Ahmed MZ, Ali N, Valis M, Kuca K and Sharma R (2023) *Front. Cell. Infect. Microbiol.* 13:1157627. doi: 10.3389/fcimb.2023.1157627

## Text correction 1

In the published article, there was an error. The error in our article is in the abstract section. Before 6.8 kcal/mol, a minus (-) sign will be added and it will be -6.8 kcal/mol. A correction has been made to **Abstract, Result, Three.**

"The mentioned derivatives demonstrated docking scores greater than 6.80 kcal/mol, and the most significant binding affinity was at -8.90 kcal/mol, even though 12 molecules had higher binding scores (-8.00 kcal/mol to -8.9 kcal/mol), and better than the standard medications".

The corrected sentence appears below:

"The mentioned derivatives demonstrated docking scores greater than -6.80 kcal/mol, and the most significant binding affinity was at -8.90 kcal/mol, even though 12 molecules

had higher binding scores (-8.00 kcal/mol to -8.9 kcal/mol), and better than the standard medications".

The authors apologize for this error and state that this does not change the scientific conclusions of the article in any way.

## Text correction 2

In the published article, there was an error. The 2nd error in our article is in the literature review section. The sub section 2.7 Effects of Curcumin on herpes simplex virus should be Effects of Curcumin on Hepatitis C virus instead of herpes simplex virus.

A correction has been made to Literature review, Effects of Curcumin on herpes simplex virus, 0.

"Effects of curcumin on herpes simplex virus".

The corrected sentence appears below:

"Effects of Curcumin on Hepatitis C virus instead of herpes simplex virus".

The authors apologize for this error and state that this does not change the scientific conclusions of the article in any way.

## Publisher's note

frontiers | Frontiers in Cellular and Infection Microbiology

TYPE Original Research
PUBLISHED 24 May 2023
DOI 10.3389/fcimb.2023.1188763



OPEN ACCESS

EDITED BY
Saadullah Khattak,
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REVIEWED BY
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RECEIVED 17 March 2023
ACCEPTED 26 April 2023
PUBLISHED 24 May 2023

CITATION
Al Mashud MA, Kumer A, Mukerjee N, Chandro A, Maitra S, Chakma U, Dey A, Akash S, Alexiou A, Khan AA, Alanazi AM, Ghosh A, Chen K-T and Sharma R (2023) Mechanistic inhibition of Monkeypox and Marburg virus infection by O-rhamnosides and Kaempferol-o-rhamnosides derivatives: a new-fangled computational approach.
*Front. Cell. Infect. Microbiol.* 13:1188763.
doi: 10.3389/fcimb.2023.1188763



# Mechanistic inhibition of Monkeypox and Marburg virus infection by O-rhamnosides and Kaempferol-o-rhamnosides derivatives: a new-fangled computational approach

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The increasing incidence of Monkeypox virus (Mpox) and Marburg virus (MARV) infections worldwide presents a significant challenge to global health, as limited treatment options are currently available. This study investigates the potential of several O-rhamnosides and Kaempferol-O-rhamnosides as Mpox and MARV inhibitors using molecular modeling methods, including ADMET, molecular docking, and molecular dynamics/MD simulation. The effectiveness of these compounds against the viruses was assessed using the Prediction of Activity Spectra for Substances (PASS) prediction. The study's primary focus is molecular docking prediction, which demonstrated that ligands (L07, L08, and L09) bind to

Mpox (PDB ID: 4QWO) and MARV (PDB ID: 4OR8) with binding affinities ranging from -8.00 kcal/mol to -9.5 kcal/mol. HOMO-LUMO based quantum calculations were employed to determine the HOMO-LUMO gap of frontier molecular orbitals (FMOs) and to estimate chemical potential, electronegativity, hardness, and softness. Drug similarity and ADMET prediction assessments of pharmacokinetic properties revealed that the compounds were likely non-carcinogenic, non-hepatotoxic, and rapidly soluble. Molecular dynamic (MD) modeling was used to identify the most favorable docked complexes involving bioactive chemicals. MD simulations indicate that varying types of kaempferol-O-rhamnoside are necessary for successful docking validation and maintaining the stability of the docked complex. These findings could facilitate the discovery of novel therapeutic agents for treating illnesses caused by the Mpox and MARV viruses.

KEYWORDS

**Monkeypox virus, Marburg virus, drug development, O-rhamnosides, Kaempferol-o-rhamnosides, admet, molecular docking, molecular dynamics simulations**

## Introduction

In the middle of the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) infection that emerged as a novel virus in 2019, and has since caused the ongoing coronavirus disease 2019 (COVID-19). Two additional zoonotic viruses, Monkeypox virus (Mpox) virus and Marburg virus (MARV), have recently re-emerged in the current years (Islam et al., 2023; Zaeck et al., 2023). With over 60,799 confirmed cases of Mpox reported from 99 countries and locations, along with 20 deaths as of September 16, 2022, the re-emergence of Mpox, a member of the family Poxviridae and genus Orthopoxvirus that also contains smallpox virus, causing monkeypox (Mpox) in humans, has posed a public health emergency of international concern (Mohapatra et al., 2022; Khattak et al., 2023). MARV, a member of Filoviridae family that also contains deadly Ebola virus, which causes Marburg virus disease (MVD), and has a fatality rate of up to 90%, re-emerged in Ghana, an African country, in July 2022, as well as it was also reported from Guinea in 2021; there have been a total of 15 MVD outbreaks, the vast majority of which have occurred in Africa (Abir et al., 2022; Aborode et al., 2022; Zhao et al., 2022). MARV was initially documented to infect people in 1967, while Mpox was first identified in 1958, with the first zoonotic human case confirmed in 1970. These two viruses have been rare and forgotten human diseases for almost 50 years. Among these, Mpox has recently become a global health emergency (Farahat and Memish, 2022; Anwar et al., 2023).

The viruses are obligate intracellular parasites that can act as living, non-loving objects (Claverie, 2006). They are very tiny in size and have a simple structure. They have the lack any metabolic activity and consist of either DNA or RNA as a nucleic acid, protein, and membrane lipids which surround them. Generally, the proteins provide the structural shape of the virus, which include capsid proteins, matrix proteins, and membrane glycoproteins (Whittaker and Helenius, 1998; Claverie, 2006). Due to obligate parasites in nature, they can act as non-living objects outside of host cells, and when they reach the physiological system or any living host cell, they are able to cross cell membranes even several other barriers in order to transport their genetics into the cytosol or nucleus of the host cell (shown in Figure 1), which ultimately results in pathogenicity or infectious disease to the host cells (Marie, 1957). The viral infection spreads quickly, and host-cell propagation occurs in three primary stages: viral particle formation in infected host cells; virus escape into the extracellular space; and finally, virus entrance into a newly exposed cell membrane (Boulant et al., 2015). Diseases caused by viruses often start in the periphery of the body, and may migrate to the internal organs, liver, kidney, heart, gastrointestinal system, neurological system of mammals, where they can directly impact the vital organs, central nervous system (CNS) and the peripheral nervous system (PNS) (Becker, 2021).

According to the recent outbreaks and findings, viruses are still the cause of a vast variety of infectious illnesses in humans and animals, as well as a substantial proportion of newly developing and reemerging infections that are considered to be of significant health concern (Fauci, 2001). Some emerging/re-emerging notable pathogenic viral infections include SARS CoV-2 (Islam et al., 2022; Shanmugaraj et al., 2022), influenza viruses including bird flu virus (H5N1), Crimean-Congo hemorrhagic fever virus (CCHFV), Zika (ZIKAV), Nipah (NiV), Hendra virus (HeV), Ebola (Coltart et al., 2017), Monkeypox (Beer and Rao, 2019), Langya Henipavirus (Akash et al., 2022) and Marburg virus (Akash et al., 2023). Due to the rarity of Mpox, it has infected a huge number of people during the current COVID-19 pandemic still has a lot of unknowns (León-Figueroa et al., 2022; Taseen et al., 2022). Infections can spread from animals to humans, making this a

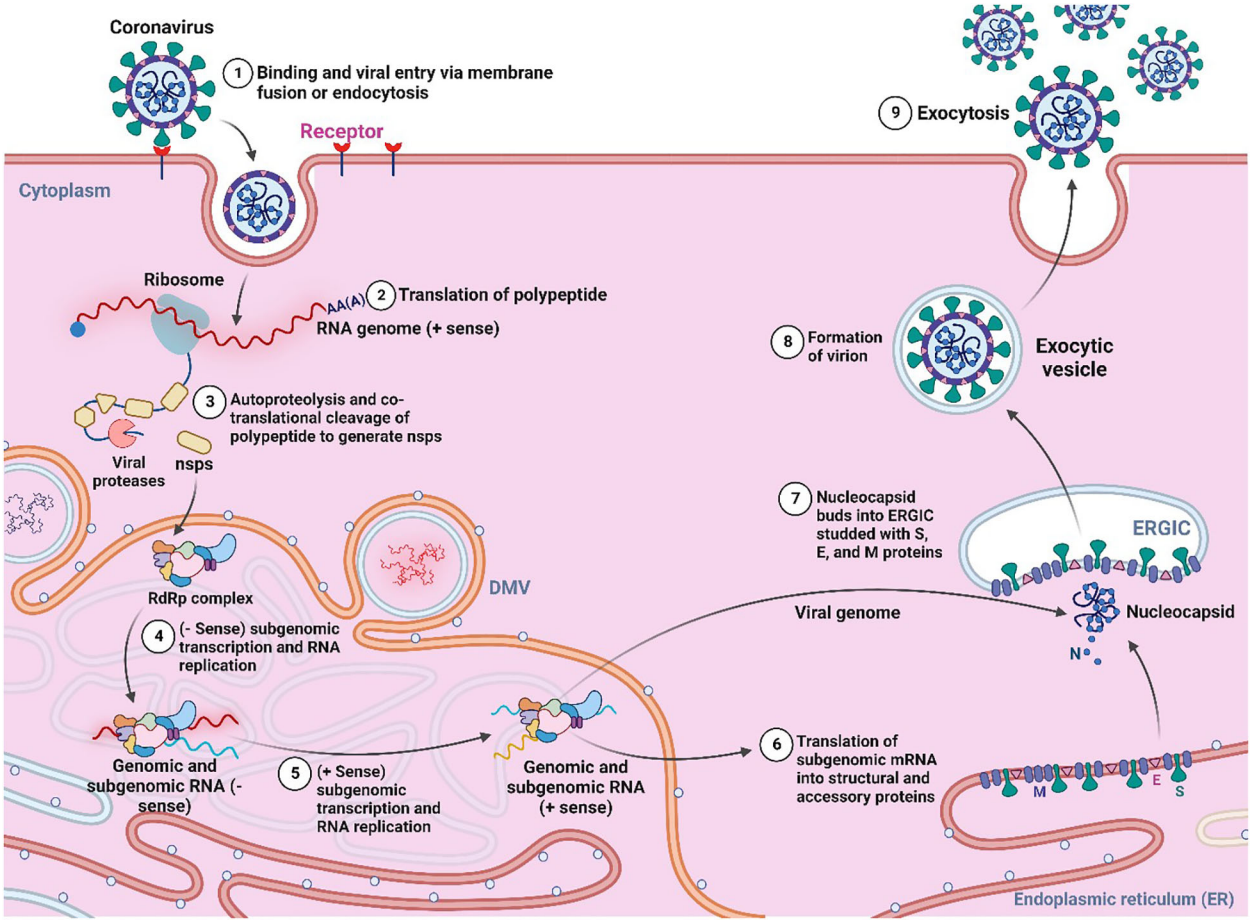


FIGURE 1
General pathogenesis and life cycle of virus (Created with BioRender.com).

zoonotic virus. Rapid human-to-human transmission has been primarily noticed with the recent re-emergence of Mpox in multiple nations, causing a worldwide health emergency. This virus is the most commonly found in Western and Central Africa (Chakraborty C. et al., 2022; Velavan and Meyer, 2022). The clinically significant illness produced by Mpox is comparable to those seen in smallpox but is not as devastating (Harapan et al., 2020; Khattak et al., 2022). This pathogenic virus was first reported in 1970 in an infant who had previously been affected by smallpox (Ladnyj et al., 1972). Despite the fact that this disease was discovered more than 60 years ago, it has lately been reported to infect SARS CoV-2 patients who have been hospitalized since the emergence of the COVID-19 pandemic (Shanmugaraj et al., 2022). Since January 2022, the World Health Organization has received reports of more than 47000 patients diagnosed with Mpox (Sharma et al., 2022). Researchers and healthcare providers are getting more concerned about the possibility of a newer pandemic of Mpox (Adalja and Inglesby, 2022; Ahmed et al., 2022). Secondly, MARV as another zoonotic pathogen has recently attracted attention of the scientists owing to its recent year's outbreaks in Ghana and Guinea (Nyakarahuka et al., 2019). In Uganda, there were reported for three separate MVD epidemics in 2007, 2012, and 2014, and thereafter from other African countries and beyond Africa, 6-8 MVD epidemics were also identified (Adjemian et al., 2011; Knust et al., 2015; Nyakarahuka et al., 2017; Zhao et al., 2021). The MARV produces severe or even life-threatening effects owing to its very high case fatality rates (Balter, 2000; Zhao et al., 2021).

There are very few approved drugs, licensed vaccine, or therapeutic without vast ranging of potential activity yet available against both the Mpox and MVD, though efforts have been made, as well currently being advanced for finding out effective prophylaxis and treatment regimens for both of the diseases (Dulin et al., 2021; Chakraborty S. et al., 2022; Hickman et al., 2022; Sah et al., 2022). However, European medical agency has approved tecovirimat drug against Mpox (Ali et al., 2023). Many researchers are trying to develop potential medication from the natural sources against mentioned disease. Currently, a number of different drugs have been shown to be effective against the Mpox when tested *in vitro*, such as Adamantane derivatives, Resveratol, Tecovirimat, Mitoxantrone, and Ribavirin. Nevertheless, their usefulness and

safety in the context of Mpox have not been shown, and further basic and clinical research is needed (Ali et al., 2023; Khan et al., 2023; Khani et al., 2023).

In addition to novel drugs, chemical ligands, broad-spectrum antivirals, and broadly neutralizing antibodies, it is worth noting that the prophylactic and therapeutic aspects of medicinal plants and herbs, their extracts and metabolites, phytochemicals, immunity-enhancing foods, and dietary nutrition have shown potentials for countering several important infectious pathogens and deadly viruses, including Mpox and MARV (Khan et al., 2023; Nguyen, 2023; Pourhajibagher and Bahador, 2023). O-rhamnosides, including Kaempferol-O-rhamnosides, are a class of polyphenolic compounds with a variety of beneficial biological effects, such as antimicrobial, antioxidant, and anti-inflammatory properties (León-Figueroa et al., 2022; Velavan and Meyer, 2022). Their propensity to bind with p-hydroxybenzoic acid in DNA may also explain why they suppress polymerase chain reaction. Virus replication is stifled as a result of this interaction, which blocks the transcription of viral DNA into RNA (Zhao et al., 2021). Therefore, Kaempferol-o-rhamnoside and its eight derivatives were chosen as the primary molecules in this investigation, and their antiviral capability was investigated against Mpox and MARV infections for their utility in treating patients with Mpox and MVD. Although these two pathogenic viruses have the feasible potential to cause another pandemic, it is unfortunate that no effective or potentially effective medication has been developed to date (Kortepeter et al., 2020). So, this investigation has been performed to identify a potential medication which may inhibit Mpox and Mpox with lower adverse effects by using Computer-Aided Drug Design (CADD) techniques and quantum chemistry of chemical descriptors. In comparison to traditional procedures, these strategies have been shown to be productive across the whole drug development process, resulting in a reduction in the amount of money and time required to produce a medicine (Rahman et al., 2012).

# Materials and methods

## Optimization and ligand preparation

O-rhamnoside is a type of carbohydrate and contains the active methyl group where one, two and three groups are attached to form the L02, L03, and L04 compounds. Kaempferol, on the other hand, is a phytochemical derived from the *Taliparitiaceae* tree and was employed as the mother compound in this investigation. Next, the Kaempferol is attached with L01, L02, L03, and L04 to form L06, L07, L08 and L09 as derivatives of Kaempferol-o-rhamnoside.

DFT functional methods were employed towards complete molecular optimization using DMol3 code from Material Studio 08 package (Kumer and Khan, 2021). To ensure accuracy, the DMol3 code was put up using the functional of B3LYP and Gaussian double zeta plus polarization function basis set (DNP). Following geometric optimization, the HOMO and LUMO molecular border orbital diagrams were split, and calculated the electron affinity, electron negativity, energy gap, chemical potential, hardness, softness, and electrophilicity index using the following equations (a-h), respectively. Finally, the optimized molecule was stored as a PDB file for future in-silico investigation, such as molecular docking, molecular dynamics, and ADMET analysis.

$$E_{gap} = (E_{LUMO} - E_{HOMO}) \ldots\ldots\ldots\ldots\ldots\ldots (a); \quad I = -E_{HOMO} \ldots\ldots\ldots\ldots\ldots\ldots (b)$$

$$A = -E_{LUMO} \ldots\ldots\ldots\ldots (c); \quad (x) = \frac{I+A}{2} \ldots\ldots\ldots\ldots (d); (\omega) = \frac{\mu^2}{2\eta} \ldots\ldots\ldots\ldots (e)$$

$$(\mu) = \frac{I+A}{2} \ldots\ldots\ldots\ldots (f); (\eta) = \frac{I+A}{2} \ldots\ldots\ldots\ldots (g);$$

$$(s) = \frac{1}{\eta} \ldots\ldots\ldots\ldots (h)$$

## termination of the data of ADMET and lipinski rule

It is critical to forecast ADMET traits during the discovery or development of a new pharmaceutical in order to prevent treatment failure throughout clinical investigations (Cheng et al., 2013). As a consequence, in-silico pharmacokinetic prediction plays a significant role. The pkCSM was utilized to create this prediction so that there was less risk of the new drug molecules failing during the trial phases and greater possibility of them making it to the final step as prospective drug candidates (Adalja and Inglesby, 2022). This prediction is based on key factors, such as molecule uptake in the human intestine, percolation capabilities of the blood-brain barrier, central nervous system, metabolism capabilities, total clearance, bioavailability, and toxicity levels.

## PASS prediction

The PASS prediction has been made to determine the capability of the ant-viral drug. The online web program PASS (http://www.pharmaexpert.ru/passonline/) was used to predict the anti-bacterial, anti-fungal, and anti-parasitic spectrum (Lagunin et al., 2000). The configurations of Kaempferol-o-rhamnoside derivatives were illustrated first, and then they were transformed into smile forms by the addition of the free online programs provided by SwissADME (http://www.swissadme.ch) (Daina et al., 2017). These programs are well-known for their ability to determine PASS spectrum by making use of the PASS web tool. PASS findings are represented by the probabilities Pa (probability for an active molecule) and Pi (probability for an inactive molecule). Pa and Pi grades may vary from 0.00 to 1.00, and Pa and Pi must be less than 1, since potentialities can be anticipated in whatever way the researcher chooses. The drug candidate should be potential and bioactive if the score of Pa > Pi (Poroikov and Filimonov, 2005).

## Preparation of protein and method for molecular docking

Molecular docking is one of the most effective ways of virtual screening, particularly when there is a computer-based drug design and three-dimensional structure of the receptor and ligand accessible. Firstly, two targeted receptor proteins, such as Mpox

(PDB ID: 4QWO), and MARV (PDB ID: 4OR8) were download from Protein Data Bank (PDB) (https://www.rcsb.org/) which were isolated by X-ray diffraction method with high stable configuration. After that, PyMol version 2021 was applied for removing all the excess heteroatom and minimized the energy by Swiss PDB viewer (Joseph and Namboothiri, 2015; Mooers and Brown, 2021). Finally, docking analysis was completed with the help of AutoDock tool and during this progres (Dallakyan and Olson, 2015), the grid box parameter was used as center X =12.4697, Y =15.9818, Z=16.0634, and Dimension (Å) X = 35.144, Y= 37.645Å, and Z= 36.966 Å for Mpox (PDB ID 4QWO). In case of MARV, center is in X = 14.267 Å, Y = 4.929, Z= 14.557, and Dimension (Å) X = 53.382 Å, Y= 48.667 Å, and Z= 66.040 Å. Three-dimensional structures of Mpox and MARV proteins are displayed in Figure 2.

## Molecular dynamics simulation

On a high-configuration computer, molecular dynamics simulation was used in live view according to the capabilities afforded by NAMD (Kumer et al., 2022b). The docking results for the most powerful drugs were validated by molecular dynamics simulations up to 100 nanoseconds for the hollow tube (drug-protein). In addition, NAMD is particularly well suited to the increasingly popular Beowulf-class PC clusters, which are quite similar to the workstation clusters for which it was originally designed. MD simulation determines the stability of molecules and verifies the docking fitting. In this study, the MD simulation was performed at 100ns and applying AMBER14 force field (Nath et al., 2021). Before that, the whole system was in the presence of a water solvent equilibrated with 0.9% NaCl at 298 K temperature in presence of liquid system with NVT ensemble having the simulation box size as boundary 1.5 and X=5, Y=5 and Z=5 which is standard for these proteins for solvation, as well as, its vector coordinate of box is at -25.06, 18.79 and -7.06, respectively. VMD evaluated the performance of this result using RMSD and RMSF. Next, the Ramachandran Plot and B-factor were evaluated to explain the validation and stability of docked complex.

# Results and analysis

## Chemistry of O-rhamnosides and Kaempferol-O-rhamnosides derivatives

In case of O-rhamnoside (L01), it is revealed that the L02, L03 and L04 are formed by attaching one, two and three O-rhamnoside rings with the original compound of O-rhamnoside, as shown in Figure 3. Moreover, the compound L01, L02, and L03 is attached with Kaempferol to form L07, L08 and L09 compounds. Finally, the computational and in silico analysis as well as SAR and comparative studies are performed on them.

## Optimized structure of derivative of O-rhamnosides and Kaempferol-o-rhamnosides

In computational chemistry, the use of quantum mechanical methods for the calculation of thermodynamic, molecular orbital, and molecule electrostatic characteristics is prevalent. The program known as Gaussian 09 has been run in order to improve the geometry and further enhance each generated derivative. The density functional theory (DFT), functional of B3LYP and Gaussian double zeta plus polarization function basis set (DNP), was utilized in order to improve and predict the molecular orbital and thermal properties of the molecules. All compounds had their dipole moment, enthalpy, free energy, and electronic energy determined. The optimized molecular structures are shown in Figure 4.

## PASS prediction

The probable biological spectrum for Kaempferol-o-rhamnoside derivatives has been predicted by applying the web server PASS. The PASS data is summarized as Pa and Pi, which are shown in Table 1. According to the presupposition in Table 1, Kaempferol-o-

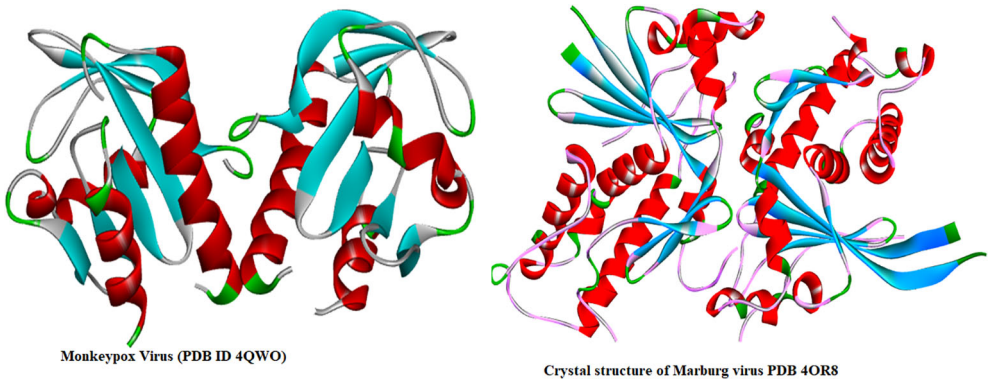


FIGURE 2
Three-dimensional protein structure of Monkeypox and Marburg viruses (Zhang et al., 2014; Minasov et al., 2022).

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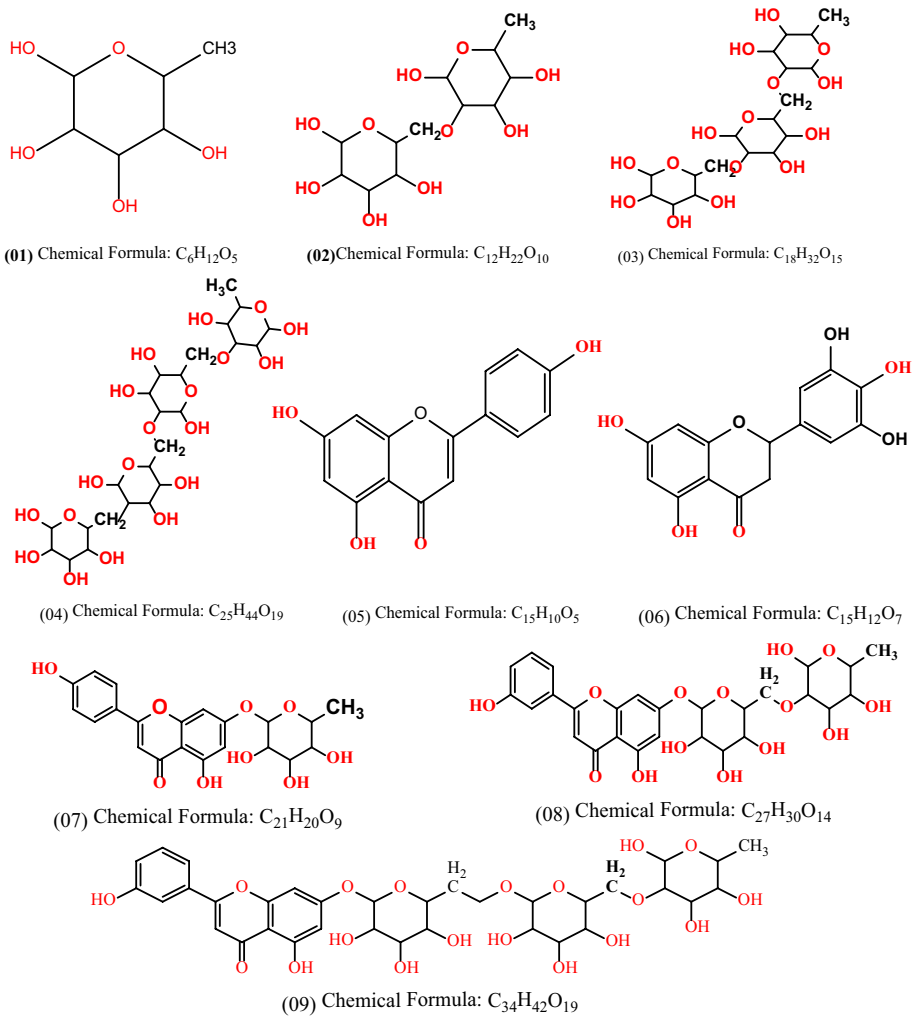


FIGURE 3
Chemical structure of derivatives of O-rhamnosides and Kaempferol-O-rhamnosides.

rhamnoside derivatives 02–09 demonstrated 0.379<Pa< 0.712 for antiviral (Influenza), 0.423<Pa<0.655 for antibacterial, 0.632< Pa< 0.755 for antifungal, and 0.214<Pa<0.391 for anti-parasitic, while Ligand 07 demonstrated Pa 0.634<pa. Consequently, the PASS assessment showed that 0.379< Pa< 0.712 for antiviral (Influenza), which indicated that the Kaempferol-O-rhamnoside derivatives have a greater potential as antiviral agent compared to other antibacterial and anti-parasitic characteristics. Although the antifungal Pa score is higher, it has been largely ignored in favor of the antiviral Pa score since researchers are more interested in discovering new antiviral drugs.

## Lipinski's rule, pharmacokinetics and drug likeness

Due to a variety of factors, the most medication candidates never become commercially available or cannot pass the clinical or preclinical stages. It is the utmost significance to create trustworthy computational approaches for the estimation of the drug-likeness of new drug candidates that increase the percentage of successful drug discovery and development attempts during the trial phase. The drug-likeness prediction model was developed by Christopher A. Lipinski in 1997, which included molecular descriptors connected to the numbers of factors that can differentiate between possible drugs and non-drugs. These factors include topological polar surface area, number of rotatable bonds, hydrogen bond acceptor, hydrogen bond donor, and molecular weight. The molecular weight of the reported Kaempferol-o-rhamnoside derivatives (L01-L09) was at 164.16-754.69, the number of rotatable bonds was 0.0-10, the hydrogen bond acceptor was 05-19, the hydrogen bond donor was 04-13, and the topological polar surface area was 90.15Å$^2$ -318.37Å$^2$. So, only Ligand of L01, L02, L06, and L07 satisfied the overall Lipinski rule (as shown in Table 2). However, the other molecules do not satisfy because they have a larger topological polar surface area, have a hydrogen bond acceptor, or are a hydrogen bond donor. Finally, the bioavailability score for ligands L01, L02, L04, and L05 is greater than 0.55, and the remaining molecules'

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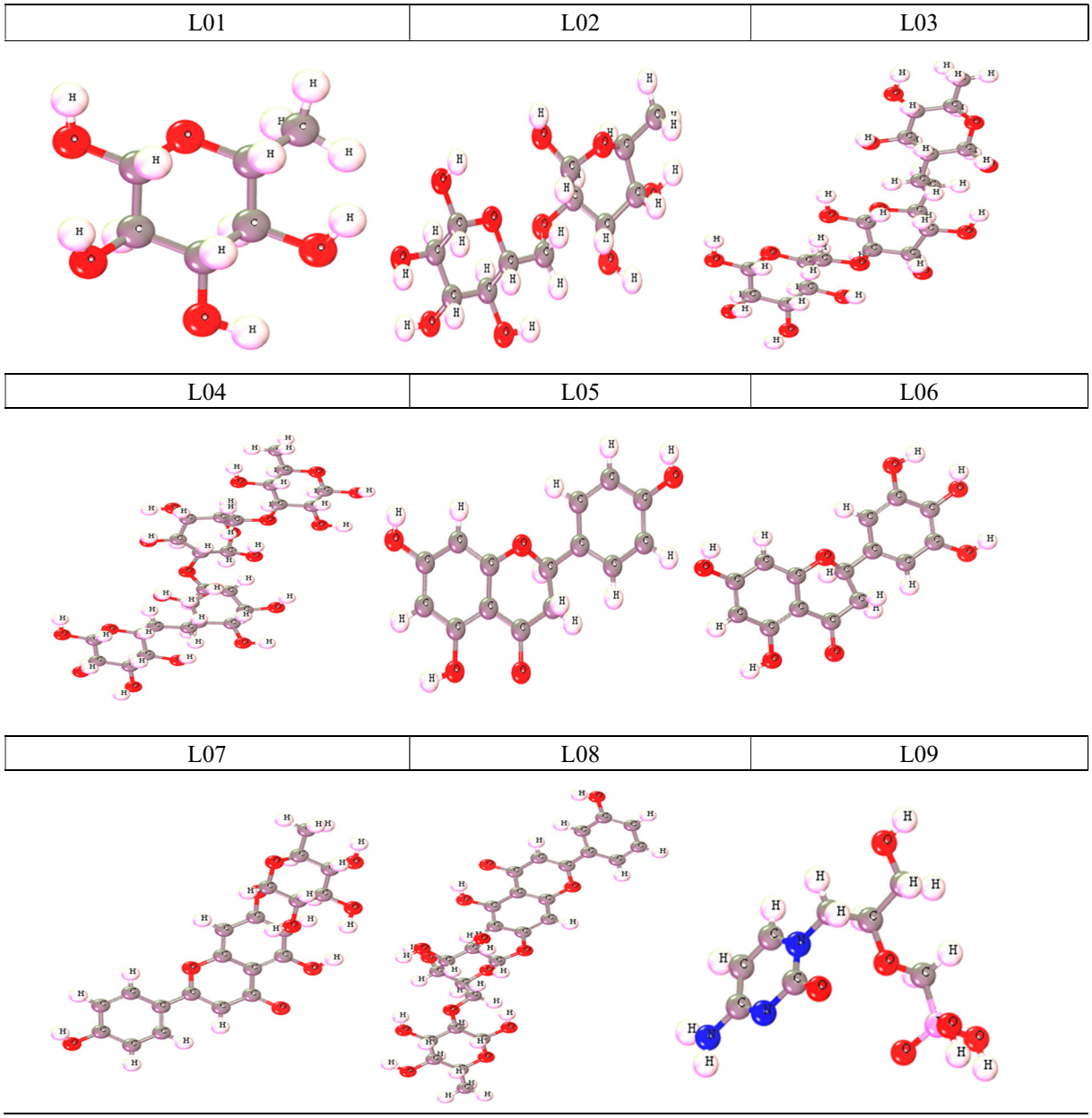


FIGURE 4
Optimized structure of derivative of O-rhamnosides and Kaempferol-o-rhamnosides.

bioavailability scores are reported at 0.17. So, the topological polar surface area or hydrogen bond acceptor and hydrogen bond donor have been abandoned.

## Quantum calculation and HOMO-LUMO and frontier molecular orbital (FMO)

Frontier molecular orbitals (FMO) are the most important orbitals in a molecule. They are used by scientists to study chemical reactivity and kinetic stability. People often talk about the highest occupied molecular orbital (HOMO) and the lowest unoccupied molecular orbital (LUMO) when talking about these molecular orbitals on the edge of the atom (LUMO). When you say "electronic absorption," you mean a change from the ground state to the first excited state. This is usually thought of as one electron moving from the HOMO state to the LUMO state (Perepichka and Bryce, 2005; Kumer et al., 2021a). A molecule's high level of chemical stability and low level of chemical reactivity are directly linked to how long its energy difference lasts (Kumer et al., 2022a). Because it makes it easier for electrons to move from one electron to another, a low energy gap is linked to low chemical stability and high chemical reactivity. Because of this, it takes a lot more energy for an electron to move from its ground state, called HOMO, to its excited state, called LUMO. Table 3 highlights the HOMO and LUMO energies, the HOMO-LUMO gap (Δ), and the hardness (η)

TABLE 1 Data of PASS prediction.

| Data of PASS Prediction | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Ligand No | Anti-viral (Influenza) | | Anti-bacterial | | Anti-fungal | | Anti-parasitic | |
| | Pa | Pi | Pa | Pi | Pa | Pi | Pa | Pi |
| L01 | 0.659 | 0.009 | 0.571 | 0.011 | 0.632 | 0.015 | 0.214 | 0.095 |
| L02 | 0.633 | 0.010 | 0.589 | 0.009 | 0.657 | 0.013 | 0.371 | 0.037 |
| L03 | 0.463 | 0.029 | 0.623 | 0.028 | 0.735 | 0.008 | 0.377 | 0.064 |
| L04 | 0.415 | 0.042 | 0.643 | 0.077 | 0.750 | 0.007 | 0.290 | 0.059 |
| L05 | 0.633 | 0.010 | 0.589 | 0.009 | 0.657 | 0.013 | 0.371 | 0.037 |
| L06 | 0.712 | 0.005 | 0.423 | 0.025 | 0.604 | 0.018 | 0.365 | 0.039 |
| L07 | 0.700 | 0.005 | 0.629 | 0.007 | 0.752 | 0.007 | 0.634 | 0.008 |
| L08 | 0.579 | 0.015 | 0.635 | 0.007 | 0.755 | 0.007 | 0.391 | 0.033 |
| L09 | 0.379 | 0.053 | 0.655 | 0.006 | 0.751 | 0.007 | 0.216 | 0.090 |

and softness (S) indexes of all derivatives of Kaempferol-o-rhamnoside, while Figure 5 highlights, for better understanding, the frontier orbital diagrams of both HOMO and LUMO, as shown using a variety of color schemes to facilitate better comprehension. In the context of HOMO, the shade of deep radish signifies the positive nodes of orbitals, while the shade of yellow corresponds to the negative node of orbitals.

As indicated in Table 3 and Figure 5, the energy gap value of Kaempferol-O-rhamnoside derivative 01-04 is consistently larger (9.524, 9.311, 9.095 and 9.303 eV) compared to other considered molecules. The range of chemical hardness lies between 4.762 and 3.477, while the softness values of the Kaempferol-O-rhamnoside derivatives extend from 0.2100 to 0.2876. It's noticeable that hardness and softness share a reciprocal relationship; a decrease in hardness consequently corresponds to an increase in softness. In the case of ligands, the least hardness is observed alongside the highest softness.

From the Figure 5, it is observed that the LUMO is scattered throughout in whole molecules for L01 and L02, but it is different for the other ligands (L03-L09). The LUMO is stayed in the O-rhamnoside for L01 to L09 but the HOMO is found in the kaempferol ring.

## Molecular electrostatic potential charge distribution mapping

The Molecular Electrostatic Potential, also known as MEP, is utilized as a global reactivity map. This map illustrates the amount of an organic molecule that is most equipped for the electrophilic and nucleophilic onslaught of charged point-like reagents (Rahman et al., 2022). It is helpful in understanding the biological recognition system and the coupling of hydrogen bonds. This MEP counter map

TABLE 2 Data of lipinski rule, pharmacokinetics and drug likeness.

| Data of Lipinski rule, Pharmacokinetics and Drug likeness | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Ligand No. | Molecular weight | Number of rotatable bonds | Hydrogen bond acceptor | Hydrogen bond donor | Topological polar surface area (Å$^2$) | Lipinski's rule | | Bioavailability Score |
| | | | | | | *Result* | *Violation* | |
| L01 | 164.16 | 00 | 05 | 04 | 90.15 | Yes | 00 | 0.55 |
| L02 | 326.30 | 03 | 10 | 07 | 169.30 | Yes | 01 | 0.55 |
| L03 | 486.46 | 06 | 14 | 10 | 239.29 | No | 02 | 0.17 |
| L04 | 648.61 | 09 | 19 | 13 | 318.37 | No | 03 | 0.17 |
| L05 | 488.44 | 06 | 15 | 10 | 248.45 | No | 02 | 0.17 |
| L06 | 304.25 | 01 | 07 | 05 | 127.45 | Yes | 00 | 0.55 |
| L07 | 416.28 | 03 | 09 | 05 | 149.82 | Yes | 00 | 0.55 |
| L08 | 578.82 | 06 | 14 | 08 | 228.97 | No | 03 | 0.17 |
| L09 | 754.69 | 10 | 19 | 11 | 308.12 | No | 12 | 0.17 |

TABLE 3 Data of chemical descriptors.

| Data of chemical descriptors | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| S/N | A=-LUMO | I=- HOMO | Energy =I-A | Chemicalpotential | Electronegativity | Hardness | Softness | Electrophilicity |
| L01 | -1.377 | -10.901 | 9.524 | 6.139 | -6.139 | 4.762 | 0.2100 | -3.957 |
| L02 | -1.461 | -10.772 | 9.311 | 6.116 | -6.116 | 4.655 | 0.2148 | -4.018 |
| L03 | -1.184 | -10.279 | 9.095 | 5.731 | -5.731 | 4.547 | 0.2199 | -3.611 |
| L04 | -0.954 | -10.257 | 9.303 | 5.605 | -5.605 | 4.655 | 0.2150 | -3.377 |
| L05 | -1.219 | -8.868 | 7.649 | 5.043 | -5.043 | 3.824 | 0.2615 | -3.325 |
| L06 | -1.276 | -9.033 | 7.757 | 5.115 | -5.115 | 3.878 | 0.2578 | -3.425 |
| L07 | -1.590 | -8.607 | 7.017 | 5.098 | -5.098 | 3.508 | 0.2850 | -3.704 |
| L08 | -1.828 | -8.783 | 6.995 | 5.305 | -5.305 | 3.477 | 0.2876 | -4.047 |
| L09 | -1.660 | -8.617 | 6.957 | 5.138 | -5.138 | 3.478 | 0.2875 | -3.795 |

offers a straightforward method for forecasting how various types of geometry could interact with one another. The significance of molecular electrostatic potential (MEP) arises from the fact that it concurrently demonstrates a molecular shape and size and also positive, negative, and neutral electrostatic potential provinces in terms of visual assessment. In addition to this, MEP is very effective in the investigation of macromolecules with physical and chemical features. On the basis of the optimized structure of the Kaempferol-O-rhamnoside derivatives (L01-L09), MEP was utilized to make a prediction about the active sites on the surface that would be susceptible to electrophilic and nucleophilic interactions. The different electrostatic potential has been shown by the red and blue hue. The red color indicates the maximum negative area, which is a significant site for electrophilic interactions; the blue color represents the largest positive region, which is an acceptable site for nucleophilic interactions; and the green color represents the neutral potential area. (See Figure 6).

## Molecular docking

The forces between molecules that occur between the ligand and the receptor during the formation of a protein-ligand complex are called binding affinity. This method has grown in popularity as a result of the research and investigation of innovative drug design (Meng et al., 2011). A binding energy of -6.00 kcal/mole has been speculated as a potential drug candidate (Kumer et al., 2021b). In this experiment, the Auto-Dock Vina program was used to investigate a set of Kaempferol-o-rhamnoside derivatives by the in-silico method in order to emphasize their putative binding energy and interaction configurations with the targeted protein of Mpox (PDB: 4QWO) and MARV (PDB: 4OR8). In light of the findings that were acquired from the docking inspection, the three derivatives with the highest binding energies were found. The results of computed analysis showed that three derivatives L07, L08, and L09 had potential docking scores of -9.2 (> -6.0), -9.0 (>-6.0) and -9.4(>-0.6) against protein Mpox (PDB: 4QWO). Also, these three derivatives L07, L08, and L09 had potential docking scores -8.1 (> -6.0), -8.4(>-0.6) and -9.0(>-0.6) against Crystal Structure of the MARV (PDB: 4OR8). Where the traditional drug Cidofovir had the docking score -6.0(=-6.0) and -5.2(<-6.0) against protein Mpox (PDB: 4QWO) and Crystal Structure of the MARV (PDB: 4OR8) respectively. It demonstrates that L07, L08, and L09 substances can firmly bind to the protein receptors' pockets than Cidofovir (as shown in Table 4). Besides, it is seen that by increasing the side chain, the binding energy is equally increased. It has been said that compared with standard antiviral drugs like Cidofovir, Ligand 03–09 shows better effectiveness.

## Protein-ligand interaction

The Kaempferol-o-rhamnoside derivatives generated a wide variety of different types of non-covalent interactions with the active sites of the microbial proteins. These observed bonds included pi-alkyl, pi-anion, pi-sigma, amide pi-stacked, and pi-donor hydrogen bonds. Based on these results, it is abundantly obvious that aromatic substituent may quickly enhance the binding ability of uridine esters as well as their potential to inhibit the growth of reported viral pathogens due to the high electron density of aromatic substituent. The influence of H-bonds had a significant impact on the selectivity of ligand binding to the receptor, drug design in chemical and biological processes, and molecular recognition and biological activity. Therefore, the H-bond surface of all derivative ligands that interact with both proteins is presented in Figure 7. The 2D structure of protein ligand interaction also illustrates how to determine how many bioactive sides are present.

## Molecular dynamics simulation

Molecular dynamics (MD) simulations are effective tools for comprehending the physiological underpinnings that encompass the structure and function of bioactive molecules (Barroso da Silva et al., 2020). The initial conception of proteins as relatively inflexible arrangements has been superseded by a dynamic model according

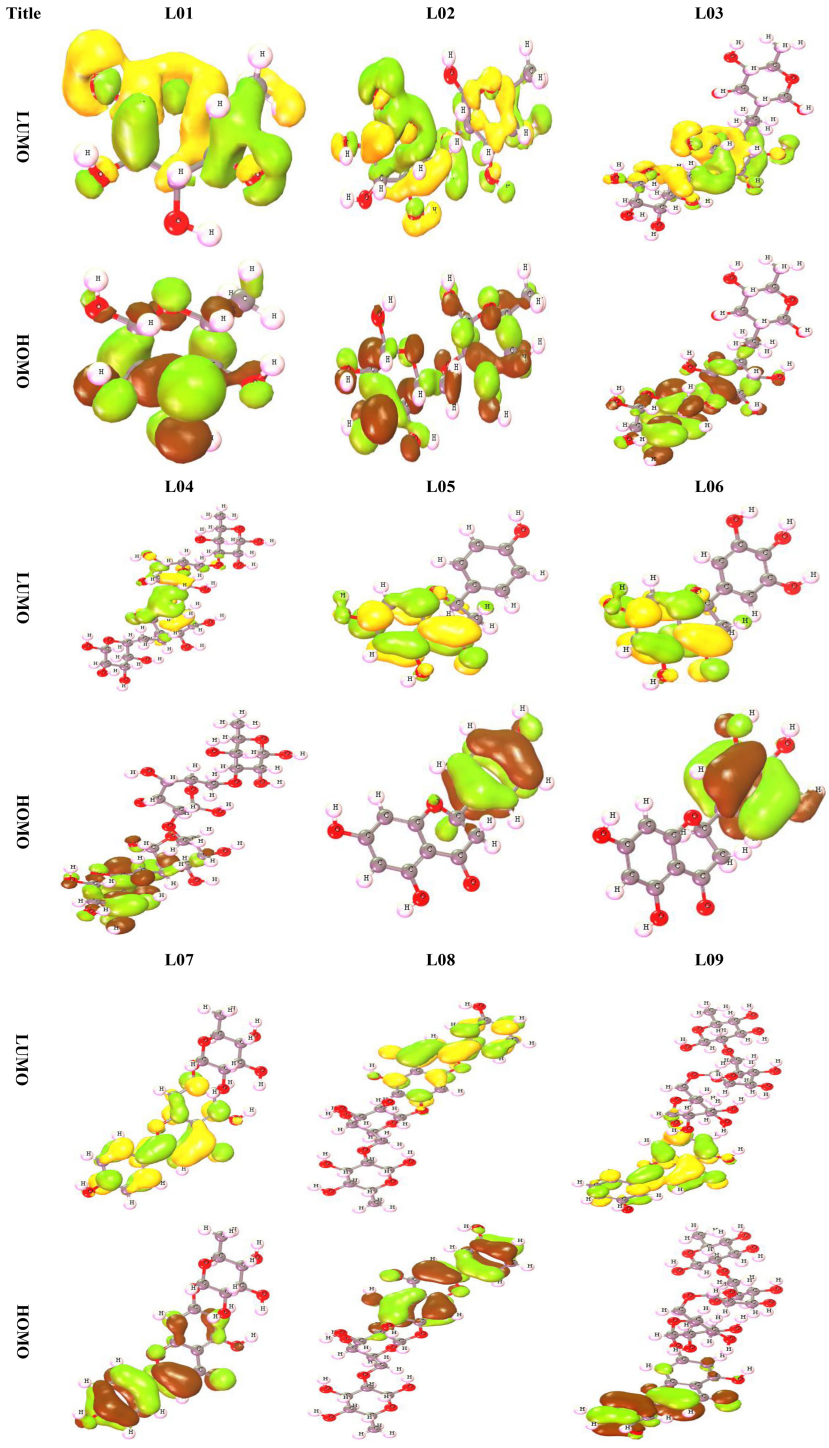


FIGURE 5
Frontier Molecular orbital for derivative of O-rhamnosides and Kaempferol-o-rhamnosides.

to which the internal movements of macromolecules and the conformational changes that follow from those movements play a significant key role in the development of new drug molecules. This MD simulation is a helpful tool for predicting the stability of drug-like molecules (Hickman et al., 2022). The outcome of MD simulation is described by RMSD and RMSF, which mean root-mean-square deviation and root-mean-square fluctuation. Stable molecules are biological molecules with a RMSD and RMSF score of less than 2.0 A° (Kawsar et al., 2022). So, based on maximum docking outcomes, Ligands of L07, and L09 have been conducted in MD simulation as well as standard Cidofovir (D1). According to the finding of MD simulation against Mpox (PDB ID 4QWO), the

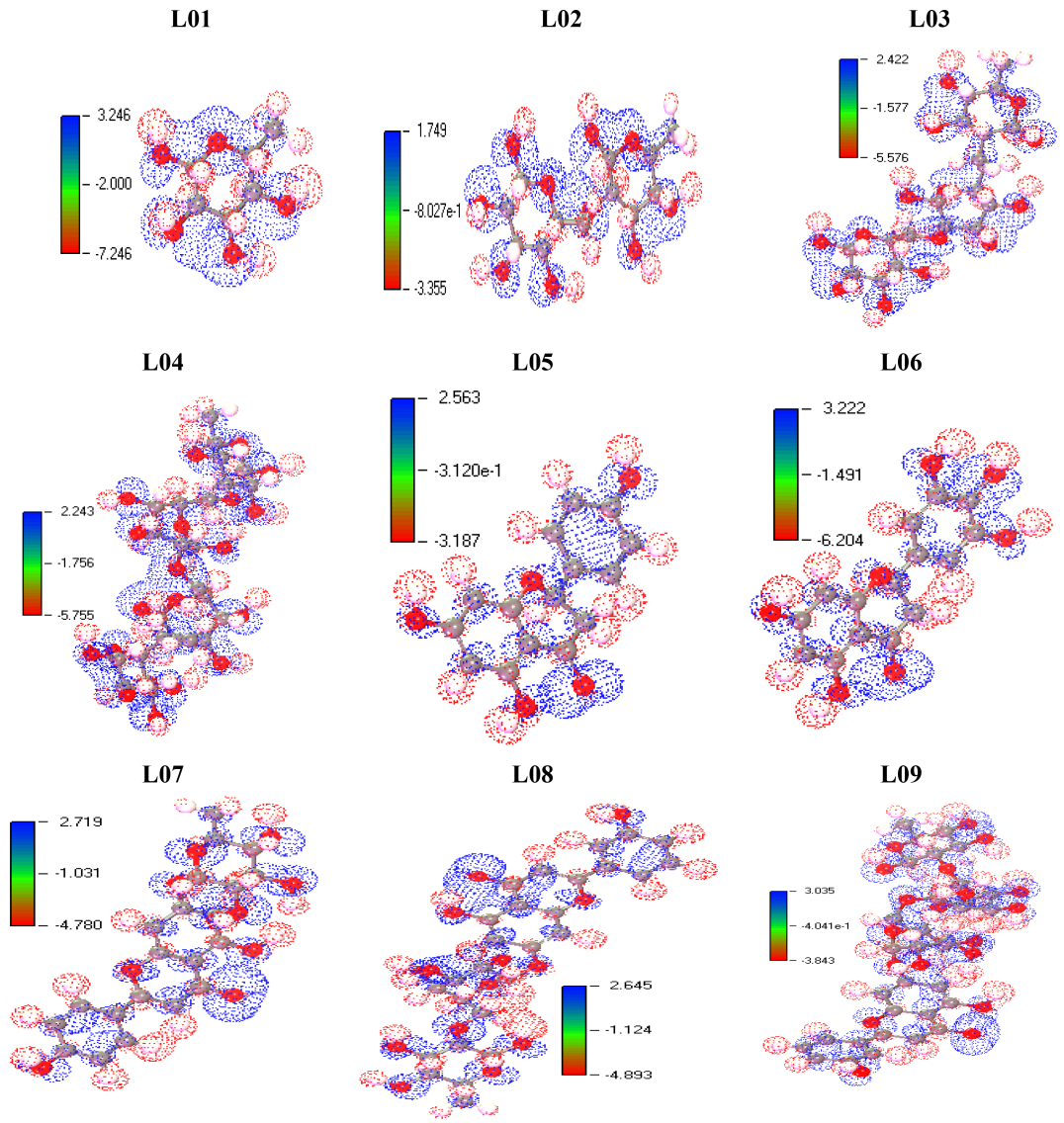


FIGURE 6
MEP mapping for the ligands after DFT optimization.

RMSD is about 0.9 Å –1.2 Å and similar outcomes were found in terms of RMSD in terms of backbone with amino acid residues. But, in RMSF, little impact has been seen in the addition of backbone with amino acid residues and there is fluctuation 0.7 Å – 0.8 Å (as shown in Figures 8A, B).

Secondly, the MD simulation has been completed for ligands 08 and 09 towards the MARV (PDB 4OR8). In this case, the RMSD and RMSF have been obtained with equivalent outcomes, and the RMSD is about 0.9 Å – 1.2 Å both time vs. backbone and backbone with amino acid residues, while the RMSF score at 0.7 Å – 0.8 Å (as shown in Figures 8C, D). In summary, the RMSD and RMSF scores are accepted and less than 2.00 Å which means these drugs could be stable after reaching biological systems.

TABLE 4 Binding affinity (kcal/mol) against Monkeypox and Marburg virus.

| Drug Molecules No. | Binding Affinity(kcal/mol) against *Monkeypox and Marburg virus* | |
|---|---|---|
| | *Monkeypox virus (4QWO)* | *Crystal structure of Marburg virus (4OR8)* |
| L01 | -5.6 | -5.0 |
| L02 | -6.2 | -5.8 |
| L03 | -8.0 | -6.4 |
| L04 | -8.1 | -7.0 |

*(Continued)*

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TABLE 4 Continued

| Drug Molecules No. | Binding Affinity(kcal/mol) against *Monkeypox and Marburg virus* | |
|---|---|---|
| | *Monkeypox virus (4QWO)* | *Crystal structure of Marburg virus (4OR8)* |
| L05 | -7.4 | -6.4 |
| L06 | -7.5 | -6.7 |
| L07 | -9.2 | -8.1 |
| L08 | -9.0 | -8.4 |
| L09 | -9.4 | -9.0 |
| Cidofovir(D1) | -6.0 | -5.2 |

## Ramachandran plot from molecular dynamic study

The Ramachandran plot is one of the most important parameters for checking chemical stability of the protein or enzyme structure satisfying both stereochemistry and steriochemical structure. In addition, it is also used for validation of docking and stability of docked complex of protein ligand. Also, this analysis illustrates the energy minimization of Botherombin and most favorable region. From the Figure 9, the Ramachandran plot is showed for the top three drugs (L07, L08 and L09), and compare with standard drugs (D1). There are 225 amino acids rasidues where 216 amino acid residues (96.0%) are out of favorable region, and 9 amino acids (4.00%) in unfavorable region for L07

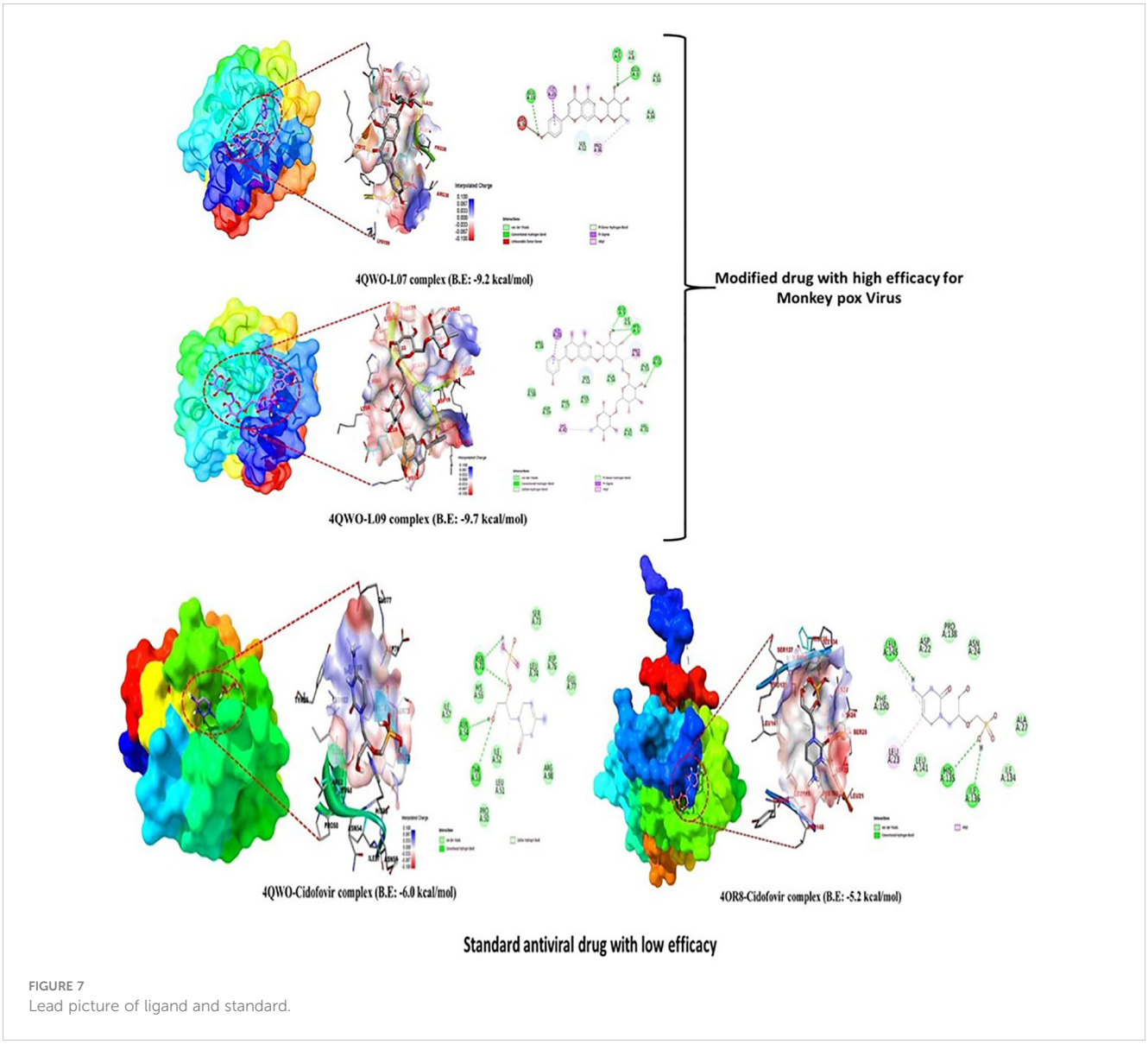


FIGURE 7
Lead picture of ligand and standard.

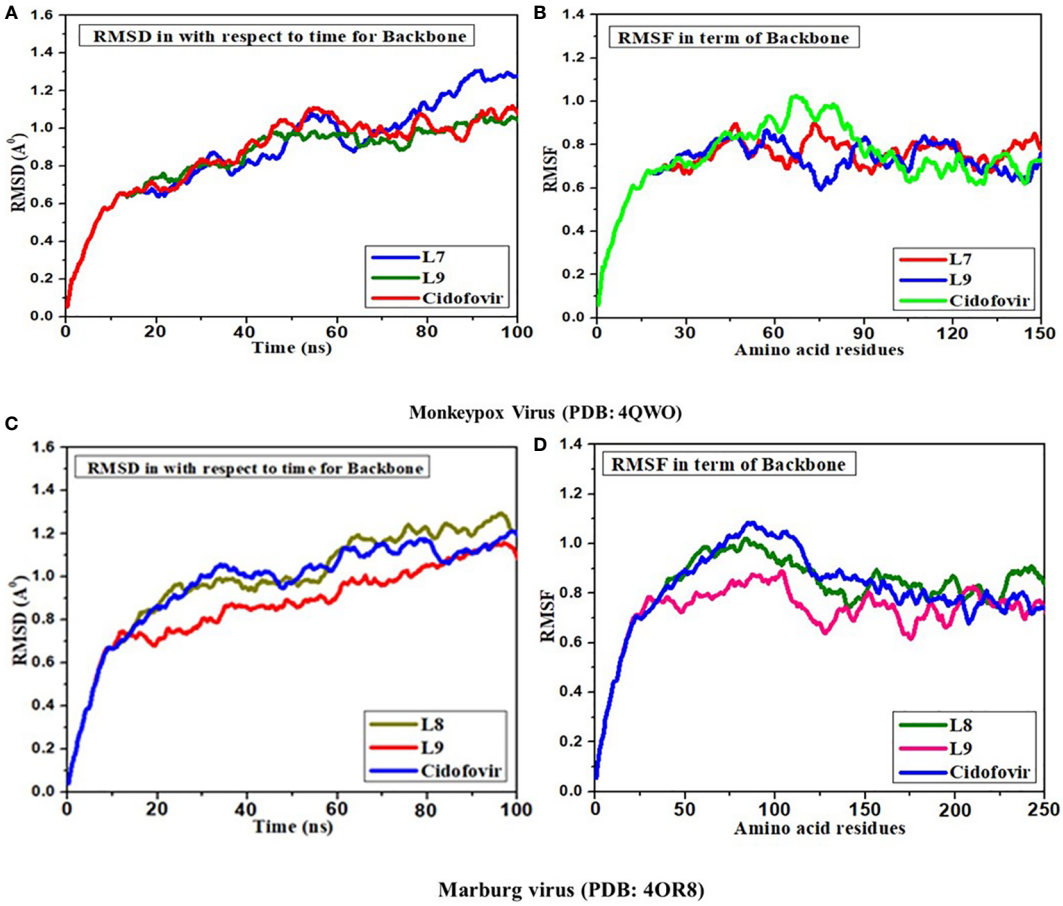


FIGURE 8
Analysis of **(A)** RMSD; **(B)** RMSF for L07, L09 and Cidofovir drugs in complex with Monkeypox virus **(C)** RMSD and **(D)** RMSF for L08, L09 and Cidofovir drugs in complex with Marburg virus.

which is almost similar trend for the L08, L09 and D1, means that there are no change after entering the ligand in protein. So that, it can be concluded that the molecular docking is valid and highly stable docked complexes were obtained after docking.

## B-factor in case of normal mode analysis (NMA) from molecular dynamic study

The expression B-factor, occasionally entitled the Debye-Waller factor, temperature factor, or atomic displacement parameter is to show by curve. In gerenal, the most significance of it is to express the higher flexibility results in larger displacements or lower electron density, indicating the lower stability. So after molecular docking, the B-factor of docked protein complex can be calculated that helps to say its deformation or stability at each of its residues by Normal Mode Analysis (NMA) of protein. From the Figure 10, the NMA of docked complex has been shown where the value of NMA is almost 0.8 or less than 1.0 for all residues for L07, L08, L09, and D1, that ensure the stability and validation of docking procedure.

## ADME and toxicity data

The present system of advanced manufacturing drug development includes the use of in silico techniques as an essential element. These tools are used to explore the absorption, distribution, metabolism, and excretion (ADME-PK) features of novel chemical substances. So, this ADMET analysis was performed to examine both the pharmacokinetic (PK) features and the toxicity of the compound. Table 5 provides an overview of the compiled data for ADMET prediction. The Water solubility Log S standard score is considered to be from -4.0 to -6.0 and -2.0 to -4.0, respectively, for minimum and maximum solubility substances (Rout et al., 2022). Our finding log S is predicted to be -0.152 to -3.543, which falls in the range of maximum aqueous solubility. Substances (L01, L04, L06, L07, and L08) have a high rate of absorption, according to the results of the human intestinal absorption test (HIA) (HIA of more than 30% is considered a high absorption rate) (Pires et al., 2015). Additionally, the Caco-2 cell permeability measure was used to assess the absorption of the substances that were investigated. According to the findings, only

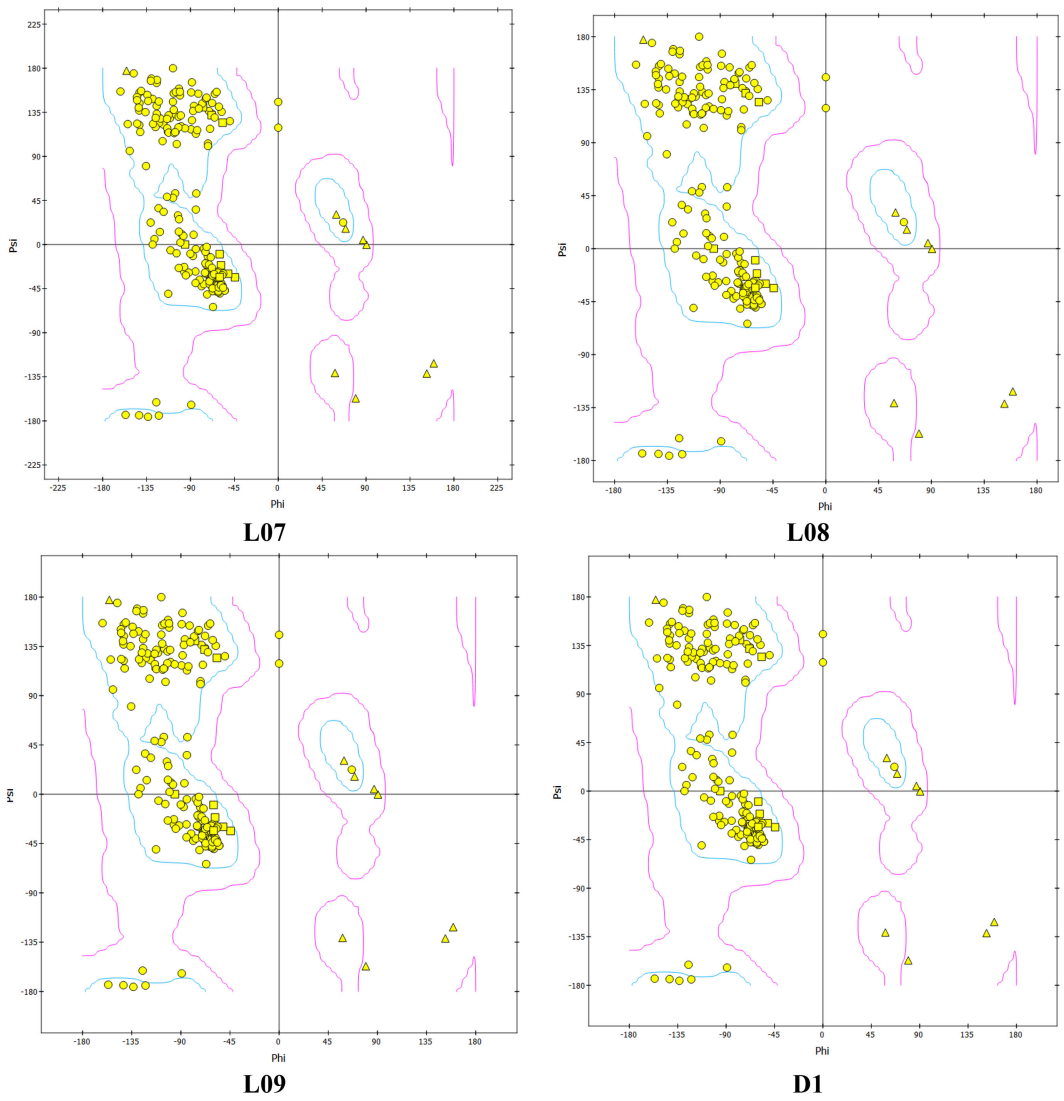


FIGURE 9
Ramachandran Plot from Molecular Dynamic Study for L07, L08, L09 and D1 for Monkeypox virus.

three of the molecules exhibit Caco-2 cell permeability in a favorable manner. The VDSs (human) have been obtained from -0.095 to 0.519, which indicates better distribution. Consequently, no single compound can cross the BBB. Furthermore, the inhibitory or substrate response of the cytochrome P450 enzymes was used to confirm the metabolic pathways of the substances that were under investigation (CYPs). This enzyme is crucial to the oxidation process and helps ease the removal of foreign organic molecules like medicines. It also serves a crucial function in the production of energy in the cell. Ligands 04, 06 and 07 found to be actively inhibited the CYP450 1A2 Inhibitor, while no compounds were found to be CYP450 2C9 Substrate. In excretion prediction, the rate of total clearance is about 0.066 ml/min/kg to -6.618 ml/min/kg, which indicates a better clearance rate than clearance rate or negative score. The results of the toxicity tests, which included the AMES toxicity test and the hepatotoxicity test, suggest that the anticipated chemicals are safe, excluding 04, 06, and 07. On the other hand, the maximum tolerated dose in humans was in the range of 0.295–2.193 mg/kg/day.

## Conclusion

Kaempferol-O-rhamnoside derivatives (L01-L09) have been studied for their potential as agonists to suppress Mpox and MARV infections; these compounds have been modeled using computational tools using DFT method, as well as molecular docking, molecular dynamics simulation, and in silico study.

In addition, investigations are conducted on pharmacokinetics, ADMET, drug-likeness, HOMO/LUMO gap, molecular electrostatic potential, and PASS prediction in an effort to offer them with an effective antiviral drug against Mpox and MARV infections. With beginning, the PASS prediction investigation outcome obtained at 0.379< Pa<0.712 for anti-viral (Influenza), 0.423< Pa<0.655 for anti-

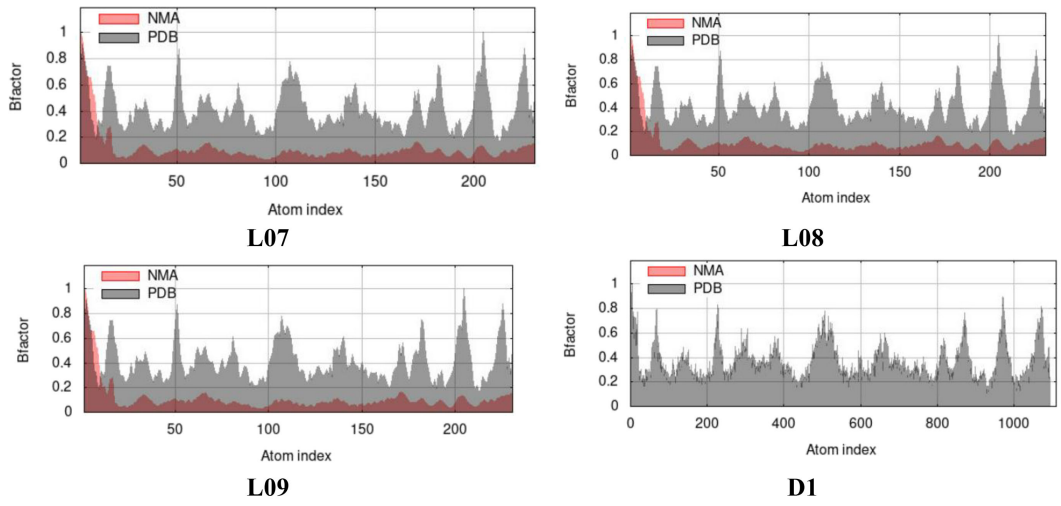


FIGURE 10
B-factor from Molecular Dynamic Study.

TABLE 5 ADME and Toxicity prediction.

| ADME and Toxicity prediction | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| S/N | Absorption | | | Distribution | | Metabolism | | Excretion | | Toxicity | | |
| | Water solubility Log S | Human Intestinal Absorption (%) | Caco-2 Permeability +/- | VDss (human) | BBB Permeability +/- | CYP450 1A2 Inhibitor | CYP450 2C9 Substrate | Total Clearance (ml/min/kg) | Renal OCT2 substrate | AMES toxicity | Max. tolerated dose (human) mg/kg/day | Hepatotoxicity |
| L01 | -0.252 | 61.19 | + | -0.095 | – | No | No | 0.585 | No | No | 2.193 | No |
| L02 | -0.152 | 15.79 | – | 0.519 | – | No | No | 1.481 | No | No | 1.375 | No |
| L03 | -1.226 | 3.49 | – | -0.156 | – | No | No | 1.642 | No | No | 0.396 | No |
| L04 | -2.892 | 100 | – | 0.008 | – | Yes | No | 6.918 | No | Yes | 0.438 | No |
| L05 | -1.177 | 0.00 | – | -0.201 | – | No | No | 1.564 | No | No | 0.523 | No |
| L06 | -3.543 | 65.97 | + | 0.13 | – | Yes | No | 0.455 | No | Yes | 0.904 | No |
| L07 | -2.892 | 83.25 | + | 0.011 | – | Yes | No | 35.56 | No | Yes | 0.438 | No |
| L08 | -2.890 | 39.94 | – | 0.026 | – | No | No | 0.131 | No | No | 0.438 | No |
| L09 | -2.881 | 11.41 | – | -0.265 | – | No | No | 0.066 | No | No | 0.295 | No |

"+ " meaning Present/Positive & "-" meaning Absent/Negative.

bacterial, 0.632< Pa<0.755 for antifungal, and 0.214< Pa<0.391 for anti-parasitic, so it can be said that they have a high possibility against viral pathogens. Next, molecular docking has been described as the most important part of this investigation, and it has achieved an acceptable docking score, which was even higher than the FDA-approved anti-viral Cidofovir drug. In accumulation, there are formed the hydrogen bonds after docking that conveys the more stability of protein as inhibitor. All of designed drugs showed the negative value of BBB permeability, CYP4501A2 inhibitor, CYP4502C9 substrate and Renal OCT2 substrate even they have no AMES toxicity. In case of quantum calculation, the energy gap is about 9.00 for 1$^{st}$ four ligands (L01-L04) which is more reactive, and it is slightly lower for next class that is about 7.0 or below. However, the softness of L01 –L04 is about 0.21 where the softness for other is about 0.25 to 0.27. However, the 2$^{nd}$ series of ligand (L05-L09) after joining is more active in case of docking but chemical more stable where L07, L08 and L09 show higher binding score. So, it can be said that with increasing the chain length, the binding capacity increases.

## Data availability statement

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding authors.

## Author contributions

AK, NM- Conceptualization, study design, writing, data acquisition. MA- Doing the simulation, DFT calculation and data analysis. AC, SM,

AG, UC, SA, AK, AAla- Writing and data acquisition. AD, RS, AG, AAle and K-TC – Review, editing, and supervision. All authors contributed to the article and approved the submitted version.

## Acknowledgments

This work was funded by the Researchers Supporting Project Number (RSP2023R339) at King Saud University, Riyadh 11451, Saudi Arabia. This study was supported by a grant (no. RA112001) from the Tainan Municipal Hospital (managed by Show Chwan Medical Care Corporation), Tainan, Taiwan. The authors extend their appreciation to their respective institutes for providing facilities to conduct this research.

## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

## Publisher's note



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frontiers | Frontiers in Cellular and Infection Microbiology

TYPE Original Research
PUBLISHED 29 May 2023
DOI 10.3389/fcimb.2023.1159389



OPEN ACCESS

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RECEIVED 05 February 2023
ACCEPTED 10 May 2023
PUBLISHED 29 May 2023

CITATION
Ajmal A, Mahmood A, Hayat C, Hakami MA, Alotaibi BS, Umair M, Abdalla AN, Li P, He P, Wadood A and Hu J (2023) Computer-assisted drug repurposing for thymidylate kinase drug target in monkeypox virus.
*Front. Cell. Infect. Microbiol.* 13:1159389.
doi: 10.3389/fcimb.2023.1159389



# Computer-assisted drug repurposing for thymidylate kinase drug target in monkeypox virus

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**Introduction:** Monkeypox is a zoonotic disease caused by brick-shaped enveloped monkeypox (Mpox) virus that belongs to the family of ancient viruses known as Poxviridae. Subsequently, the viruses have been reported in various countries. The virus is transmitted by respiratory droplets, skin lesions, and infected body fluids. The infected patients experience fluid-filled blisters, maculopapular rash, myalgia, and fever. Due to the lack of effective drugs or vaccines, there is a need to identify the most potent and effective drugs to reduce the spread of monkeypox. The current study aimed to use computational methods to quickly identify potentially effective drugs against the Mpox virus.

**Methods:** In our study, the Mpox protein thymidylate kinase (A48R) was targeted because it is a unique drug target. We screened a library of 9000 FDA-approved compounds of the DrugBank database by using various in silico approaches, such as molecular docking and molecular dynamic (MD) simulation.

**Results:** Based on docking score and interaction analysis, compounds DB12380, DB13276, DB13276, DB11740, DB14675, DB11978, DB08526, DB06573, DB15796, DB08223, DB11736, DB16250, and DB16335 were predicted as the most potent. To examine the dynamic behavior and stability of the docked complexes, three compounds—DB16335, DB15796, and DB16250 —along with the Apo state were simulated for 300ns. The results revealed that compound DB16335 revealed the best docking score (-9.57 kcal/mol) against the Mpox protein thymidylate kinase.

**Discussion:** Additionally, during the 300 ns MD simulation period, thymidylate kinase DB16335 showed great stability. Further, *in vitro* and *in vivo* study is recommended for the final predicted compounds.

KEYWORDS

monkeypox, homology modeling, molecular docking, MD simulation, drugs development

# 1 Introduction

The Mpox virus is widespread primarily in West and Central Africa and is a member of the Orthopoxvirus genus, which also include cowpox, vaccinia, variola, and smallpox (MacNeil et al., 2009). In Africa, various strains of Mpox have fatality rates ranging from 3.6% to 10.6% (Bunge et al., 2022), raising the possibility of increasing lethality. Recently, travelers have confirmed cases of monkeypox exported to Singapore (Yong et al., 2020), the United Kingdom (MacNeil et al., 2009), Israel, and the United States (Erez et al., 2019). Mpox is spread through direct contact with an animal's body secretions or through animal bites. However, it can also be transmitted through breathing droplets during close and prolonged face-to-face contact, through direct contact with an infected person's body fluids, or *via* objects contaminated with virus particles (Farahat et al., 2022; Lam et al., 2022).

Monkeypox is considered a sexually transmitted disease (STD) in the current outbreak. The smallpox vaccine is 85% effective at preventing monkeypox (Vallée et al., 2022). Malaise, rash, headaches, and fever between 38.5 and 40.5 degrees Celsius are some of the symptoms of monkeypox. The presence of hard, deep, and umbilicated lesions along with swollen lymph nodes are also some of the symptoms (MacNeil et al., 2009). The incubation period for Mpox lasts from one week to 17 days, with the fever going away after three days following the appearance of the rash. The lesions are described as painful, stiff, and swollen. It has been proposed that lymphadenopathy, which occurs in Mpox but not smallpox, causes a higher immune response than smallpox (Lam et al., 2022). Sepsis caused by lesions has been recorded, but it is generally believed to be uncommon (Reynolds et al., 2017). The central regions of the Mpox genome, which contain crucial enzymes and proteins, have 96.3% similarity with the smallpox genome, according to a prior study (Shchelkunov et al., 2001). The genome of Mpox is linear and has double double-stranded DNA with a genome size of 197 kb (Kugelman et al., 2014). A48R is a thymidylate kinase that has previously been found to bind with thymidine diphosphate. It is a unique target because no recognized drugs currently target it. The substantial structural difference from human thymidylate kinase at the active site makes it an attractive target (Caillat et al., 2008). According to reports, smallpox immunizations are 85% effective against monkeypox (Fine et al., 1988) (Grant et al., 2020) (Reynolds and Damon, 2012),,. Since the illness was eradicated in 1980, smallpox immunization programs have ceased (Belongia and Naleway, 2003). Although the use of smallpox medications against monkeypox is advised (Rizk et al., 2022), their safety and efficacy in human beings have not yet been determined (Sherwat et al., 2022). The present surge, therefore, indicates the urgent need for developing Mpox-specific drugs and therapies.

Finding new drugs requires several steps, including target identification, lead identification, animal studies, and clinical trials, which can typically take at least ten to twelve years to complete. Consequently, drug repurposing is a desirable substitute in the epidemic's situation (Yong et al., 2020). The strategy of repurposing drugs has numerous advantages, including a significant decrease in testing time (Sherwat et al., 2022). In particular, drugs that have already received approval for treating different illnesses have undergone comprehensive toxicity testing and can thus be given safely to the general public (Yong et al., 2020). The drug discovery process now includes computational methods that allow the screening of small molecule libraries to identify lead candidates that can be optimized to find potential medications for clinical testing (Ashburn and Thor, 2004) (Sliwoski et al., 2014),. The crystal structure of the TMPK enzyme of Mpox was not available in the PDB database so we developed a homology model of the TMPK drug target. Furthermore, a total of 9000 FDA-approved drugs retrieved from the drug bank database were screened against the drug target. In order to computationally evaluate the stability of the ligand-protein complexes, we then carried out molecular dynamics (MD) simulations for the top three inhibitors predicted by the docking studies. In summary, our *in-silico* research explores whether drug that have already been approved can be good candidates against Mpox.

# 2 Materials and method

## 2.1 Structure prediction

In computational biology, predicting protein structure has been a significant scientific challenge for a long time. Regardless of the size of a protein, homology modeling has proved a relatively quick method for predicting a protein's structure based on experimental structures available in the Protein Data Bank. AlphaFold is a trained neural network-based deep learning method for homology modeling. The most recent version of this software, AlphaFold 2,

revealed extremely high accuracy in predicting protein structures (Cramer, 2021). In the present study, Google Colab was used for the prediction of the 3D structure of thymidylate kinase (TK). For the purpose of evaluating Ramachandran plots to confirm the stereochemical accuracy of the predicted protein structure, PROCHECK (Suganya et al., 2014) was used. ERRAT (Zheng et al., 2022) was also used for model validation.

## 2.2 Structure-based virtual screening and docking

### 2.2.1 Structure preparation

It is crucial to employ precise protein structures in structure-based molecular modeling. In this study, the model developed by AlphaFold 2 was used for virtual screening. The structure preparation wizard of MOE (Molecular Operating Environment 2016) software was used to prepare the structure (Ahmed et al., 2022). The protein structure was 3D protonated to add hydrogen atoms, and then the MOE software default parameters were utilized to minimize the energy of the structure.

### 2.2.2 Ligands preparation

Over 9000 compounds documented in the drug-bank database were retrieved from the drug-bank database. All of the compounds in the drug-bank database were subjected to three-dimensional protonation using the MMFF94x force field and energy minimization using MOE software with an RMS gradient of 0.05 (Rauf et al., 2022).

### 2.2.3 Molecular docking

Structure-based virtual screening (SBVS) techniques require the 3D structures of the drug target (receptor) and ligands in the database. We used molecular docking to evaluate the binding patterns of drug-target proteins and ligands in order to discover novel potential inhibitors. The interactions between ligands and receptors can be predicted by molecular docking approaches. The molecular docking investigations were carried out using the MOE docking program (Taj et al., 2022). The retrieved compounds were docked with the active site of thymidylate kinase to predict the active compounds against thymidylate kinase (A48R). For each compound, a total of five conformers were generated, and the top-ranked conformation for each compound was analyzed for interaction analysis. Using the rigid receptor docking protocol and GBVI/WSA scoring function, molecular docking (Taha et al., 2023) of all FDA-approved drugs was performed. AutoDock Vina software was used to carry out the docking operation (Ali et al., 2023) of the lowest docking score hits obtained from the MOE software to validate the docking protocol of the MOE software. Adding polar hydrogen atoms and assigning partial charges to each atom were all done using the AutoDock software, and the structure of ligands and receptors were saved in PDBQT format. The grid spacing was set to 1 Å and the box size was 16×20 ×24. Twenty conformations were set in the docking output. The binding poses of the molecule with the best docking energies were visualized using the PyMol software (Du et al., 2023).

## 2.3 MD simulation

Molecular dynamics simulations were performed using the AMBER 20 software suite (Ajmal et al., 2022). The best docking score complexes were used as the starting structures for the MD simulations. The AMBER force field FF14SB was used for the protein, and the generic AMBER force field (GAFF) was used for the ligands (Wadood et al., 2022). The topology files and atomic charges of ligands were generated using the Antechamber suite in the AMBER 20 package (Mahmood et al., 2023). The topology and coordinate data for the entire system were generated using the tleap module of the AMBER 20 software. The entire system was soaked in a TIP3P water box at a margin distance of 8 Å. An appropriate quantity of chloride ions was introduced in order to neutralize the system's charge. During the simulation, the particle mesh Ewald (PME) was used to handle the long-range electrostatic interactions, and the cut-off distance for nonbonded interactions was adjusted to 10 Å. The SHAKE algorithm (Poli et al., 2022) was employed to constrain the hydrogen-containing bonds. Each system underwent two stages of energy minimization: the first stage involved the use of the algorithms (10,000 steps of the steepest descent and 10,000 steps of the conjugate gradient) with restraint, and the second stage involved the use of the same algorithms without restraint. Then, each system was gradually heated from 0 to 300 K. The system was then brought up to equilibrium at 300 K and constant pressure. Finally, a production process lasting 200 ns was carried out under conditions of constant temperature and pressure (NTP). Finally, the CPPTRAJ was used for trajectory analysis (Junaid et al., 2018).

### 2.3.1 Principal component analysis

Proteins' high-amplitude movements can be captured by using principal component analysis (PCA). The PCA analysis in this study was performed using the cpptraj package (Ajmal et al., 2022). The covariance matrix was generated based on the Cartesian coordinates of the carbon alpha of each system to evaluate the dynamic behavior of each system. The covariance matrix's eigenvalues and eigenvectors were obtained by diagonalization. The eigenvectors and eigenvalues, respectively, showed the direction of high-amplitude motion and their mean square fluctuation. The PC1 and PC2, for each system, were calculated and plotted to track their movements.

## 2.4 Binding energy calculation

The free energy of binding between ligands and protein complexes was computed by the MMGBSA and MMPBSA techniques. For the calculation of binding energy, the last 100 frames were used. Two effective methods for analyzing the free energy of binding are MM/GBSA and MM/PBSA. The values of MM/PBSA strongly correlate with experimental methods (33).

Here, we calculated the binding free energy using both the MMPBSA and MMGBSA method. The following equation was employed to calculate the free energy:

$$Gbind = \Delta Gcomplex - [\Delta Greceptor + \Delta Gligand]$$

In the above equation, *Gbind* stands for the total binding free energies, *ΔGcomplex* for complex free energies, and the remaining terms stand for the corresponding free energies of the receptor protein and ligand.

# 3 Results

## 3.1 Protein sequence retrieval

The protein sequence of the Mpox virus thymidylate kinase was retrieved from the NCBI database.

## 3.2 Homology modeling

In addition, the 3D structure was predicated by the AlphaFold2 server as presented in Figure 1. A per-residue confidence rating between 0 and 100 was produced by AlphaFold2. There may be some disorder regions in the predicted structure which can be represented by a low pLDDT value. In the generated model, most of the residues have very high confidence scores that represent the model accuracy (pLDDT > 90, Figure 1). The dark green region in the predicted aligned error plot is ideal, indicating the accuracy of the model, but light green is bad (indicating a high error).

## 3.3 Structure validation

The structure was validated by PROCHECK and ERRAT servers. PROCHECK is used for three-dimensional model validation. The Ramachandran plot is shown in Figure S1A. In the plot, residues were determined in three various regions such as favored region (94%), allowed region (4.3%), and outlier region (0.5%). Furthermore, the ERRAT server was used to check the quality of the model. ERRAT validated the model by using statistical relation of non-bonded interactions between various atom types on basis of atomic interaction. The values around 95% and higher than 95% indicate standard high-resolution structures; however, values around 91% indicate low-resolution structures. Figure S2B illustrates a 97% ERRAT score, indicating the best model according to the ERRAT plot.

## 3.4 Molecular docking analysis

In the present study, molecular docking was performed to evaluate the interaction of FDA-approved drugs against the drug target thymidylate kinase. By employing the site finder option of MOE software, active site residues were identified. The docking scores of all the compounds were arranged in ascending order. Twenty percent of the data was further used for interaction analysis. Among these compounds, five compounds exhibited the most active compounds against thymidylate kinase. As noted in Figure 2A, compound DB15796 mediates a docking score of -7.49 kcal/mol. Compound DB15796 made six hydrogen bonds with the active site residues of thymidylate kinase (Asp13, Asp92, Glu145, Lys17, Arg41, Arg93), and one π-H (Thr18) interaction (Table 1). Moreover, in Figure 2B, the compound DB08223 exhibited six hydrogen bonds with Lys17, Gly16, Thr18, and Arg93 with a docking score of -7.12 kcal/mol. The docking complex of DB11736 and A48R exhibits hydrogen bonds with Thr18, Lys14, and Gly16, one ionic (Glu142), and one Pi-cation (Arg93) interaction with a docking score of -7.29 kcal/mol as depicted in Figure 2C. In addition, the complex of DB16250 and A48R protein, as shown in Figure 2D, mediates one hydrogen bond donor (Asp13), three hydrogen bonds acceptor (Gly16, Lys17, Arg93),

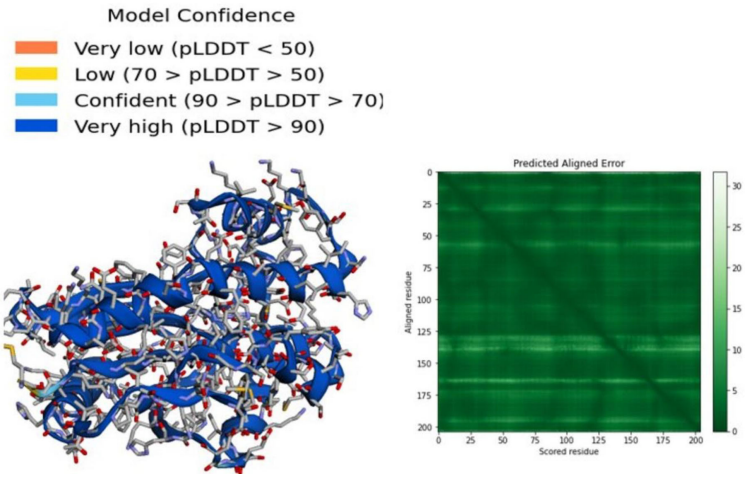


FIGURE 1
3D structure of thymidylate kinase, a drug target in Mpox, generated by AlphaFold 2 server.

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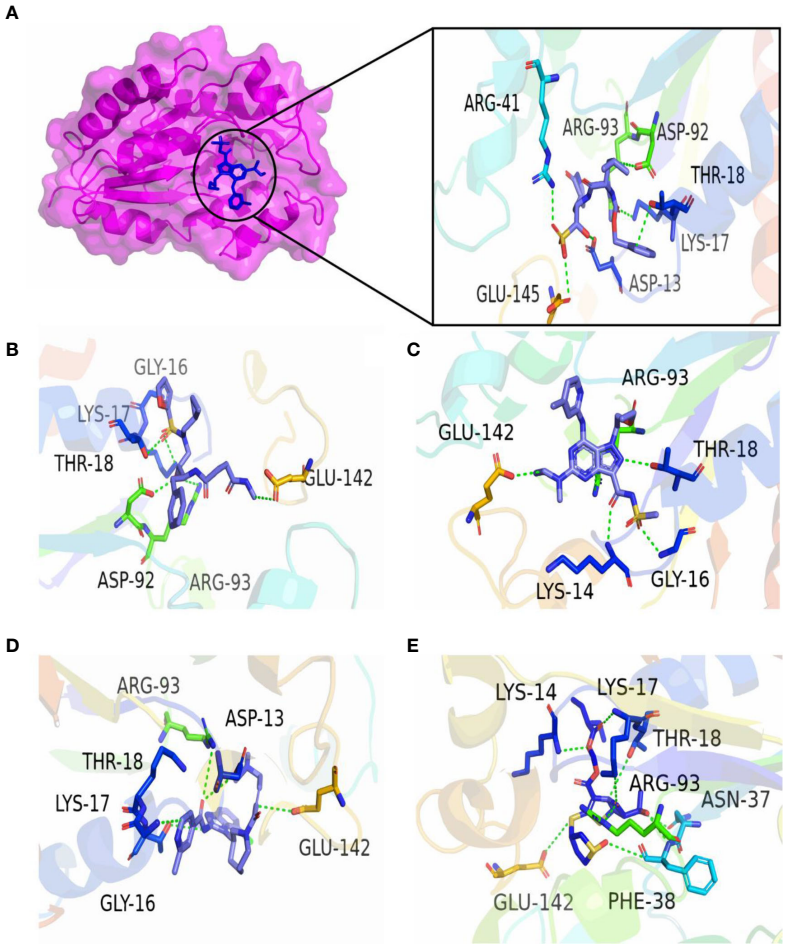


FIGURE 2
3D interactions of top five final compounds: **(A)** DB15796; **(B)** DB08223; **(C)** DB11736; **(D)** DB16250; and **(E)** DB16335 in complex with thymidylate kinase. The green dotted line represents the bond.

**TABLE 1** Docking score and protein-ligands interaction of best-scored compounds.

| Compound ID | Docking score (S) | Receptors | | | Interactions | Distance | E(kcal/mole) |
|---|---|---|---|---|---|---|---|
| DB15796 | -7.49 | OE1 | GLU | 145 | H-bond | 3.14 | -1.3 |
| | | OD2 | ASP | 92 | H-bond | 3.20 | -0.7 |
| | | OD1 | ASP | 13 | H-bond | 2.77 | -0.8 |
| | | NH2 | ARG | 41 | H-bond | 2.86 | -4.8 |
| | | NZ | LYS | 17 | H-bond | 2.87 | -11.8 |
| | | NE | ARG | 93 | H-bond | 3.01 | -2.0 |
| | | N | THR | 18 | π-H | 4.35 | -0.6 |
| DB08223 | -7.12 | OD2 | ASP | 92 | H-bond | 2.94 | -3.5 |
| | | OE2 | GLU | 142 | H-bond | 2.95 | -1.4 |
| | | NE | ARG | 93 | H-bond | 2.20 | 98.8 |
| | | NZ | LYS | 17 | H-bond | 2.90 | -5.8 |
| | | N | THR | 18 | H-bond | 2.84 | -3.8 |
| | | N | GLY | 16 | H-bond | 3.23 | -1.5 |
| DB11736 | -7.29 | OG1 | THR | 18 | H-bond | 2.89 | -3.6 |
| | | N | LYS | 14 | H-bond | 3.03 | -3.8 |
| | | N | GLY | 16 | H-bond | 2.73 | -3.3 |
| | | OE2 | GLU | 142 | Ionic | 3.33 | -2.6 |
| | | NE | ARG | 93 | π-cation | 3.93 | -2.1 |

*(Continued)*

TABLE 1 Continued

| Compound ID | Docking score (S) | Receptors | | | Interactions | Distance | E(kcal/mole) |
|---|---|---|---|---|---|---|---|
| DB16250 | -8.65 | OD2 | ASP | 13 | H-bond | 3.51 | -1.0 |
| | | NH2 | ARG | 93 | H-bond | 2.73 | -5.7 |
| | | NZ | LYS | 17 | H-bond | 3.40 | -1.6 |
| | | N | GLY | 16 | H-bond | 2.93 | -1.0 |
| | | OE2 | GLU | 142 | Ionic | 3.92 | -0.7 |
| | | N | THR | 18 | π-H | 4.10 | -1.6 |
| DB16335 | -9.57 | O | PHE | 38 | H-bond | 2.84 | -1.6 |
| | | OE2 | GLU | 142 | H-bond | 2.57 | 0.5 |
| | | CB | ASN | 37 | H-bond | 2.64 | 1.2 |
| | | NZ | LYS | 17 | H-bond | 3.50 | -2.4 |
| | | NE | ARG | 93 | H-bond | 2.96 | -1.0 |
| | | N | LYS | 14 | H-bond | 3.22 | -3.0 |
| | | N | THR | 18 | H-bond | 3.26 | -1.3 |

one ionic bond (Glu142), and one π-cation (Thr18) interactions. The docking score of the compound DB16250 was predicted as -8.65 kcal/mol. Finally, the compound DB16335 exhibits the best docking score of -9.57 kcal/mol and made seven hydrogen bonds with Lys 14, lys17, Thr 18, Asn37, Arg93, Phe38, and Glu142 residues of thymidylate kinase (Figure 2E) (Table 1). A number of studies reported that the success rate of virtual screening can be increased by combining the results of different docking software (36). To increase the success rate of virtual screening, the top best docking score hits obtained from the MOE software were further docked by the AutoDock software. The binding energy values obtained from AutoDock Vina are presented in Table 2. The binding energy obtained from AutoDock and the S score obtained from the MOE software is almost similar and ranges from -7.5 to -9.4 kcal/mol. The docking results also revealed that by performing the docking with MOE and AutoDock software, the residues including Asp13, Lys17, Thr18, Arg93, Arg41, and Gly16 were involved in the hydrogen bond formations with the ligands atoms.

## 3.5 Molecular dynamic simulation analysis

Molecular dynamic simulation helps to study conformational changes of compounds (ligands) into the binding pocket of a protein. On the basis of docking score and interactions, the top three compounds—DB16335, DB15796, DB16250—and Apo state of thymidylate kinase were subjected to MD simulation in which dynamic behavior and stability of complexes were examined by utilizing Amber 20 software. Further, RMSD values were analyzed to evaluate the stability of complexes.

### 3.5.1 Stability analysis

In the Apo state, initially, RMSD was drastically high at 2.0 Å up to 70 ns. After that, the RMSD value decreases to 1.4 Å from 80 to 230 ns. The RMSD value then gradually increases to 2.2 Å up to 300 ns. The average RMSD of the Apo-state was found to be 2.2 Å. The thymidine kinase DB16335 compound has a minimum RMSD value of 1.2-1.5 Å up to 160 ns, representing maximum stability in trajectories, however during 160 to 170 ns, small fluctuations were observed, and then after 180 to 300 ns, the complex revealed great stability and remained stable throughout the 300 ns (Figure 3A). The thymidine kinase DB15796 initially showed stable behavior with an RMSD value of 1.2 Å up to 70 ns, then the RMSD value gradually increases to 2.2 Å up to 80-250ns, and finally, becomes stable at 300 ns (Figure 3B). The thymidylate kinase DB16250 shows a steady state behavior with an RMSD value of ~1.5 Å at 25 ns up to 230 ns. After that, the RMSD increases to 2.4 Å up to 270 ns and then reaches a stable condition at 300 ns (Figure 3C). Among all the complexes, the RMSD of the thymidine kinase DB16335 complex was more stable.

### 3.5.2 Residues flexibility index analysis

RMSF analysis was performed in order to evaluate the contributions of every amino acid to the stability of complexes. The residues from 50-53 and -150-153 show major fluctuations in all complexes during simulation (Figure 4). Smaller fluctuations represent a stable complex. The overall result of RMSF indicates

TABLE 2 The binding energy and interacted residues of the docked compounds obtained from the AutoDock vina software.

| Drug bank ID | Binding energy | Interacting residues |
|---|---|---|
| DB15796 | -8.1 | ASP 13, LYS 17, THR 18, ARG 93 |
| DB08223 | -8.4 | ASP 13, GLU 145, PHE 38, LYS 14, ARG 41, ARG 93 |
| DB11736 | -7.6 | THR 18, THR 19, ASN 37, ARG 93 |
| DB16250 | -7.5 | PRO 39, LYS 14 |
| DB16335 | -9.4 | ASP 13, THR 18, ARG 41, GLY 16 |

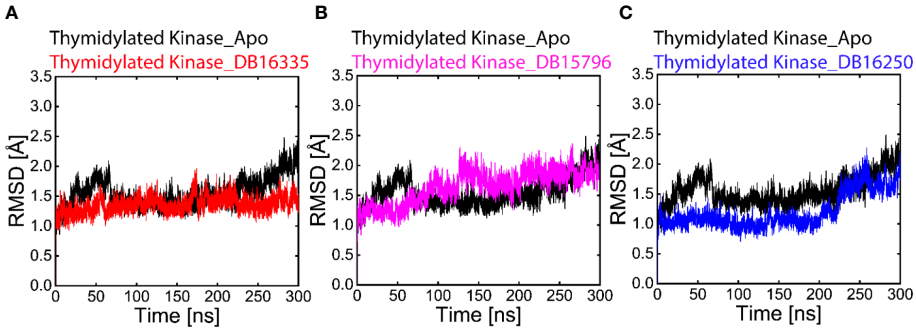


FIGURE 3
Black-colored lines in all graphs represent the Apo state. **(A)** Root-mean-square deviation (RMSD) graph of thymidylate kinase DB16335, **(B)** thymidylate kinaseDB15796, **(C)** graph of thymidylate kinase DB16250.

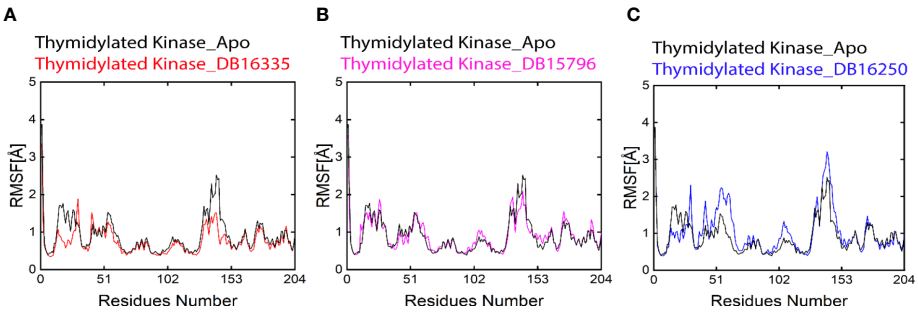


FIGURE 4
Black-colored lines in all graphs represent the Apo state. **(A)** Root-mean-square deviation (RMSD) graph of thymidylate kinase DB16335; **(B)** thymidylate kinase DB15796; **(C)** thymidylate kinase DB16250.

that thymidine kinase DB16335 and thymidine kinase DB15796 show low fluctuations compared with Apo; however, thymidylate kinase DB16250 shows more similar fluctuations to that of the Apo state. Residues Asp 50, Asp51, Tyr 52, Leu 53, Gln 151, Lys 152, and Val 153 revealed high fluctuations during the 300 ns MD simulation. The RMSF plot is shown in Figure 4.

### 3.5.3 Compactness analysis

Folded and unfolded states of protein complexes were predicated by RoG. Our result revealed that the final three complexes along with the Apo state represent change behaviors throughout molecular dynamic simulations. In addition, we also analyze the radius of gyration (RoG) that helps to measure the compactness of the complexes. The average ROG values of thymidine kinase DB16335, thymidine kinase DB15796; thymidylate kinase DB16250; and Apo were 16.5 ± 16.7; 16.5 ± 16.9; 16.6 ± 17.0; and 16.7± 17.1 A, respectively (Figure 5). Among all the complexes as well as the Apo state, the complex thymidine kinase DB16335 revealed a highly compact complex.

### 3.5.4 Principal component analysis

A dimensionality reduction technique such as principal component analysis was performed to examine the mobility of the proteins and the clusters of the relevant structural frames.

A statistical technique known as principal component analysis (PCA) combines several correlated variables with a smaller set of uncorrelated variables called principal components. The PCA was calculated and is displayed in Figure 6 in order to properly evaluate the effect of the drug binding on protein mobility. The first two principal components, known as PC1 and PC2, were plotted against one another. Colors ranging from red to blue reflect how one conformation changes into another. Each dot in Figure 6 corresponds to a single frame of the trajectory. The coordinate covariance matrix, which was derived from the time series of 3D positional coordinates of the complexes over the course of the 200 ns MD simulation, served as the input for principal component analysis. The Apo state displayed a slightly dispersed form of motion, while the drug complexes displayed a cluster type of motion. Among all the complexes TK DB16335 revealed more cluster types of motion.

## 3.6 Binding energy calculations

Finally, we calculated ΔG binding in order to refine ligands predicted through virtual screening by using MM/PBSA and MM/GBSA techniques. The MMGBSA analysis composed of electrostatic energy, van der Waals energy, surface area energy, and electrostatic contribution to the solvation-free energy was

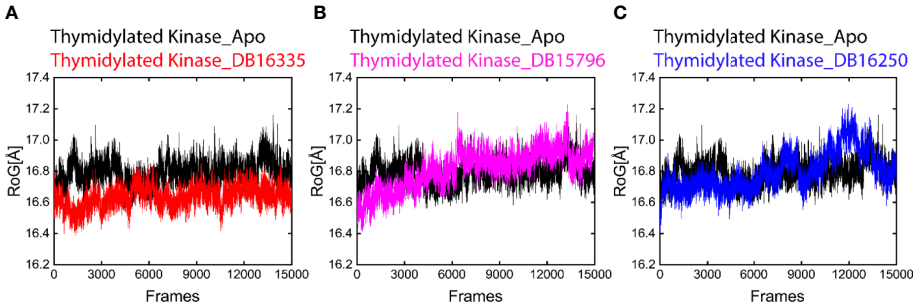


FIGURE 5
Radius of gyration (RoG) plot of Apo (black) in all graphs. **(A)** ROG graph of thymidylate kinase DB16335; **(B)** thymidylate kinase DB15796; **(C)** thymidylate kinase DB16250.

calculated as presented in Table 3, while Table 4 represents the MMPBSA analysis. Among all the complexes, DB16335 in complex with thymidylate kinase revealed lower binding energy and high binding affinity for thymidylate kinase, a drug target in the Mpox virus predicted by both MMPBSA and MMGBSA methods.

## 4 Discussion

Mpox virus, a member of Orthopoxvirus genus that includes smallpox, has become endemic to Africa and throughout the world. No vaccines against Orthopoxviruses have been developed in the last four decades, after the eradication of smallpox. The reemergence of monkeypox in unprepared and unvaccinated populations is an emergency demanding attention from the scientific community. Instead of infeasible mass vaccination campaigns in a short time period, specific therapeutics against Mpox can provide sustainable solutions for affected patients (Potter et al., 2007). Approximately 49 genes are common among all the members of the provirus family from the total 150 genes encoded by poxviruses (Llanos et al., 2021). Currently, no approved drug is available for the treatment of monkeypox virus (Anwar et al., 2023).

Drug repurposing is best way to determine effective therapeutics against Mpox. Therefore, the present study aims to identify the best

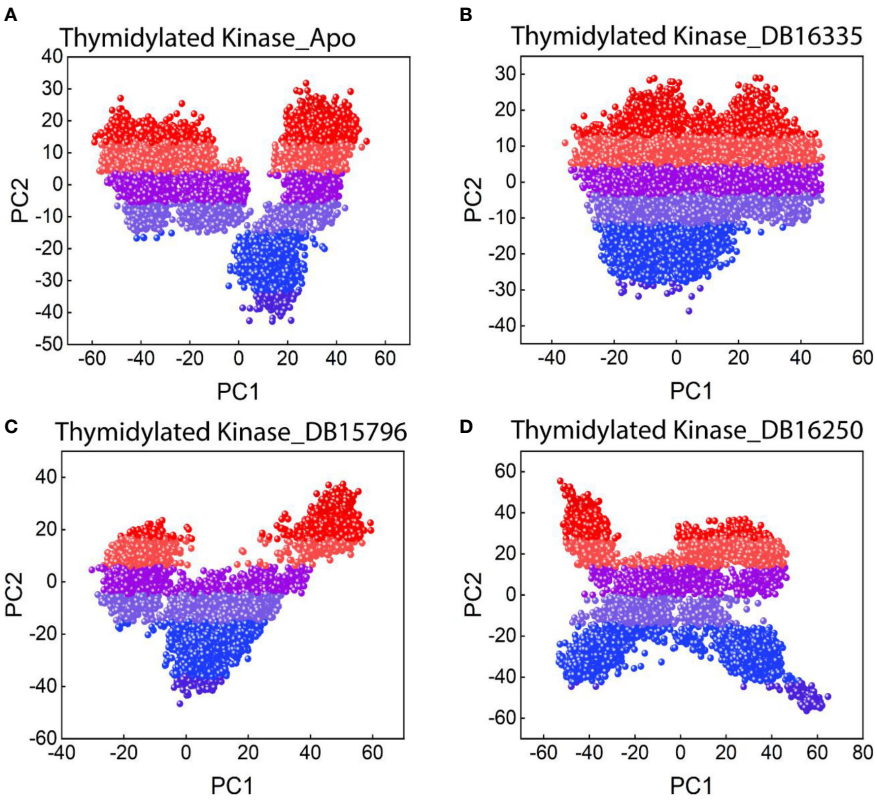


FIGURE 6
PCA plot for **(A)** thymidylate kinase-Apo state; **(B)** thymidylate kinase DB16335; **(C)** thymidylate kinase DB15796; **(D)** thymidylate kinase DB16250.

TABLE 3 MMGBSA analysis reported in kcal/mol.

| Complexes | VDW | EEL | ESURF | EGB | $\Delta G^{Total}$ |
|---|---|---|---|---|---|
| DB16335 | -63.6066 | -3.5111 | -6.7332 | 13.7796 | -60.0723 |
| DB15796 | -27.2213 | -19.2840 | -2.9749 | 29.3219 | -20.1555 |
| DB16250 | -46.2392 | 0.5465 | -5.4155 | 15.6348 | -35.4764 |

TABLE 4 MMPBSA analysis reported in kcal/mol.

| Complexes | VDW | EEL | ENPOLAR | EDISPER | EPB | $\Delta G^{Total}$ |
|---|---|---|---|---|---|---|
| DB16335 | -63.6066 | -3.5111 | -33.8273 | 65.3356 | 20.5451 | -15.0652 |
| DB15796 | -27.2213 | -19.2840 | -15.7229 | 30.8764 | 31.6112 | 0.2622 |
| DB16250 | -46.2392 | 0.5465 | -26.2276 | 53.4729 | 24.8560 | 6.4056 |

drugs against Mpox by using different computational approaches such as homology modeling, virtual screening, molecular docking, and MD simulation. For this purpose, we collected FDA-approved drugs, and 3D structure of protein was modeled. The model structure was subjected to molecular docking against a curated library of FDA-approved drugs. Afterwards, by identifying binding energy and interaction, various compounds were selected in which drugs DB12380, DB13276, DB13276, DB11740, DB14675, DB11978, DB08526, DB06573, DB15796, DB08223, DB11736, DB16250, and DB16335 mediate significant binding energy and binding interaction with thymidylate kinase (A48R). Among these compounds, the compounds having the lowest binding energy—DB15796, DB08223, DB11736, DB16250, and DB16335, with -7.49, -7.12, -7.29, 8.65, and -9.57 kcal/mol, respectively—were recognized as the best compounds (Table 1).

Finally, compounds DB16335, DB15796, and DB16250 along with Apo were subjected to 300 ns MD simulation to evaluate the stability of the compounds. Among the Apo and selected compounds, DB16335 was found to be active and showed strong binding against Mpox. DB16335 is an antibiotic drug that is under a clinical trial NCT03354598) and is used to treat complicated and uncomplicated urinary tract infections, intra-abdominal infections, pneumonia, and acute pyelonephritis. In addition, it is also active against Gram-positive and Gram-negative bacteria (Dunne et al., 2023). The selected compounds have low RMSD and low amino acid residue fluctuations compared with Apo, which indicates that the binding of selected compounds to the active site of Mpox protein makes the protein more stabilized. In addition, the ROG also indicates that thymidylate kinase DB16335 has a low value of RoG (Figure 5). The binding of these compounds to Mpox can destroy its activity and provide an unfavorable situation for Mpox.

## 5 Concluding remarks

Monkeypox has rapidly spread through the world, but still, no suitable vaccine or drug is available. The present study is designed to determine a potent drug against Mpox by using computational methods. Our result shows that all three compounds were notable because of good interaction and binding energy. Among all the drugs, DB16335 was identified as the most potent compound in terms of docking score, interactions, and binding energy calculation. Overall, compound DB16335 could bind and inhibit the drug target thymidylate kinase, which can be helpful to reduce infections associated with the Mpox virus. However, further *in vitro* and *in vivo* experiments are required to validate the findings of our study.

## Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

## Author contributions

AA, AM and CH performed experiments, drafted the manuscript. MAH performed some of the experiments and revised the manuscript and discussion. BSA, PL, MU, ANA and PH drafted the manuscript and critically revised the manuscript. AW and JH conceptualized, designed, and supervised the study and revised the manuscript. All authors contributed to the article and approved the submitted version.

## Funding

The authors would like to thank the Deanship of Scientific Research at Umm Al-Qura University for supporting this work by Grant Code: 23UQU4331128DSR001. This study is also funded by the Incubation Fund of SSL Central Hospital of Dongguan (YJ002).

## Acknowledgments

The authors thank Dr. Ajmal, University of Nizwa, Sultanate Oman for providing technical support for the computational study.

## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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## Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcimb.2023.1159389/full#supplementary-material

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frontiers | Frontiers in Cellular and Infection Microbiology

TYPE Systematic Review
PUBLISHED 29 June 2023
DOI 10.3389/fcimb.2023.1179885



OPEN ACCESS

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RECEIVED 17 April 2023
ACCEPTED 05 May 2023
PUBLISHED 29 June 2023

CITATION
Rani I, Goyal A, Shamim MA, Satapathy P, Pal A, Squitti R, Goswami K, Sah R, Barboza JJ and Padhi BK (2023) Prevalence of mpox viral DNA in cutaneous specimens of monkeypox-infected patients: a systematic review and meta-analysis. *Front. Cell. Infect. Microbiol.* 13:1179885. doi: 10.3389/fcimb.2023.1179885



# Prevalence of mpox viral DNA in cutaneous specimens of monkeypox-infected patients: a systematic review and meta-analysis

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**Background:** Human monkeypox (mpox) disease is a multicountry outbreak driven by human–human transmission which has resulted in an international public health emergency. However, there is limited evidence on the positivity rate of skin lesions for mpox viral DNA. We aim to fill this gap by estimating the pooled positivity rate of skin samples with mpox viral DNA from mpox patients globally.

**Methods:** In this systematic review and meta-analysis, seven databases and several preprint servers have been extensively searched until 17 January 2023 according to a prospectively registered protocol (PROSPERO: CRD42023392505). Articles including the positivity rate of skin samples with mpox viral DNA in mpox-confirmed patients were considered eligible. After a quality assessment, a random-effect meta-analysis was used for pooled prevalence. To explore and resolve heterogeneity, we used statistical methods for outlier detection, influence analysis, and sensitivity analysis.

**Findings:** Among the 331 articles retrieved after deduplication, 14 studies were finally included. The pooled positivity rate of the skin samples was 98.77% (95% CI: 94.74%–99.72%). After the removal of an influential outlier, $I^2$ for heterogeneity dropped from 92.5% to 10.8%. Meta-regression did not reveal any significant moderator.

**Conclusion/interpretation:** The present findings reinforce that skin lesions act as a reservoir of mpox viral DNA and contribute to a high infectivity risk. This may be a prevailing basis of prompt transmission during the current multicountry outbreak and also needs further investigation. The present imperative outcome may benefit in producing valuable preventive and management procedures in an appropriate health strategy.

KEYWORDS

**monkeypox, mpox viral DNA, skin lesion, cutaneous, meta-analysis, infectivity potential, transmission**

## 1 Introduction

Previous decades have witnessed multiple outbreaks of mpox (formerly known as monkeypox) infection in the Democratic Republic of the Congo (DRC), Nigeria, and Gambia (Ladnyj et al., 1972; MacNeil et al., 2009). With the growing multinational outbreak, the World Health Organization (WHO) declared mpox disease a potential "public health emergency of international concern (PHEIC)" on 23 July 2022 (World Health Organization (WHO), 2022a). According to Centers for Disease Control and Prevention (CDC) data, 85,922 cases of mpox and 96 deaths have been diagnosed globally since 1 February 2023. So far, mpox cases have been reported in 110 member states of all six WHO regions (CDC, 2023).

The mpox virus is a double-stranded DNA virus and belongs to an Orthopoxvirus genus of the Poxviridae family that includes the smallpox virus (MacNeil et al., 2009). The clinical manifestations of mpox are analogous to smallpox, but it is commonly less severe (Rubins et al., 2011). Even though mpox is a rare disease, it has a devastating impact on infected individuals. Furthermore, no suitable diagnostic test, precise therapy, or vaccine is still available (Shamim et al., 2023b). The clinical patterns observed in the current outbreak are different from the earlier African outbreaks. The clinical manifestations of mpox infection are mostly characterized by headaches, fever, chills, fatigue, myalgia, flu-like symptoms, skin lesions/rash, and lymphadenopathy. However, in the recent outbreak, atypical patterns of clinical symptoms have been reported in many cases (Huhn et al., 2005; Satapathy et al., 2022; Thornhill et al., 2022a; Gandhi AP. et al., 2023; Gandhi PA. et al., 2023). Most patients with moderate mpox do not require antiviral therapy or hospitalization (Huhn et al., 2005; Adler et al., 2022; Català et al., 2022). Importantly, the spread of the mpox virus occurs by direct or indirect close contact via sores, scabs, respiratory droplets or body fluids, and possibly contaminated surfaces or fomites (Thornhill et al., 2022a).

Indeed, international agencies and organizations are deeply concerned about the current epidemic. The effects of the transmission are alarming and staggering. Globally, future outbreaks may cause more severe mortality, morbidity, and broad economic impacts. Therefore, it is crucial to identify the route of transmission of the infection to implement approaches for empowering health strategies and social and environmental interventions.

In this regard, the positivity rate of viral particles in biological samples may provide an estimate of infectivity potential. A recent meta-analysis study revealed that skin lesions are the dominant clinical features of the current mpox outbreak (Liu et al., 2023). Consistently, other studies have evaluated the viral burden in skin samples of mpox patients (Peiró-Mestres et al., 2022; Thornhill et al., 2022a; Palich et al., 2023) and suggested that it increases with the severity of the disease (Huhn et al., 2005; Hennessee et al., 2022). According to these data, viral contents may be higher from skin lesions and predict the risk and severity of mpox infection. Validating if the prevalence of mpox viral DNA predicts the disease severity and infectivity might lead to treatment development, set up schemes, or even put in place activities to control community transmission. Additionally, cutaneous specimens are more easily accessible and minimally invasive than other biological specimens and, hence, are considered more suitable for diagnostic and prognostic purposes.

Keeping this in view, we performed a systematic review and meta-analysis of articles published till 17 January 2023 on the frequency of positive cutaneous specimens with mpox viral DNA of mpox patients. The results of this study can afford valuable insights into the illness and progress development of effective actions to restrain the spread of infection. Importantly, this novel information may be favorable for applying appropriate social measures to curtail the spread of the endemic infection.

## 2 Methods

This systematic review and meta-analysis is in accordance with the Meta-analysis of Observational Studies in Epidemiology (MOOSE) reporting guidelines (Stroup, 2000) and the 2020 Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement (Page et al., 2021). The review protocol was registered on the PROSPERO International Prospective Register of Systematic Reviews (CRD42023392505).

## 2.1 Search strategy

This systematic and meta-analysis search strategy was designed according to PECOS criteria (refer to Supplementary Annex 2) with the research question "What is the prevalence (or positivity rate; %) of skin samples with viral DNA in mpox patients." Seven databases, namely, the Cochrane Library, EBSCOhost, EMBASE, ProQuest, PubMed/MEDLINE, Scopus, and Web of Science were searched for eligible articles till 17 January 2023 according to PECOS criteria (see Supplementary Annex 2 for the search strategy and Annex 1 for the PECOS criteria). A search strategy was prepared (IR) for PubMed with truncations, Boolean operators, and Medical Subject Heading (MeSH) terms. This was peer-reviewed by a second co-author (AG) in accordance with the Peer Review of Electronic Search Strategies: 2015 Guideline Statement (McGowan et al., 2016). The following terms were used: (mpox OR monkeypox OR mpxv) AND (skin OR cutaneous*) AND (lesion* OR swab OR sample). In addition, preprint servers (bioRxiv and medRxiv) were also examined to detect potentially eligible articles. This approach was further combined with manual exploration of citations in relevant articles, alongside checking forward citations. Google/Google Scholar was also searched for supplementary studies overlooked during the automated search.

## 2.2 Inclusion and exclusion criteria

The inclusion criteria were as follows: cases with mpox virus infection confirmed by real-time polymerase chain reaction (PCR). The confirmed cases were selected regardless of age, ethnicity, and gender. Observational studies such as cross-sectional, cohort, and case series published till 17 January 2023 were included in this study (Supplementary Annex 1). It is significant to indicate that relevant reports, communications, and editorials that provided the positivity rate of skin samples were also considered. Also, the exclusion criteria were as follows: suspected or probable subjects with mpox infection. Any irrelevant studies, abstracts, qualitative, randomized controlled trials (RCTs), policy, case reports, reviews, opinion reports, and articles without available full texts were excluded.

## 2.3 Selection criteria

All articles resulting from the electronic search were further imported into the reference management tool (Mendeley desktop V1.19.5) to manage the references and coordinate the review process. Furthermore, duplicate documents were eliminated by software function and also by manual reading of the title, authors, and journal name (IR and AG). Moreover, three randomized controlled trials (RCTs) from the electronic search were also removed. Clinical studies regarding mpox disease were separated by two authors (IR and AG) independently by reading the titles and abstracts of acquired studies by applying the eligibility criteria, and they selected 17 articles for full-text screening. Those articles concerning the prevalence of mpox DNA in cutaneous samples were selected by further reading the full text (IR and AG). Any differences between the two researchers (IR and AG) during the screening process were resolved through communication to preserve synchronization and decided consistently on the eligibility. The third author (MAS) decided on the unresolved doubts.

## 2.4 Data extraction and management

Two authors (IR and AG) independently extracted data from literature information in a Microsoft Excel spreadsheet, and any inconsistency at any stage was resolved by the authors through negotiation and discussion to build harmony. The third author (MAS) decided on the unsettled doubts. The subsequent information extracted from each of the included studies is given as follows: bibliographic details of the reports, characteristics of the study (study design, region where the study was conducted), characteristics of the participants (number of mpox confirmed cases from whom skin specimens were taken, age, gender), and summary measures (% of skin samples positive for mpox DNA).

The entire process of literature examination, screening, data extraction, systematic review, and meta-analysis was explained using the Preferred Reporting Standard of Systematic Reviews and Meta-Analyses (PRISMA-2020) flowchart and checklist to certify scientific precision (Figure 1).

## 2.5 Quality assessment

Two authors (IR and AG) independently assessed the risk of bias in the included literature via the quality assessment tools suggested by the National Institutes of Health (NIH) (National Institute of Health (NIH), 2021). The case series, cross-sectional, and cohort studies were evaluated with the NIH quality assessment tool. Any disparity between the authors (IG and AG) concerning the risk of bias in any of the studies was resolved by discussion. The third author (MAS) settled the unexplained ambiguities. The overall and rating scores for each study are explained in Supplementary Annex 3.

## 2.6 Statistical analysis

In the included studies, we extracted data on the percentage of mpox patients (diagnosed via any sample) whose skin samples also tested positive for mpox DNA. Heterogeneity was assessed using $I^2$, $H$, $\tau^2$, and Cochran's $Q$, apart from the prediction interval (Begg and Mazumdar, 1994; Higgins and Thompson, 2002). Prediction interval helps predict the effect size in a future study and does not merely provide the average effect across the available studies (Spineli and Pandis, 2020). It has been estimated based on a t-distribution. The choice of a fixed-effects model or a random-effects model is made depending upon factors including the observed heterogeneity. For synthesizing the results, a random intercept

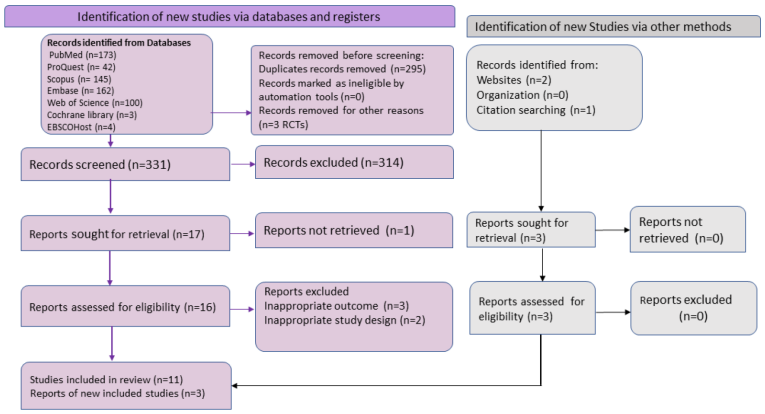


FIGURE 1
The Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) flowchart summarizing the literature search and giving reasons for the exclusion of studies.

logistic regression model with logit transformation of proportions has been used.

In case of high heterogeneity as we encountered here, we will explore the cause behind heterogeneity and try to resolve or reduce it. Outlier detection will be done. Next, to detect influence, we will run a chain of statistical methods including the Baujat plot, influence diagnostics, leave-one-out meta-analyses, and graphical display of heterogeneity (GOSH) plots. We will also perform meta-regression using sample size and the average age of the participants as moderators (Sterne and Egger, 2005). This will be reported by the omnibus test of moderators employing a mixed-effects model and depicted visually using bubble plots. Publication bias and small-study effects will be assessed using the Doi plot and LFK index as these have been shown to be better suited for the meta-analysis of proportions (Furuya-Kanamori et al., 2018; Shamim et al., 2023a). All statistical analyses were performed using meta and metafor packages in the R programming language (v4.2.2) (R Core Team, 2020). The distribution of true effect size computations was carried out using Comprehensive Meta-Analysis Version 4 (Borenstein et al., 2022). A $p$-value <0.05 (two-sided) was considered statistically significant.

# 3 Results

## 3.1 Selection criteria

We identified 629 possibly relevant articles from the systematic search, among which 295 overlapping articles and 3 RCTs were excluded. After title and abstract screening of 331 articles, we retrieved 17 articles for the further review process. Out of 17, full-text screening was performed on 16 reports, while the full text of one article was not available and was eliminated (Gaspari et al., 2023). During the full-text screening, five articles did not fulfil the inclusion criteria and, thus, were not considered. Additionally, three other articles were also considered to be eligible as per inclusion criteria through related bibliography of the included reports and websites. Finally, a total of 14 studies were included in the meta-analysis for an overall pooled proportion (%) of mpox viral DNA in skin specimens (Table 1). The selection of literature is illustrated in the PRISMA flowchart (Figure 1). All 14 included studies were of good quality. The quality assessment of the included studies is depicted in the supplementary data (Supplementary Annex 3A, B).

## 3.2 General study characteristics

The baseline characteristics of the 14 studies included in the systematic review and meta-analysis consisting of three case series (Thornhill et al., 2022a; Thornhill et al., 2022b; Palich et al., 2023), two cross-sectional studies (García-Piqueras et al., 2022; Ouafi et al., 2023), eight prospective observational studies (Loconsole et al., 2022; Mailhe et al., 2022; Nörz et al., 2022; Peiró-Mestres et al., 2022; Tarín-Vicente et al., 2022; Ubals et al., 2022; Veintimilla et al., 2022; Silva et al., 2023), and one retrospective study (Hasso et al., 2022) are explained in Table 1. Most of the studies were conducted in mpox non-endemic countries such as Spain (5/14, 35.71%), France (3/14, 21.42%), Italy (1/14), Germany (1/14), Canada (1/14), and Brazil (1/14). On the other hand, 2 studies out of 14 were carried out in both mpox non-endemic and endemic countries at the same time, for example, approximately 15 countries in Europe, America, and Africa as well as 16 countries in Europe, Americas, Africa, Asia, and Australia, respectively (Thornhill et al., 2022a; Thornhill et al., 2022b). Most of the cases were adults above 18 years. The sample size of these included studies ranged from 10 (Loconsole et al., 2022) to as high as 528 (Thornhill et al., 2022a). Furthermore, 5 out of 14 studies reported travel history in mpox participants (García-Piqueras et al., 2022; Loconsole et al., 2022; Peiró-Mestres et al., 2022; Thornhill et al., 2022a; Silva et al., 2023). Though we searched for studies from any time, we only found reports from the current epidemic. In all the included studies, mpox was confirmed by diagnostic testing such as real-time PCR for mpox DNA. Most of the cases were men consisting mostly of MSM (men who have sex with men) in all the included studies except one case series study (Thornhill et al., 2022b) where the majority of the participants were women (cis females, trans females, and non-binary individuals). In the case series

TABLE 1 Baseline characteristics of the included studies that reported the frequency of skin samples positive with mpox viral DNA [based on positivity rate (%)] in mpox patients ($n$ = 14).

| Authors (YOP) | Study design | Number of mpox confirmed cases from whom skin samples were taken | Outcome measure: frequency of positive skin samples with mpox viral DNA (%) | Age (years) (median) | Gender distribution | Geographical region |
|---|---|---|---|---|---|---|
| García-Piqueras P et al. (2022) (García-Piqueras et al., 2022) | C/Sl | 53 | 100% | 36 | 52 males including MSM or homosexuals and 1 female | Madrid, Spain |
| Hasso M et al. (2022) (Hasso et al., 2022) | R | 78 | 43.60% | 38[a] | All males | Ontario, Canada |
| Loconsole D et al. (2022) (Loconsole et al., 2022) | PO | 10 | 100% | 36.7 | 8 males (6 MSM) and 2 females | Southern Italy |
| Mailhe et al. (2022) (Mailhe et al., 2022) | PO | 258 | 98% | 35 | Majority of males including MSM except 1 female and 1 transgender female | France |
| Nörz D et al. (2022) (Nörz et al., 2022) | PO | 16 | 100% | 37 | All males (MSM) | Germany |
| Ouafi M et al. (2022) (Ouafi et al., 2023) | C/Sl | 116 | 100% | 37 | All males (including mostly MSM) except 1 female | Northern France |
| Palich R et al. (2023) (Palich et al., 2023) | C-S | 50 | 88% | 34 | All males including 49 MSM and 1 MSW | Paris, France |
| Peiró-Mestres A et al. (2022) (Peiró-Mestres et al., 2022) | PO | 12 | 100% | 38.5 | All males (MSM) | Barcelona, Spain |
| Silva MST et al. (2022) (Silva et al., 2023) | PO | 188 | 96.30% | 33 | All cisgender males (majority MSM) except 8 cisgender females | Brazil |
| Tarín-Vicente EJ et al. (2022) (Tarín-Vicente et al., 2022) | PO | 180 | 99% | 37 | Majority of males including gay, bisexual, MSM except for a few heterosexual males or females | Madrid and Barcelona, Spain |
| Thornhill JP et al. (2022) (Thornhill et al., 2022b) | C-S | 123 | 100% | 34 | Majority of trans females and cis females and five non-binary individuals | 15 countries in WHO regions of Europe, the Americas, and Africa |
| Thornhill JP et al. (2022) (Thornhill et al., 2022a) | C-S | 528 | 97% | 38 | All males except one trans or non-binary (heterosexual, homosexual, or bisexual) | 16 countries in Europe, the Americas, Africa, Asia, and Australia |
| Ubals M et al. (2022) (Ubals et al., 2022) | PO | 49 | 100% | 33.5 | All males | Spain |
| Veintimilla C et al. (2022) (Veintimilla et al., 2022) | PO | 37 | 97% | 31 | All males (MSM) | Madrid, Spain |

The prevalence of skin mpox viral DNA is presented in percentage. Age (years) is presented in median.
YOP, year of publication; n, number; DNA, deoxyribonucleic acid; MSM, men who have sex with men; MSW, men who have sex with women; %, percentage; C-S, case series; C/Sl, cross-sectional; PO, prospective observational; R, retrospective.
[a]Represents mean value.

with the largest sample size by Thornhill et al., 96.4% (509 out of 528) of mpox-confirmed cases were MSM (Thornhill et al., 2022a). The common systemic symptoms or manifestations presented by most of the mpox patients comprised rash, fatigue, headaches, myalgia, fever, and lymphadenopathy, while others were tonsillitis, proctitis, pharyngitis, odynophagia, epiglottitis, and asthenia.

## 3.3 Summary measure and heterogeneity

A systematic review and meta-analysis of all 14 studies was carried out to assess the pooled prevalence of mpox viral DNA. Among the 1,754 confirmed mpox patients in the studies, skin samples were taken from 1,698, out of which 1,616 had mpox viral positivity in the skin samples. The pooled prevalence was 98.77% (95% CI: 94.74%–99.72%) using a random-effects model. There was significant heterogeneity in the results with an $I^2$ value of 92.5% (95% CI: 89.1%–94.8%). The studies show a relatively wide prediction interval of 39.22% to 99.99%. The individual study results, the methods used for the meta-analysis, and other results are summarized in Figure 2A.

## 3.4 Meta-regression

The bubble plots are produced after a simple meta-regression with continuous moderators and are shown in Figure 3. The effect size does not show a significant dependence on the moderator variables. The omnibus test of moderators yields $Q_M$ values of 0.24 ($p = 0.63$) and 0.64 ($p = 0.42$) for meta-regression based on sample size and age, respectively.

## 3.5 Influence analysis

We detect outliers by observing the confidence interval (CI) of the individual study estimates. If the CI of a study does not overlap at all with the CI of the pooled effect, we declare the said study an outlier. In this case, one study (Hasso et al., 2022) fulfills this condition as can be observed in Figure 2B.

The Baujat plot shows that this study (Hasso et al., 2022) overly contributes to both the overall heterogeneity and the effect size of the summary estimate (Supplementary Annex 4A). In influence diagnostics (Supplementary Annex 4B), we see that the externally standardized residual of this study is more than four units away. The difference in fits suggests a considerable influence. Cook's distance that depends both on residual and leverage also shows a high influence. A covariance ratio of even less than 1 indicates the need for the removal of the study for a sensitivity analysis. Here, the covariance ratio is less than 0.5 for this study. The leave-one-out $\tau^2$ and Cochran's $Q$ plots show a massive dip in heterogeneity after excluding this study. The hat values and the study weight plots also indicate a considerable influence of this study. The leave-one-out meta-analysis sorted by $I^2$ shows a clear trend wherein $I^2$ varies between 91% and 93% for all the studies but is 10.8% for this case (Supplementary Annex 4C).

For the GOSH plots, instead of omitting one study at a time (as in the leave-one-out meta-analysis), we build meta-analytic models of all possible subsets of the included studies. Therefore, we have fit 8,192 meta-analytical models with the given studies and plotted the results (Supplementary Annex 4D). This helps identify clusters and highly influential studies. Supplementary Annex 4E shows the influence of this study (Hasso et al., 2022). All the fitted subsets are demonstrated, and the colored points correspond to only those models where this study was included. We can see that the presence of this study leads to a different cluster altogether. This cluster has much higher heterogeneity as indicated by the $I^2$ estimate in the $Y$-axis. Overall, we can easily conclude that this study is overly influential.

## 3.6 Sensitivity analysis

We found this study (Hasso et al., 2022) to be an overly influential outlier in our preceding analysis. So, we conducted a sensitivity analysis after excluding this study. The heterogeneity has dropped significantly. The previous $I^2$ of 92.5% (95% CI: 89.1%–94.8%) dropped to 10.8% (95% CI: 0.0%–49.7%). The prediction

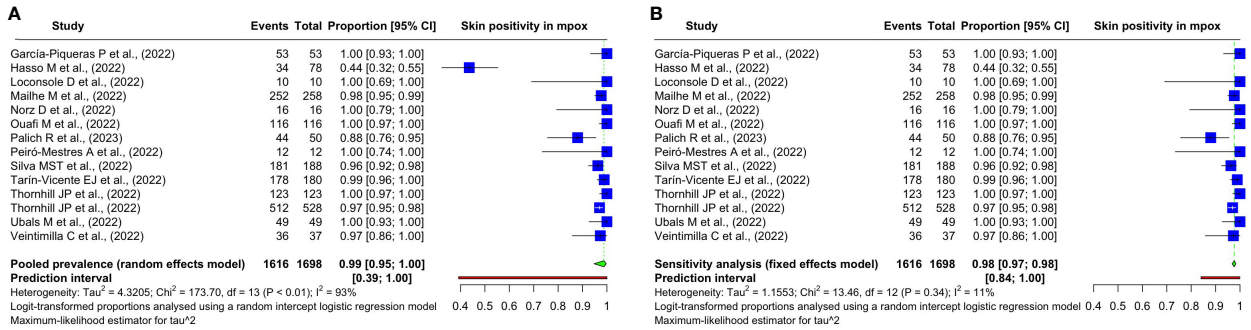


FIGURE 2
Forest plots showing the **(A)** overall pooled prevalence and its 95% confidence interval and heterogeneity statistics of the prevalence of mpox DNA in the skin samples of patients with mpox based on positivity rate (%) and **(B)** sensitivity analysis on the included studies.

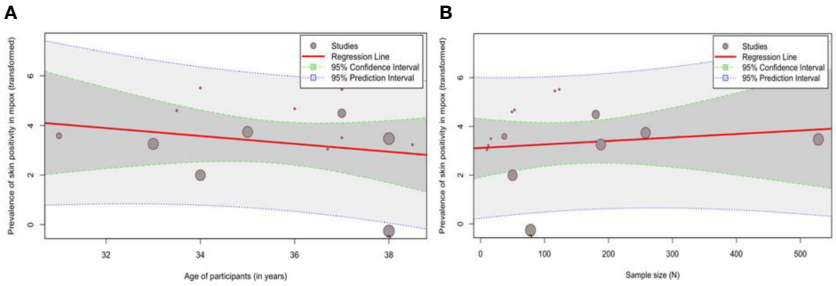


FIGURE 3
Bubble plots showing the meta-regression analysis of **(A)** age (years) and **(B)** sample size (*N*).

interval considerably narrowed from 39.22%–99.99% to 84.05%–99.90%. Given the homogeneity among these studies and a substantially decreased heterogeneity, we employed a common-effects model to meta-analyze the pooled prevalence in the sensitivity analysis. It has changed from 98.77% (95% CI: 94.74%–99.72%) to 97.65% (95% CI: 96.79%–98.29%). The findings are summarized in Figure 2B.

## 3.7 Publication bias

To assess small-study effects and publication bias, we calculated the LFK index performed in addition to the visual inspection of a Doi plot (Supplementary Annex 5). Most of the studies fall within the right limb. Moreover, the LFK index is 2.7 falling outside the limits of −1 to +1. This suggests asymmetry of the study findings.

## 3.8 Distribution of true effect size and prediction interval

If we assume that the true effects are normally distributed (in logit units), we can estimate that the prediction interval is 0.265 to 1.000. The true effect size in 95% of all comparable populations falls in this interval (Figure 4).

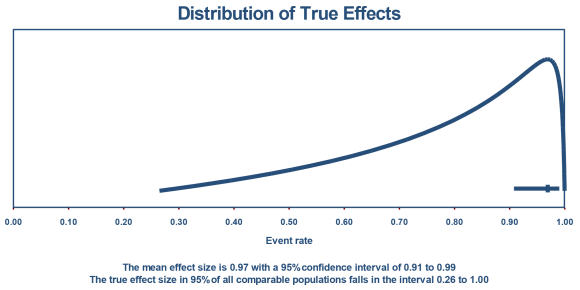


FIGURE 4
Distribution of true effect size computations.

# 4 Discussion

The main result of this study is that the pooled proportion (%) of skin samples with mpox viral DNA was 98.77% (95% CI: 94.74%–99.72%) yielded from a total pool of 1,616 patients. To the best of our knowledge, the present systematic review and meta-analysis is the first study to evaluate the overall viral positivity rate in the cutaneous samples of mpox-infected cases.

Importantly, a few articles included in the meta-analysis have also mentioned a high viral load [lower cycle threshold (Ct) value] in the skin specimens of mpox-infected patients. The authors confirmed that a lower Ct value is predictive of a higher probability of skin samples being positive for mpox DNA. The current outcomes are also in concordance with a larger prospective study depicting a high viral burden in skin samples responsible for transmission which most likely occurs through direct body contact rather than through the respiratory route or contact with body fluids (Palich et al., 2023). Similarly, another *in vivo* study observed a remarkable correlation between viral DNA load and infectivity in the BSC-1 cell line with epithelial morphology. Moreover, an mpox-infected patient with lesions is considered infectious till the crust from the crusty lesions falls off (Ouafi et al., 2023). Notably, this extremely envisages a higher risk of transmission of infection from dermal lesions.

The results of this study also confirm earlier findings from a meta-analysis displaying high mpox viral load in skin samples than in other biological samples (Martins-Filho et al., 2022). The current data extends the previous results yielded from studies with a small sample size published through August 2022. We performed the meta-analysis on a large number of studies available worldwide till 17 January 2023 in order to assess the pooled proportion of mpox patients' cutaneous specimens being positive with mpox viral DNA. Likewise, the current quantitative results considerably validated the high viral positivity rate in dermal specimens according to the latest several studies published worldwide. Especially, Noe et al. observed the highest viral concentrations (copy number/ml) in skin swabs of the first two mpox patients in Germany (Noe et al., 2023). These researchers were able to isolate mpox only from the skin pustules and proposed that skin (close) contact is the main route of transmission.

Furthermore, Suner and colleagues (Suñer et al., 2022) have revealed that skin lesions had a high median of viral DNA content of at least 2 orders of magnitude [7.3 $\log_{10}$ copies/ml (IQR 6.5–8.2)] compared with all the other clinical samples during the course of the disease. Moreover, the replication-competent viruses with high DNA levels (>6.5 $\log_{10}$ copies/ml) were isolated from dermal specimens. Moreover, these lesions had the longest median time [25 days (95% CI: 23–28)] of viral clearance from symptom onset than other clinical samples (Suñer et al., 2022).

Another longitudinal study on viral DNA load kinetics revealed that higher mpox viral load in skin lesion swabs was observed at the late stages of the disease. Despite this, all skin lesion samples were positive for mpox viral DNA during the entire time course as compared with the oropharyngeal samples (Nörz et al., 2022). Thus, contact transmission via mpox skin lesions may be a dominant route of mpox infection.

The current approach also highlights that skin lesion swabs are a suitable and reliable source of specimens for diagnostic purposes: they can be easily assessed using the real-time PCR technique. In line with these results, the WHO guidelines have recommended skin lesions as suitable diagnostic specimens for laboratory mpox confirmation (World Health Organization (WHO), 2022b; Jiang et al., 2022). Most of all, these samples are easy to collect from the roof or fluid from vesicles, pustules, and dry crusts of the skin lesions. A recent study has observed no statistically significant difference in the viral positivity rate of skin swabs among the self- or physician-collected samples (Ubals et al., 2022). This is suggestive of adopting self-sampling policies which will definitely benefit patients as well as disease control.

In the present study, Hasso and colleagues (Hasso et al., 2022) have reported that 43.6% of skin samples of mpox patients had mpox viral DNA. This study is observed to be an overly influential outlier during the preceding analysis, but this outlier only changes the pooled proportions from 98.77% (95% CI: 99.74%–99.72%) to 97.65% (95% CI: 96.79%–98.29%). However, Hasso et al. (2022) observed that skin lesions were most frequently positive (92.3%) in mpox patients who were analyzed for >1 skin specimen than other samples, indicating that testing multiple skin samples may increase the sensitivity of this test.

Taken alongside the data from earlier studies, our study suggests that skin lesions can play a main role in the transmission of mpox, either directly through cutaneous contact or indirectly through contaminated materials. Our data might be translated into informed decision-making regarding guidelines for mpox patients and for preventive as well as containment measures and can be implemented to prevent the spread of the infection in multinational outbreaks. Notably, this study has some shortcomings such as the restricted sample size or the limited number of selected studies. Preferably, a bigger sample size would assure good accuracy in records assessment. However, the current outcomes are based on consistent literature; thus, they appear reliable. Furthermore, the correlation of skin viral positivity rate with a high risk of infectivity potential is established upon the particular features of mpox infection. For example, most of the included studies had male mpox cases of all ages mainly from non-endemic regions. It would be a better method to emphasize the role of cutaneous viral burden in the severity and infectivity of illness according to age, gender, and endemic and non-endemic regions. Another limitation involves the lack of data about testing samples from multiple sites such as mucocutaneous or body fluids, and facts from these clinical specimens may improve diagnostic sensitivity and reduce false-negative test results. Indeed, extensive studies are required to attain a logical understanding of transmission such as factors that have allowed the surprising penetration of active mpox infection into human communities globally.

## 5 Conclusion

The present study provides an estimate of the pooled positivity rate of skin samples from mpox patients. It provides novel and reliable evidence regarding the potential role of direct skin-to-skin contact in mpox transmission, relating to a high risk of transmission of infection from dermal lesions. This new knowledge can allow focusing on mitigation and containment measures to flatten the peak of mpox infection during future spreads.

## Author contributions

Substantial contributions to the conception or design of the work: BKP, IR, AG, MAS, PS, and JJB. Acquisition, analysis, or interpretation of data for the work: BKP, MAS, IR, and AG. Drafting the work: IR, AG, PS, MAS, and BKP. Revising the manuscript critically for important intellectual content: RS, BKP, AP, KG, RoS, IR, AG, and JJB. Final approval of the version to be published: all authors (IR, PS, AG, MAS, AP, RoS, KG, RS, JJB, and BKP). Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved: all authors (IR, PS, AG, MAS, AP, RoS, KG, RS, JJB, and BKP).

## Acknowledgments

The authors acknowledge the management of Maharishi Markandeshwar College of Sciences and Research (MMCMSR), Sadopur, Ambala, Haryana, India. They also acknowledge the organizing committee of the National Workshop on Systematic Review and Meta-analysis, Department of Community Medicine and School of Public Health, Postgraduate Institute of Medical Education and Research (PGIMER), Chandigarh, 160012 India, for providing tremendous guidance during the preparation of the manuscript. Authors also acknowledge Global Center for Evidence Synthesis, Chandigarh, India.

## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

## Publisher's note



## Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcimb.2023.1179885/full#supplementary-material

SUPPLEMENTARY ANNEX 1
Inclusion and Exclusion Criteria according to PECOS.

SUPPLEMENTARY ANNEX 2
The adjusted search terms as per the PECOS framework (prevalence of mpox DNA in skin samples of patients with monkeypox virus infection): searched electronic databases [as of 17.01.2023].

SUPPLEMENTARY ANNEX 3
Risk of Bias assessment of included studies using NIH tools **(A)** for Case series; **(B)** Cross-sectional studies.

SUPPLEMENTARY ANNEX 4
Influence analysis for confirming the meta-analysis conducted for the prevalence of mpox viral DNA in skin samples: **(A)** Baujat plot showing the studies which overly contribute to the overall heterogeneity, **(B)** Influence diagnostics, **(C)** Leave-one-out study method sorted by $I^2$, **(D)** GOSH plot showing the estimate and heterogeneity of meta-analysis of all possible subsets of included studies, **(E)** GOSH plot showing the contributions of the influential outlying study.

SUPPLEMENTARY ANNEX 5
Doi plot and LFK index to show asymmetry of study findings.

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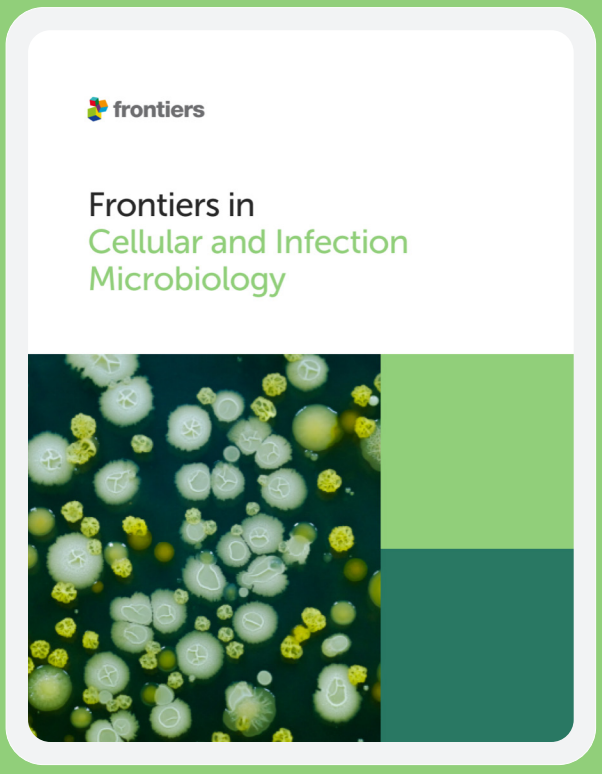


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