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Computational Design of a Phytochemical Drug Candidate Targeting Monkeypox Virus D8l and D4r/A20r Complex: An *in Silico* Study of Pharmacophore and Molecular Prediction

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ABSTRACT

Due to its rapid spread and the absence of approved antiviral treatments, Monkeypox virus (MPXV) has been declared as a global health emergency. This study aimed to identify possible drug candidates targeting MPXV vaccinia proteins D8L which is involved in host cell entry, and D4R/A20R Complex responsible for viral DNA replication and host cell entry. Phytochemical and pharmacophore compounds were assessed against MPXV target proteins using an in silico quantitative method that combined computational screening, molecular docking, and protein–ligand interaction analysis. Protein and ligand libraries were obtained from RCSB PDB, NCBI BLASTp, PubChem, and DrugBank. Drug-likeness and pharmacokinetic properties were assessed using ADMETLab 3.0. This screening reduced the initial number of 1,854 phytochemical and 808 pharmacophore compounds to 168 phytochemical and 11 pharmacophore candidates. The compounds that satisfied the former criterion were subjected to molecular docking and cavity assessment using CB-Dock which was then followed by an interaction analysis using PLIP. The leading ligands were then further evaluated using a criterion derived from docking rank, hydrogen bonds, and hydrophobic interactions gained. Among the three (3) methods, Artonol A and Camptothecin showed identical strong binding affinities of −9.1 kcal/mol and achieved the highest scores against their respective proteins. These findings support further experimental validation by in vitro and in vivo tests as possible choices for treating MPXV.

INTRODUCTION

Emerging infectious diseases (EIDs) are a major global health concern due to their unpredictable outbreaks, high transmissibility, and often minimal options for prevention or treatment. These diseases typically originate from viruses or bacteria that are transmitted from animals to humans or that evolve, becoming more virulent and spreading to immunodeficient individuals (Kobayashi, 2018 & Chen, 2022). One instance of an infectious disease is the monkeypox virus (MPXV), a DNA virus that belongs to the same viral family as smallpox and is part of the orthopoxvirus group. Similar to other orthopoxviruses, MPXV spreads through close contact with an infected person and can also be transmitted from animals to humans through bites, scratches, or direct contact (World Health Organization, 2024). Currently, there is no confirmed effective antiviral treatment for MPXV. Although several vaccine and clinical trials are ongoing to assess efficacy and safety, studies and results on drug or vaccine development for MPXV remain limited (Preet *et al.*, 2022). Furthermore, these vaccines are still in the experimental stage, and no officially verified drug or vaccine exists at present. Emerging infectious diseases such as MPXV highlight global vulnerability, as no confirmed treatment or approved vaccine is yet available despite ongoing research efforts (Kobayashi, 2018; Chen, 2022; World Health Organization, 2024; Preet *et al.*, 2022)

MPXV was declared a public health emergency in 2024 following its resurgence in 2022, which led to more than 91,000 confirmed cases and over 150 deaths reported across 115 countries (Chen, 2022). The impact of the virus is not uniform: epidemiological studies show that the Central African clade (clade I) has a much higher fatality rate of 10.6% compared with 3.6% for the West African clade (clade II) (Bunge *et al.*, 2022). Earlier outbreaks reflect this same variability, such as the 2017–2018 Nigerian epidemic, which records 122 confirmed or probable cases across 17 states (Yinka-Ogunleye, 2019). Even though smallpox has a lower fatality rate, MPXV's continuous spread remains a global public health challenge (Bhardwaj *et al.*, 2024). Therefore, MPXV is regarded as a persistent global health concern rather than a temporary one, emphasizing the urgent need for preventive measures, particularly effective vaccination (World Health Organization, 2024 & Bhardwaj *et al.*, 2024).

Understanding the molecular mechanisms of MPXV viral entry and replication is important to preventing this threat, as it provides possible targets for prevention and treatment. Among these targets, the D4R/A20R complex is essential for orthopoxvirus replication, and the D8L protein allows viral attachment to host cells by binding to chondroitin sulfate through a positively charged region (Riccardo & Pablo, 2023). The D4R protein selects other proteins necessary for viral DNA synthesis, while A20R

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stabilizes this interaction (Yu *et al.*, 2025). Blocking these interactions is considered to be a possible antiviral drug strategy to prevent orthopoxvirus infections (Burmeister *et al.*, 2024). Since MPXV and other orthopoxviruses share homologous proteins due to genetic similarity, they use conserved mechanisms for entry and replication, which explains why vaccines or drugs developed for smallpox may also demonstrate effectiveness against MPXV (Bhardwaj *et al.*, 2024). Building on this concept, phytochemical classes such as terpenoids, flavonoids, alkaloids, phenolic compounds, tannins, steroids, and saponins are studied as possible treatments because they may block viral entry and replication by regulating viral proteins and affecting the MPXV life cycle (Ndayambaje *et al.*, 2025; Ojo *et al.*, 2025; Bhambhani *et al.*, 2021; Stachelska *et al.*, 2025; Fraga-Corral *et al.*, 2021; Dominguez-Arca *et al.*, 2025; Hajdas *et al.*, 2025). Studies on phytochemicals and drug reuse offer possible strategies to block infection and support antiviral drug development (Kulkarni *et al.*, 2023 & Pokorny *et al.*, 2024).

MPXV spreads efficiently by relying on key steps in viral entry and replication, wherein the D8L protein facilitates viral attachment and fusion, while the D4R/A20R complex plays a crucial role in DNA replication, making both proteins important targets for antiviral drug development (Rosa *et al.*, 2025; Abdi *et al.*, 2022). However, studies that explore phytochemical- and pharmacophore-based approaches to identify inhibitors targeting the D8L protein and the D4R/A20R complex of MPXV remain limited.

In order to bridge this gap, this study aimed to design drug candidates using phytochemicals and pharmacophores that target MPXV proteins, specifically the D8L and the D4R/A20R complex. Specifically, this study: (1) developed protein and ligand libraries for the MPXV vaccinia D8L protein and D4R/A20R complex through data collection; (2) evaluated phytochemicals for drug-likeness, pharmacokinetics, and toxicity using ADMET profiling; (3) identified phytochemical ligands with the highest binding affinity to the D8L protein and D4R/A20R complex through molecular docking; and (4) analyzed molecular interactions between lead ligands and target proteins using interaction profiling. Using an in silico pipeline, this approach incorporates phytochemicals and target proteins with less reliance on costly laboratory experiments while maintaining ethical procedures. This work directly contributes to the Sustainable Development Goal three (Good Health and Well-being) by advancing drug innovation to promote health and well-being for all.

MATERIALS AND METHODS

Materials

The databases used for this study were RCSB Protein Data Bank (RCSB PDB), PubChem, and DrugBank, while the different tools were the NCBI Basic Local Alignment Search Tool, Protein (BLASTp), ADMETlab 3.0, CB-Dock, and Protein-Ligand Interaction Profiler (PLIP).

Research Design

The study implemented an in silico quantitative research design conducted using purely online databases and computational tools (Monteiro *et al.*, 2021) from August 2025 until November 2025. This design was selected to identify lead phytochemicals with strong potential to inhibit the D8L and D4R/A20R complex proteins, thereby contributing to drug development through safer and more sustainable alternatives. Within this period, databases, namely RCSB PDB, PubChem, and DrugBank, were accessed. Computational tools were used to generate, analyze, and validate results under controlled parameters. The independent variables were the target proteins, phytochemical and pharmacophore compounds, and docking parameters, while the dependent variables included drug-likeness and pharmacokinetic predictions from ADMETlab 3.0, molecular docking outcomes such as Vina scores and cavity sizes obtained using CB-Dock, and interaction profiles, specifically hydrogen bonding and hydrophobic interactions analyzed through PLIP, with sequence homology between Vaccinia and monkeypox virus proteins verified using BLASTp before docking. Reliability was ensured by performing each ADMET screening once for initial verification and conducting molecular docking in a single independent run to obtain consistent binding scores and interaction data.

Procedure

Preparation of Protein and Phytochemical Ligand Library

Protein Retrieval

To retrieve the target protein, first open the RCSB Protein Data Bank (PDB) and search for the *Vaccinia D8L ectodomain structure* or its corresponding UniProt entry (4E9O) and the *crystal structure of the vaccinia virus DNA polymerase holoenzyme subunit D4 in complex with the A20 N-terminus* or its corresponding UniProt entry (4OD8). The three-dimensional structures of both the D8L and D4R/A20R complexes were then downloaded by selecting *Legacy PBD Format* under the *Download Files* option and saved for future use. The selection criteria for the proteins included a resolution of 2.5 Å or lower, absence of mutations, and free binding sites suitable for molecular docking (Vittorio *et al.*, 2024).

Sequence Alignment and Homology Validation

The protein structures were initially retrieved from the RCSB Protein Data Bank using their corresponding UniProt entries. The FASTA sequences were obtained by selecting the "Display Files" option for each structure. These sequences were subsequently submitted to the NCBI BLAST platform by selecting BLASTp (protein–protein BLAST), pasting the retrieved FASTA sequences, and searching against the non-redundant protein sequences (nr) database. The organism filter was restricted to *Monkeypox virus (taxid: 10244*), while all other parameters were set to their default values. After running BLASTp, the top hit was evaluated based on

two (2) criteria: percent identity and percent similarity. Percent identity was obtained directly from the BLAST alignment results. Percent similarity was then assessed by opening the alignment of the top hit and examining the "Positives" value. If the proteins satisfied both identity and similarity thresholds, they were considered homologous and sufficiently similar (Johnson *et al.*, 2008).

Phytochemical Ligand Selection

Phytochemical ligands from plants were collected through the PubChem database, with a focus on compounds belonging to the terpenoids, flavonoids, alkaloids, phenolic compounds, tannins, steroids, and saponins, as these classes are known to exhibit antiviral activity. First, the PubChem database was opened, and the names of the selected phytochemicals were entered in the input box (Amjid *et al.*, 2025). The phytochemicals were then accessed by selecting the compounds from the best match module. Only phytochemicals reported in the literature to exhibit antiviral, antimicrobial, anti-inflammatory, or immunomodulatory activity were considered. Next, ligands were required to have complete and well-annotated chemical information in the PubChem database, including PubChem CID and an available 3D conformer structure. Once the phytochemical interface was opened, the *.sdf format from the 3D Conformer section was downloaded. Finally, compounds with extreme molecular weights or highly reactive functional groups that could compromise docking reliability or drug-likeness were excluded.

Pharmacophore-related Antiviral Compounds

This procedure began with a systematic review of literature to identify ten potential drugs for each MPXV protein, focusing on existing and investigational drugs with reported antiviral or related bioactivities. Each drug was evaluated using clinical and toxicity data to establish safety profiles, assess long-term risks, and ensure suitability for vulnerable populations. DrugBank, a widely used database for access to information on approved and experimental drugs, drug targets, mechanisms, and clinical trials (Knox *et al.*, 2024) was then used to review pharmacological activity, clinical trial outcomes, formulation details, stability, shelf life, and approval status, following FDA (Benet *et al.*, 2016), EMA (Clinical Efficacy and Safety Guidelines, 2024), WHO (Research CFDEA, 2025), and ICH (Santos *et al.*, 2025) guidelines, alongside ethical standards such as Good Clinical Practice (GCP) (Wang *et al.*, 2022) and informed consent. Once these criteria are met, each drug is cross-checked in DrugBank to verify its origin, determine whether it is plant-derived, and confirm its phytochemical components through literature. The identified phytochemicals were retrieved through PubChem, and the same process for phytochemical ligands was applied.

ADMET and Drug-Likeness Profiling

Drug-Likeness Evaluation

For each phytochemical, Canonical SMILES formats were retrieved from PubChem (2.1.4 SMILES section) and uploaded to ADMETLab 3.0 under the ADMET Evaluation menu. Compounds were evaluated for drug-likeness using multiple criteria, including Lipinski, Pfizer, and GSK rules, as well as the Golden Triangle guidelines in the Medicinal Chemistry module. Lipinski's Rule of Five assesses oral absorption potential, requiring molecular weight $\leq$ 500 Da, LogP $\leq$ 5, hydrogen bond donors $\leq$ 5, and hydrogen bond acceptors $\leq$ 10 (Hajduk *et al.*, 2005). The Pfizer Rule focuses on safety and bioavailability, flagging molecules with LogP $>$ 3 combined with a topological polar surface area $<$ 75 Å^2 (Lipinski *et al.*, 2001). The GSK Rule identifies compounds with a lower probability of clinical success, flagging molecules with molecular weight $>$ 400 Da or LogP $>$ 4 due to poor solubility and bioavailability (Hughes *et al.*, 2008). The Golden Triangle highlights compounds with favorable pharmacokinetics, selecting those with 200 $\leq$ MW $\leq$ 500 and $-2 \leq$ LogD (pH 7.4) $\leq$ 5 (Gleeson, 2008). If a compound passed these rules, ADMETLab 3.0 showed Accepted. Once a compound passed all these criteria were considered to have potential for good oral absorption and pharmacokinetic suitability, making it a candidate for further evaluation.

Pharmacokinetics

In the same ADMETLab 3.0 interface used for drug-likeness evaluation, phytochemicals were analyzed for their pharmacokinetic (ADME) properties in the *Absorption, Distribution, Metabolism, Excretion,* and *Toxicity* modules, which helped identify compounds likely to behave favorably in the human body (Johnson *et al.*, 2009). Virtual pharmacokinetic simulations were performed using mouse and rat models to predict compound behavior. Absorption was assessed to estimate oral bioavailability, with high permeability indicated by Caco-2 $>$ -5.15. Distribution predicted how a compound spreads through the body, with a steady-state volume of distribution (VDss) $>$ 0.45 L/kg considered acceptable. Metabolism evaluated chemical modification by liver enzymes, requiring human liver microsomal (HLM) stability $>$ 30 minutes. Excretion predicted elimination efficiency, with compounds showing high (green) or moderate (yellow) plasma clearance (CLplasma) considered suitable. Toxicity screening was also performed in the same interface, with compounds required to have skin sensitization $<$ 1 to pass (Kamiya *et al.*, 2021).

Selection of Safe Candidate

After checking the compounds' drug-likeness and pharmacokinetics, only those that meet the Drug Likeness Evaluation criteria and exhibit good ADMET behavior were retained. These remaining phytochemicals were considered safe and effective as a potential drug candidate.

Molecular Docking

Phytochemicals that passed ADMET and drug-likeness

profiling, including pharmacophore-based compounds from DrugBank, were subjected to molecular docking. The three-dimensional (.pdb) structures of the D8L protein and D4R/A20R complex were retrieved from the RCSB Protein Data Bank and uploaded to CB-Dock together with ligand (.sdf) files. Protein preparation was performed automatically using CB-Dock, which removed heteroatoms, including water molecules and co-crystallized ligands, and converted the structures into AutoDock-compatible PDBQT format for docking with AutoDock Vina (Liu *et al.*, 2020). The CB-Dock server generated the top five docking poses with corresponding Vina scores (-kcal/mol) and cavity sizes. Only results with cavity sizes within the threshold range of 160 to 800 Å3 were considered for ranking, while those outside this range were excluded (Loving *et al.*, 2014). The accepted Vina scores and cavity sizes were ranked and compiled in an Excel (*.xlsx) file, as presented in Appendix AN.

Protein-Ligand Interaction Analysis

The top five (5) protein-ligand complexes based on the Vina scores and cavity size fit, with their corresponding *.pdb files, including those from pharmacophore ligands, were then uploaded to the PLIP web server for detailed interaction analysis. PLIP automatically detected the hydrogen bonds and hydrophobic interactions in the form of tables and images (*.png). The hydrogen bonds and hydrophobic interactions were counted and totaled. The top five (5) leading Vina scores for both proteins were analyzed using PLIP to identify their hydrogen bonds and hydrophobic interactions count, with hydrogen bonds between −1.5 and −4.7 kcal/mol and hydrophobic interactions between 1 and 2 kcal/mol considered significant (De Freitas & Schapira, 2017; Nittinger *et al.*, 2017). The ligands were then subjected to an evaluation criterion and ranked based on a score comprising docking rank (50%), hydrogen bond count (30%), and hydrophobic interactions (20%) (Swain, 2019; Du *et al.*, 2016; Freitas & Schapira, 2017; Kozakov *et al.*, 2015; Tobin, 2024).

Data Analysis

Data collection began with the construction of protein and ligand libraries for the D8L protein and D4R/A20R complex through systematic retrieval, duplicate removal, and preparation of SMILES formats and three-dimensional conformers. The phytochemical library was evaluated for drug-likeness and pharmacokinetic properties, and compounds that passed ADMET screening were subjected to molecular docking to predict binding affinity and cavity compatibility. The ligands that passed the ADMET screening and cavity assessment were ranked; those failing the criteria were excluded from further analysis. The top five (5) drug-like compounds were selected as the focus. Their results, such as ADMET profiles, docking scores, interaction counts, and evaluation criteria, were briefed in tables, while diagrams of the target proteins and CB-Dock results were shown as figures.

Ethical Consideration

To ensure the responsible management of information, the study followed established ethical guidelines in data handling with emphasis on data minimization, data privacy, and intellectual property rights. Data minimization was practiced by collecting only the necessary data required for the research objectives to reduce the risk of unnecessary exposure. All data sources were ethically sourced, and proper attribution was given to the original creators to uphold academic integrity. The researchers also prioritized readily accessible data while maintaining transparency throughout the entire process. Respecting intellectual property and ensuring proper dissemination of results helped make the study evidence-based and ethical (Tobin 2024). In addition, the tools and databases underwent validation by the researchers to ensure their reliability. The preparation of documents and the writing of formal letters to the principal were also undertaken as part of the study requirements to secure approval and ensure adherence to institutional protocols.

RESULTS AND DISCUSSION

Protein Library

In Figure 1, the Vaccinia D8L ectodomain structure (UniProt entry 4E9O) was selected because of its role in viral attachment and host cell interaction (Riccardo & Pablo, 2023). This structure met the selection criteria, which comprised high experimental resolution (1.42 Å), absence of reported mutations, and well-defined binding sites suitable for molecular docking analyses (RCSB Protein Data Bank, 2023). In a similar way, the crystal structure of the Vaccinia virus DNA polymerase holoenzyme subunit D4 in complex with the A20

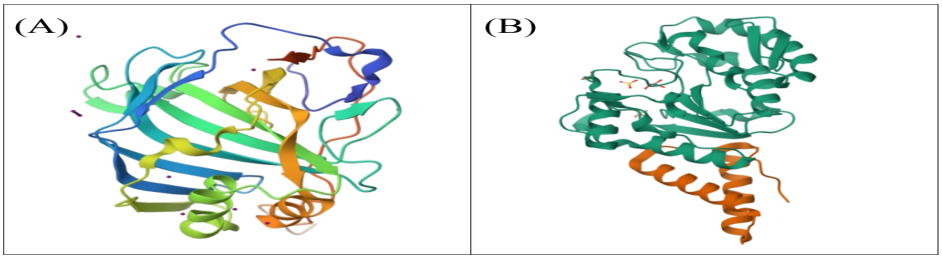

Figure 1: Vaccinia Virus Structures Used in Molecular Docking

Note1. (A). Vaccinia D8L ectodomain Chain A; and (B) Vaccinia DNA polymerase D4R/A20R complex Chain A and B. Source: RCSB PDB

N-terminus (UniProt entry 4OD8) was picked to be included in the protein library because of its critical role in viral DNA replication (Yu *et al.*, 2025). This structure also satisfied the selection criteria: a high-resolution structure (1.85 Å), no reported mutations, and free binding sites for docking (RCSB Protein Data Bank, 2023). These viral protein structures as entry and replication-related targets provide a reliable starting point for evaluating potential drug-like phytochemicals.

Sequence Alignment and Percent Identity

Table 1 shows the percentage of sequence alignment and percent identity between the Vaccinia virus proteins and their MPXV homologs.

In Table 1, both Vaccinia virus proteins showed high sequence alignment and percent identity with their MPXV counterparts as shown by their greater than 90% identities; meanwhile, sequences with percent identities between 95% and 99% were considered near-identical and highly conserved. The similarity percentages, which reflect both identical and conservatively substituted amino acids, indicate that essential biochemical and structural characteristics are preserved. These findings are consistent with previous studies showing strong conservation among orthologous poxvirus proteins, suggesting that essential functions have been maintained. These characteristics directly support the reliability of homologous models and subsequent molecular docking analyses to predict protein–ligand interactions (Shen-Gunther *et al.*, 2025).

Table 1: Sequence Alignment and Percent Identity Between Vaccinia Virus Proteins and MPXV Homologs

Target Protein	Structures Used for Docking (PDB ID)	MPXV Homolog	Identity (%)	Similarity (%)
D8L	4E9O	E8L	94.27%	96.95%
D4R/A20R Complex	40D8	E4L/A22R	96.00%	96.00%

Note. Identity percentage represents exact AA matches, while percent similarity includes conservative substitutions

Ligand Library

Table 2 summarizes the number of phytochemical and pharmacophore ligands utilized for the ligand library.

The ligand library was developed to provide a collection of plant-derived compounds for in silico drug discovery. As indicated in Table 1, a total of 1,854 phytochemicals were consolidated, with alkaloids (409), flavonoids (404), and terpenoids (399) forming the largest groups. These classes are well-established in the literature as major sources of biologically active scaffolds because of their structural diversity and capacity to engage multiple biological targets (Lautie *et al.*, 2020). The addition of phenolic compounds, tannins, steroids, and saponins further strengthens the library by ensuring that compounds with antiviral, antioxidant, anti-inflammatory, antimicrobial, and antimutagenic properties are well represented.

Table 2: Number of Phytochemical and Pharmacophore Ligands

Phytochemical Class	Number of Phytochemical Ligands	Pharmacophore Ligand	Number of Pharmacophore Ligands
Alkaloids	409	Curcumin	122
Flavonoids	404	Quercetin	95
Terpenoids	399	Silibinin	95
Phenolic Compounds	248	Ellagic Acid	91
Tannins	191	Eugenol	87
Steroids	123	Glycyrrhizic Acid	80
Saponins	80	Andrographolide	66
-	-	Baicalein	63
-	-	Berberine	57
-	-	Resveratrol	52
Subtotal:	1854	Subtotal:	808
Total: 2,662			

Note. Rows are ordered by decreasing sample size.

Alongside the phytochemical class, 808 pharmacophore ligands based on well-established reference compounds such as curcumin (122), quercetin (95), and silibinin (95) were incorporated. These compounds are commonly used in virtual screening studies because they show favorable drug-like properties and acceptable pharmacokinetic behavior (Lipinski *et al.*, 2001). By combining a large and chemically diverse phytochemical dataset with a pharmacophore, the ligand library improves the reliability of early-stage screening and increases the chance of

identifying promising lead ligands.

Drug-Likeness Test Results and Pharmacokinetic Results

Table 3 shows the results of the drug-likeness screening and pharmacokinetic evaluation of phytochemical and pharmacophore ligands for the D8L and D4R/A20R complex.

Early-stage screening is essential in drug development to increase the likelihood of identifying safe and effective candidates. The results of drug-likeness and pharmacokinetic evaluations demonstrate that only a small portion of the initially screened compounds are predicted to possess favorable oral bioavailability and ADMET properties. This highlights the rigid nature of early-stage virtual screening and the importance of computational filtering in efficiently prioritizing promising candidates for further evaluation. Similar trends have been observed in other studies, where a small proportion of initially drug-like compounds meet strict pharmacokinetic criteria, underscoring the challenges in identifying compounds with both drug-like and favorable ADMET characteristics (Dubey *et al.*, 2025).

Table 3: Number of Ligands that Passed the Drug-Likeness and Pharmacokinetic Evaluations

Screening Stage	Ligand Type	Total Screened, n	Passed, n (%)
Drug-Likeness Evaluation a	Phytochemical Ligand	1,854	628 (33.87%)
Drug-Likeness Evaluation a	Pharmacophore Ligand	808	113 (13.99%)
Pharmacokinetic (ADMET) Evaluation b	Phytochemical Ligand	628	168 (26.75%)
Pharmacokinetic (ADMET) Evaluation b	Pharmacophore Ligand	113	11 (9.74%)

a Drug-likeness evaluation followed Lipinski's, Pfizer's, GSK's, and the Golden Triangle rules

b Pharmacokinetic (ADMET) evaluation assessed the absorption, distribution, metabolism, excretion, and toxicity profile

Docking Simulations

Table 4 (columns 1–5) presents the docking results (binding energies and cavity sizes) for the top five ligands against D8L and D4R/A20R complex proteins

The docking simulations predicted that Artonol A and Camptothecin emerged as the leading candidates among the screened phytochemicals against D8L and D4R/A20R complex proteins, each exhibiting a docking score of –9.1 kcal/mol and occupying relatively large binding cavities (776 Å³ for Artonol A in D8L; 389 Å³ for Camptothecin in D4R/A20R). In molecular docking, more negative binding energy values indicate stronger predicted binding affinity, while cavity size refers to the volume of the predicted binding pocket; ligands that exhibit both favorable scores will rank higher during

Table 4: Comprehensive Docking Affinities and Molecular Interaction Profiles of Top-ranking Ligands Against D8L and D4R/A20R Proteins

Target	Rank	Ligand	Binding Energy (kcal/mol	Cavity size (Å³)	H-Bonds	H-Bonds (AA Residues)	Hydrophobic Interactions	Hydrophobic Interactions (AA Residues)
D8L	1	Artonol A	-9.1	776	5	Tyr69, Tyr104, Ile174, Asn175 (2)	3	Leu42, Tyr69, Asn175
	2	Evodiamine	-8.6			Tyr104, Ser177	6	Lys41, Leu42, Tyr104, Ile174, Ile174, Asn175
	3	Sesamin	-8.6	776	2	Arg44, Ser64, Tyr69, Arg220	1	Leu42
	4	Sophoraisoflavanone A	-8.3	776	4	Arg44, Val64, Ser65, Tyr69 (2), Tyr104	7	Asn92, Val94, Ile116, Thr173, Ile174, Ile174, Trp182
	5	Strychnine	-8.3	776	6	N/A	6	Arg20, Leu21, Phe56, Thr168, Leu170, Val181

D4R/ A20R	1	Camptothecin	-9.1	260	0	Thr130, Lys131	4	
	2	Sophoraflavanone B	-8.2	389	2	Ile67, Asp68, Lys87, Ser88, Thr130	9	Tyr70, Phe79, Phe79, Thr130
	3	Isoxanthohumol	-8.1	389	5	Ile67, Asp68 (2), Lys87, Ser88, Thr130	8	Ile67, Tyr70 (3), Pro71, Phe79, Lys86 (2), Lys87
	4	Lupiwighteone	-8.1	389	6	Ile67, Asp68, Lys87, Ser88 (2), Thr130	5	Ile67, Tyr70 (3), Pro71, Phe79, Lys86, Lys87
	5	Licoagrodione	-7.9	389	6	Lys87, Ser88 (2), Thr130	4	Tyr70 (2), Phe79, Lys87, Thr130
				616	4			Tyr70, Pro71, Phe79, Lys86

the virtual screening. Whereas the cavity size threshold (160 to 800 Å3) was based on reported mammalian cavity size (Loving *et al.*, 2014). Accordingly, Artonol A and Camptothecin were prioritized based on their combination of favorable docking scores and geometric complementarity within the predicted binding pockets (Harigua-Souiai *et al.*, 2015).

Figure 2 illustrates the predicted binding poses of the top-ranked ligands listed in Table 4. Artonol A is positioned in a region of neutral to negatively charged electrostatic surfaces, consistent with the presence of predicted hydrogen bonds and hydrophobic contacts. Camptothecin is similarly oriented in the D4R/A20R pocket, aligning with the cavity geometry and mainly neutral surfaces. In each panel, the left image shows the overall protein-ligand complex with electrostatic surfaces (blue: positive; red: negative; white: neutral), while the right highlights the binding pocket and key interacting

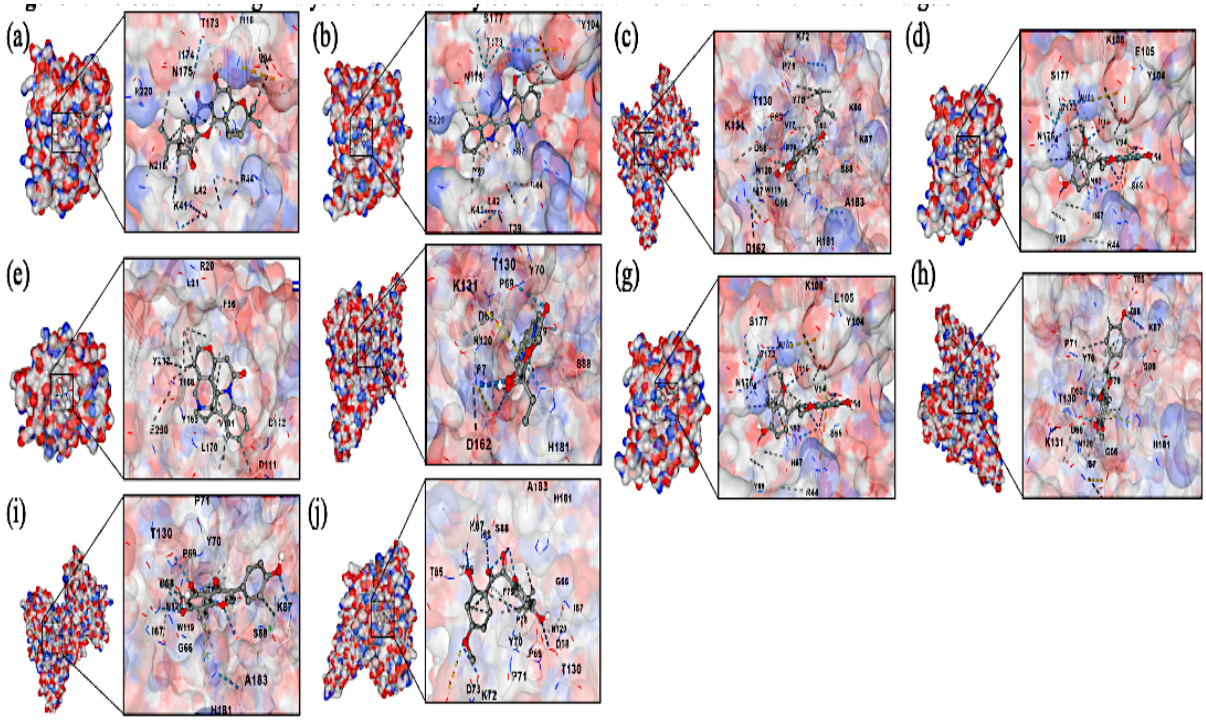

Figure 2: Molecular Docking Analysis of Selected Phytochemicals with D8L and D4R/A20R Protein Targets

Note 1. (a) Artonol A – D8L, (b) Evodiamine – D8L, (c) Sesamine – D8L, (d) Sophoraisoflavanone A – D8L, (e) Strychnine – D8L, (f) Camptothecin – D4R/A20R, (g) Sophoraflavanone B – D4R/A20R, (h) Isoxanthohumol – D4R/A20R, (i) Lupiwighteone – D4R/A20R, (j) Licoagrodione – D4R/A20R

Note 2. For each panel, the left image shows the overall protein-ligand complex in an electrostatic surface, while the right image highlights the binding pocket and key interacting residues

Note 3. Electrostatic surfaces color: blue (positively charged surfaces), red (negatively charged surfaces), and white (neutral areas)

residues. These results reflect predicted poses and relative affinities only and do not demonstrate biological activity or antiviral efficacy. Thus, the ligands should be considered preliminary in silico screening outcomes, requiring experimental validation to assess their potential relevance as MPXV inhibitors (Pinzi & Rastelli, 2018).

Protein–Ligand Interaction Analyses

Table 4 (columns 1, 3, 6–9) summarizes the leading ligands for each MPXV target together with their interaction profiles.

Interaction profiling was performed to identify hydrogen bonds, hydrophobic contacts, and other stabilizing interactions within the protein-ligand complexes (Harigua-Souiai *et al.*, 2015). Hydrogen bonds and hydrophobic interactions were emphasized because they are among the most frequently observed atomic contacts in protein–ligand binding (Pinzi & Rastelli, 2018). These interactions provide a descriptive measure of how the ligands fit within the binding pockets and potentially stabilize the complexes, though no formal energetic calculations were performed. Artonol A formed five (5) hydrogen bonds and three (3) hydrophobic interactions, while Camptothecin formed two (2) predicted hydrogen bonds and four (4) hydrophobic interactions. Compounds with more hydrogen bonds and hydrophobic interactions are likely to form more stable interactions with the protein, which may correspond with the observed docking scores; however, there are no formal energetic calculations performed to quantify these contributions.58 Both ligands presented similar docking scores (–9.1 kcal/mol), suggesting that scoring functions may weigh different interaction types differently and may not accurately reflect their true energetic contributions.

The cavity size analysis showed that the ligands occupied the binding areas of different sizes, which were related to minor differences in their interaction profiles. These observations are descriptive and do not imply a direct relationship between the cavity size and interaction behavior. The ADMET evaluation supports the potential of the lead ligands as safe drug candidates, as they exhibited favorable pharmacokinetic properties and low predicted toxicity. Overall, the interaction profiling provides a clearer understanding of how the ligands interact with their target proteins at the molecular level. These profiles highlight interaction features that may contribute to ligand binding and complex stability. This information establishes a foundation for further computational studies or experimental validation, such as molecular dynamics (MD) simulations, to validate and assess the functional relevance of these interactions.

Evaluation Criteria Results

Table 5 presents the final scores of the top 5 lead ligands for D8L and D4R/A20R complex from the evaluation criteria.

Out of the 1,854 phytochemical and 808 pharmacophore ligands that went through screening, Artonol A, a flavonoid, obtained the highest final score for D8L inhibition which possibly indicates a stable interaction within the D8L pocket. This interaction may be biologically relevant since it is consistent with flavonoids' antiviral properties because this may influence protein activities related to viral attachment or entrance (Stachelska *et al.*, 2025). Similarly, Camptothecin, an alkaloid that is dominantly synthesized for therapeutic uses demonstrated stable interaction within the D4R/A20R complex binding pocket. This suggests a possible

Table 5: Lead Ligand for MPXV Vaccinia D8L and D4R/A20R Complex Based on Evaluation Criteria

Final Rank	Ligands for D8L	Final Score (100%)	Ligands for D4R/A20R Comple	Final Score (100%)
1	Artonol A	61.67%	Camptothecin	65.09%
2	Strychnine	46.67%	Sophoraflavanone B	39.64%
3	Evodiamine	44.79%	Isoxanthohumol	38.33%
4	Sesamin	31.88%	Licoagrodione	32.13%
5	Sophoraisoflavanone A	20.00%	Lupiwighteone	16.90%

interference with protein functions related to viral replication (Bhambhani *et al.*, 2021). Raw Camptothecin is highly cytotoxic to humans and is primarily used in chemotherapy treatment due to its ability to target rapidly dividing cells. Its natural form would not be safe for general antiviral us (Wang *et al.*, 2023). Nevertheless, Camptothecin may be chemically modified to reduce toxicity while retaining biological activity. This makes it a useful scaffold for the development of safer antiviral derivatives, rather than a direct therapeutic.

The final scores for the lead compounds which were 61.67% and 65.09%, were derived from a weighted evaluation framework that integrates binding affinity (50%), hydrogen bonding (30%), and hydrophobic interactions (20%) (Swain, 2019; Du *et al.*, 2016; Freitas & Schapira, 2017; Kozakov *et al.*, 2015; Tobin, 2024). Benchmark studies that employed the Protein–Ligand Interaction Profiler PLIP showed that the recovery of hydrogen bonds and hydrophobic interactions in docking poses is limited and often remains below 50% when compared with crystallographic reference structures (Lai *et al.*, 2024). With this limitation, the interaction portions of the criterion contribute only half of their actual weight which results in a maximum score of ~75%. Therefore,

the observed scores above 60% indicate strong predicted interactions and consistent performance across all evaluated criteria rather than weak binding.

It is important to note that these ligands serve primarily as reference scaffolds for drug design rather than finalized therapeutic agents. Further optimization and testing are required before any clinical application. Collectively, these findings support the potential of Artonol A and Camptothecin as candidate compounds for further in vitro and in vivo tests to clarify their antiviral relevance for MPXV.

CONCLUSION

Based on the findings of the study, the researchers concluded that 1) a protein and phytochemical-ligand library is compiled through systematic data collection, consisting of MPXV vaccinia D8L protein and the D4R/A20R complex, 1,854 phytochemicals, and 808 pharmacophore ligands for virtual screening; 2) 168 phytochemicals and 11 pharmacophore ligands meet drug-likeness standards through pharmacokinetic analysis; 3) There are compounds with strong binding affinities and complementary cavity fit with the proteins. Specifically, Artonol A (−9.1 kcal/mol) shows the highest docking score for D8L, while Camptothecin (−9.1 kcal/mol) scores highest for the D4R/A20R complex. Likewise, 4) PLIP analysis confirms stable hydrogen bonds and hydrophobic interactions between these compounds and their target proteins. These results identify Artonol A and Camptothecin as promising candidates for MPXV vaccinia D8L and the D4R/A20R complex and support their potential for further in silico and in vivo validation.

Recommendations

Based on the conclusions of the study, the researchers recommend that 1) integrate Molecular Dynamics (MD) simulation into the study to further understand the interactions between potential drug compounds and biological targets; 2) future studies should move toward in vitro and in vivo experiments to support the computational findings, wherein in vitro tests can determine whether the compounds block the target proteins and in vivo tests can assess safety and behavior in the body; 3) improve docking methods by repeating each test multiple times and using the mean score to reduce bias from a single run and ensure consistent ranking of top compounds; 4) study how several viral proteins work together to facilitate MPXV infection and test strategies that simultaneously target multiple proteins; and 5) apply the computational methods used in this study to other MPXV or orthopoxvirus proteins to support efficient and rapid antiviral drug discovery and preparedness for future outbreaks.

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