Research Article

Integrated *In silico* and *In vitro* Evaluation of the Antiviral Potential of *Hibiscus sabdariffa* Ethanolic Extract Against Tobacco Mosaic Virus

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Abstract | Tobacco mosaic virus (TMV) is a major plant pathogen causing significant agricultural losses. Roselle-derived phytochemicals, including flavonoids and phenolic acids, are recognized for their broad-spectrum antiviral properties. This study aimed to evaluate the antiviral potential of *Hibiscus sabdariffa* ethanolic extract against TMV using integrated *in silico* and *in vitro* approaches. In addition, the bioactive compounds gallic acid, hesperidin, kaempferol, and quercetin were investigated through molecular docking and Absorption, Distribution, Metabolism, Excretion, and Toxicity (ADMET)/pharmacokinetic profiling against TMV coat, movement, and replicase proteins. Molecular docking was performed to assess binding affinities and interaction patterns of selected compounds with TMV proteins. ADMET analysis (Absorption, distribution, metabolism, excretion, and toxicity analysis) evaluated drug-likeness, absorption, and toxicity profiles relative to acyclovir. Gallic acid, kaempferol, and quercetin complied with Lipinski's Rule of Five and exhibited a favorable absorption, whereas hesperidin showed poor gastrointestinal absorption and potential toxicity risks. Docking revealed that hesperidin had the strongest binding affinity (replicase BA = −9.1 kcal/mol), followed by quercetin (−8.5 kcal/mol), and kaempferol (−7.7 kcal/mol), indicating potential inhibition of viral RNA synthesis and systemic spread. Gallic acid displayed moderate affinity (−6.5 kcal/mol), while acyclovir exhibited the weakest binding. Interaction analysis showed extensive hydrogen bonding, π-interactions, and hydrophobic contacts for Roselle compounds, particularly hesperidin, which formed multiple bonds with the catalytically relevant residues. Root Mean Square Deviation (RMSD) values of zero confirmed stable docking conformations. ADMET profiling highlighted possible CYP enzyme inhibition by kaempferol and quercetin, suggesting a caution for drug–drug interactions. Overall, Roselle compounds demonstrated stronger and more diverse interactions than acyclovir, indicating multi-target antiviral potential. *In vitro*, the ethanolic Roselle extract showed a strong, dose-dependent antiviral effect against TMV on *Datura metel* leaves, supporting its potential as a natural source of bioactive compounds for plant viral control. Roselle-derived phytochemicals, especially hesperidin, kaempferol, and quercetin, exhibited a promising antiviral activity against TMV through stable, multi-type interactions with the viral coat, movement, and replicase proteins. Their favorable pharmacokinetic profiles (except hesperidin) and superior docking performance compared to acyclovir support their development as natural antiviral agents for plant protection. Further *in vivo* validation, formulation, optimization, and toxicological studies are recommended to confirm the efficacy, safety, and bioavailability of *H. sabdariffa* bioactive compounds as potential antiviral agents against TMV under field conditions.

Received | April 12, 2026; **Revised** | May 17, 2026; **Accepted** | May 25, 2026; **Published** | June 04, 2026
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Citation | Abo Saif, A.M., A.I. Ahmed, A.S. Sadik, A.A. Megahed and S.K. Ali. 2026. Integrated *in silico* and *in vitro* evaluation of the antiviral potential of *Hibiscus sabdariffa* ethanolic extract against Tobacco mosaic virus. *Novel Research in Microbiology Journal,* 10(3): 286-310.
DOI | https://dx.doi.org/10.17582/journal.nrmj/2026/10.3.286.310
Keywords | ADMET profiling, Roselle-derived phytochemicals, Tobacco mosaic virus, Molecular docking, Natural antiviral agents, Plant protection

Introduction

Plant viruses, particularly Tobacco mosaic virus (TMV), present remarkable challenges to global agriculture, causing substantial yield losses in crops such as tobacco, tomatoes, and peppers (Megahed *et al.*, 2019; Jones, 2021; Petrov *et al.*, 2024; Angira *et al.*, 2025). TMV is a single-stranded RNA virus that encodes several proteins essential for its replication and systemic movement within the plant tissues (Peña and Heinlein, 2012). Among these, the coat protein (CP), movement protein (MP), and replication proteins are critical for viral infectivity and represent prime targets for antiviral interventions (Dorokhov *et al.*, 2020; Schlicksup and Zlotnick, 2020).

While the chemical pesticides have traditionally been used to manage plant viral infections, concerns over their environmental impact and the development of resistance have driven the search for alternative eco-friendly solutions (Souto *et al.*, 2021; Chowdhury *et al.*, 2024). Phytochemicals derived from plants have emerged as promising candidates due to their bioactivity, biodegradability, and sustainability (El-Dougdoug *et al.*, 2013; Megahed *et al.*, 2023; Beyari, 2025; Hamden *et al.*, 2026). *Hibiscus* species, particularly *H. sabdariffa* (roselle) and *H. rosa-sinensis*, are rich sources of bioactive compounds, including flavonoids, phenolic acids, and anthocyanins (Jabeur *et al.*, 2017; Duque-Soto *et al.*, 2023; Mejía *et al.*, 2023; Raza *et al.*, 2025). These compounds are known for their antioxidant, anti-inflammatory, antimicrobial, and antiviral potentials (El-Shiekh *et al.*, 2020; Bala *et al.*, 2022; Alharbi *et al.*, 2024).

Recent studies suggest that *Hibiscus* extracts can modulate plant-pathogen interactions and enhance plant defense mechanisms, making them potential natural agents for managing viral infections (Calefi *et al.*, 2025). For instance, aqueous extracts of *H. sabdariffa* calyces have been shown to substantially reduce viral titers in various plant viruses, with bioactive compounds such as ferulic acid, protocatechuic acid, and malic acid playing key roles in these antiviral effects (Davidova *et al.*, 2024). Different natural plant extracts reduced TMV activity, likely through direct interactions between their phytochemicals and the viral proteins (Jing *et al.*, 2012; Islam *et al.*, 2018). In line with this, Abdelkhalek *et al.*, (2021) demonstrated that the methanol extract of *Paronychia argentea* effectively reduced TMV accumulation by 77.88% in tomato plants under greenhouse conditions. Moreover, this extract induced systemic resistance, as reflected by increased activities of antioxidant enzymes and upregulation of defense-related genes. Collectively, these findings highlight the potential of plant-derived extracts as safe and effective sources of bioactive compounds for the management of viral infections in plants, offering a promising alternative to chemical control strategies.

Given the promising antiviral potential of *H. sabdariffa* (Roselle) phytochemicals, this study aimed to evaluate their potential anti-TMV activity through integrated *in silico* and *in vitro* approaches. Molecular docking and ADMET analyses were performed to predict the interactions of selected phytochemicals with TMV coat, movement, and replication proteins, while experimental *in vitro* assays were conducted to validate their antiviral activity. This integrated approach may contribute to the development of novel plant-based antiviral strategies for the sustainable management of plant viral diseases.

Materials and Methods

All experiments included in this study were conducted during the period 2025–2026 at the Virology Laboratory, Department of Agricultural Microbiology, and the Plant Clinic, Department of Plant Pathology, Faculty of Agriculture, Ain Shams University.

Molecular docking analysis

Selection of bioactive ligands

A literature survey was conducted to identify bioactive compounds from *H. sabdariffa* with reported antiviral activity, with the aim of selecting promising candidates for subsequent *in silico* studies (Hassan *et al.*, 2017; Jabeur *et al.*, 2017; El-Shiekh *et al.*, 2020). Based on these findings, five compounds were selected: Gallic acid, hesperidin, kaempferol, and quercetin-derived from *H. sabdariffa* along with acyclovir, which was included as a reference antiviral drug. These compounds were selected due to their documented antiviral, antioxidant, and anti-inflammatory properties.

Prediction of pharmacokinetics and ADMET profiles

The pharmacokinetic and ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) properties of the selected compounds were evaluated

using the SwissADME web server (http://www.swissadme.ch). SMILES codes from PubChem were used as input. Parameters assessed included molecular weight, number of rotatable bonds, hydrogen bond donors and acceptors, gastrointestinal absorption, blood-brain barrier penetration, Cytochrome P450 enzyme inhibition (CYP1A2, CYP2C19, CYP2C9, CYP2D6, CYP3A4), compliance with Lipinski's Rule of Five, and predicted toxicity. Results were compiled to compare drug-likeness, oral bioavailability, metabolic stability, and safety profiles. All compounds met Lipinski's criteria and were predicted to be non-toxic.

Preparation of ligand structures

2D chemical structures were retrieved from PubChem and converted to 3D models using Open Babel (GitHub, openbabel.org). Energy minimization was performed with the MMFF94 force field to optimize the geometry. The ligands were saved in PDBQT format for molecular docking analysis (Daina *et al.*, 2017; Abdel Razek *et al.*, 2025).

Modeling of TMV target proteins

Three TMV proteins were selected as targets: Replicase (WCO04976.1), Movement protein (WCO04978.1), and Coat protein (WCO04977.1). Sequences were obtained from NCBI and 3D structures, and were generated *via* homology modeling using SWISS-Model (https://swissmodel.expasy.org) and AlphaFold (https://alphafold.com). Structural quality was validated through Ramachandran plots and PROCHECK to ensure accuracy and reliability.

Molecular docking procedure

Molecular docking simulations were performed using AutoDock Vina. Protein structures were prepared by removing water molecules, adding polar hydrogen atoms, and assigning Kollman charges using AutoDock Tools. Ligands were prepared by assigning Gasteiger charges and defining rotatable bonds to ensure conformational flexibility. The docking grid boxes were generated based on the predicted active sites of the target proteins, with coordinates and dimensions selected to fully encompass the catalytic or ligand-binding regions. Active-site residues were identified based on reported literature and structural analysis of the conserved functional domains. All docking calculations were performed using default AutoDock Vina parameters, and binding affinities (kcal/mol) were recorded for each ligand–protein complex.

Assessment of docking performance

Docking results were analyzed by comparing binding affinities and interaction profiles of all tested compounds against the TMV proteins. RMSD values were used to evaluate the reliability and the reproducibility of the docking poses. RMSD values ≤ 2.0 Å were considered indicative of stable and consistent binding conformations, while lower values reflected higher docking precision.

Visualization and interaction analysis

Docking poses were visualized using PyMOL (https://pymol.org) and Discovery Studio Visualizer (https://discover.3ds.com/discovery-studio-visualizer-download) to analyze ligand orientations and interaction patterns within the binding pockets. Hydrogen bonds, hydrophobic interactions, and other non-covalent interactions were identified, and the key interacting amino acid residues were recorded to highlight the most promising antiviral candidates.

In vitro evaluation of anti-TMV activity of H. sabdariffa ethanolic extract

Plant material

Dried leaves of *H. sabdariffa* were obtained from a locally authenticated market (Cairo, Egypt). The plant material was thoroughly washed with distilled water, shade-dried at room temperature, and ground into a fine powder using a sterile electric grinder. The powdered material was stored in airtight containers at room temperature until further use.

Preparation of ethanolic extract

The dried powdered material was homogenized in 70% ethanol (1:5, w/v) under aseptic conditions using a mortar and pestle. The mixture was incubated at room temperature for 48 h with occasional stirring, followed by filtration through sterile gauze and centrifugation at 800 xg for 5 min. The supernatant was concentrated by evaporation at low heat using a ventilated setup to remove ethanol. The resulting crude extract was reconstituted in 10 ml sterile distilled water and stored at 4 °C until further use (Elhalag *et al.*, 2025).

Preparation of extract dilutions

Serial two-fold dilutions of the ethanolic extract were prepared (1:2, 1:4, 1:8, 1:16, 1:32, and 1:64) by mixing equal volumes of the extract and sterile distilled water

in a stepwise manner under aseptic conditions.

Virus inoculum preparation

Tobacco mosaic virus infected sap was obtained from the Virology Laboratory, Faculty of Agriculture, Ain Shams University. The inoculum was prepared 14 days post-inoculation as previously described by Ahmed *et al*. (2024), and maintained at 4 °C until use.

Treatment preparation

Equal volumes (750 µl) of TMV-infected sap were mixed with each extract dilution and incubated at 4°C for 24 h to allow interaction between the viral particles and the plant extract constituents prior to inoculation.

Leaf inoculation and experimental design

For bioassay evaluation, 250 µl of each treatment mixture were mechanically applied to the surface of Datura metel leaves cultivated in the greenhouse of the Virology laboratory, Department of Agricultural Microbiology, Faculty of Agriculture, Ain Shams University, Egypt, a local lesion host for TMV. Young *Datura metel* plants at the 4–5 leaf stage were used to ensure uniform physiological age and consistent susceptibility to the viral infection. Treatments were arranged in a completely randomized design with three biological replicate per treatment. Inoculated plants were maintained under controlled greenhouse conditions and the development of necrotic local lesions was recorded daily for 10 days (Megahed *et al*., 2013a).

Data collection and statistical analysis

The number of developing necrotic local lesions (NLL) per leaf was recorded for each treatment and mean values were calculated. Percentage inhibition of TMV infection was determined relative to the untreated infected control (Megahed *et al*., 2013b). Data were expressed as mean±standard deviation (SD). Statistical analysis was performed using SAS software (version XX; SAS Institute Inc., Cary, NC, USA). Prior to analysis, data were tested for normality and homogeneity of variance to ensure that the assumptions of parametric testing were satisfied. Differences among treatment means were evaluated using one-way analysis of variance (ANOVA). When significant differences were observed ($P \leq 0.05$), Tukey's honestly significant difference (HSD) test was applied for post-hoc pairwise comparisons among treatment groups. Dose–response relationships were analyzed using probit analysis. Inhibition percentages were converted into probit units and concentrations were log-transformed. A linear regression model ($Y = a + bX$) was applied, where Y represents the probit response and X represents the logarithm of concentration, a= the intercept (constant term), and b= the slope (regression coefficient). Effective doses (ED_{50} and ED_{90}) were estimated from the regression equations and the goodness of fit was assessed using the coefficient of determination (R^2). All statistical tests were considered significant at $P < 0.05$.

Results and Discussion

Selection of bioactive ligands from H. sabdariffa

The literature survey presented in Table 1 highlights the broad-spectrum antiviral potential of the selected bioactive compounds derived from *H. sabdariffa*, particularly gallic acid, hesperidin, kaempferol, and quercetin. These compounds have been previously reported to exhibit antiviral activities against a diverse range of RNA and DNA viruses, including human pathogens such as influenza A (H1N1) (You *et al*., 2018), hepatitis C virus (HCV) (Govea-Salas *et al*., 2016), herpes simplex virus (HSV) (Ürményi *et al*., 2016; Alhemaly and Sofy, 2024), and Zika virus (ZIKV) (Wong *et al*., 2017), along with plant viruses such as TMV (Malhotra *et al*., 1996; Chojnacka *et al*., 2021; Li *et al*., 2021; Sun *et al*., 2021). This diversity in antiviral targets suggests that these phytochemicals may act through conserved or multi-target mechanisms, enhancing their potential as antiviral agents. Overall, the integration of literature-based evidence with molecular docking findings strengthens the hypothesis that these bioactive compounds can act as effective antiviral agents. Their structural diversity, multi-target potential, and favorable binding characteristics make them promising candidates for further experimental validation, including *in vitro* or *in vivo* studies. Accordingly, these compounds were selected as potent antiviral candidates and subsequently utilized in the present study to evaluate their potential inhibitory activity against the target viral protein using *in silico* molecular docking approaches.

Computational assessment of H. sabdariffa bioactive compounds targeting TMV proteins

ADMET analysis and antiviral potential of H. sabdariffa compounds

The ADMET and pharmacokinetic profiles

presented in Table 2 highlight the potential drug-likeness characteristics of several *H. sabdariffa*-derived phytochemicals, compared to the reference antiviral compound acyclovir, which was used as a standard control for benchmarking binding affinity and interaction profiles in the molecular docking studies. Although acyclovir is primarily active against plant RNA (Ahmed *et al.*, 2024; Mahmoud *et al.*, 2025; Megahed *et al.*, 2025) and DNA (Abdel Razek *et al.*, 2025; Elshaer *et al.*, 2025) viruses, it had been widely employed in docking-based studies as a reference compound to provide a consistent comparative framework for evaluating the ligand–protein interactions across the different viral systems (Lipinski, 2004; Daina *et al.*, 2017). Among the tested compounds, gallic acid, kaempferol, and quercetin demonstrated compliance with Lipinski's Rule of Five (Kitchen *et al.*, 2004) and exhibited favorable predicted absorption properties, suggesting their potential suitability for oral administration. In contrast, hesperidin showed less favorable pharmacokinetic characteristics, including high molecular weight and an increased number of hydrogen bond donors and acceptors, which may contribute to its predicted poor gastrointestinal absorption and potential toxicity risk (Meng *et al.*, 2011). Overall, these findings provide preliminary insights into the pharmacokinetic behavior and safety profile of the investigated compounds, supporting their further evaluation as potential antiviral agents in the plant-based therapeutic strategies.

Table 1: *Antiviral activities of selected bioactive compounds from H. sabdariffa against various human and plant viruses based on literature survey.*

Bioactive compounds	Antiviral activities	References
Gallic-acid	Human rhinoviruses (HRVs)	Choi *et al.* (2010)
	Influenza A (H1N1) virus	You *et al.* (2018)
	Hepatitis C virus (HCV)	Govea-Salas *et al.* (2016)
	Tobacco mosaic virus (TMV)	Chojnacka *et al.* (2021)
Hesperidin	Herpes simplex virus (HSV)	Alhemaly and Sofy (2024)
	Feline Calicivirus	Choi *et al.* (2025)
	Tobacco mosaic virus (TMV)	Sun *et al.* (2021)
Kaempferol	HSV-1 and HSV-2	Ürményi *et al.* (2016)
	Murine norovirus (MNV)	Shahrajabian *et al.* (2022)
	Tobacco mosaic virus (TMV)	Li *et al.* (2021)
Quercetin	Zika virus (ZIKV)	Wong *et al.* (2017)
	Varicella-zoster virus (VZV)	Kim *et al.* (2020)
	Tobacco mosaic virus (TMV)	Malhotra *et al.* (1996)

Table 2: *ADMET, pharmacokinetic and toxicity profiles of H. sabdariffa bioactive compounds vs. acyclovir.*

Property	Gallic acid	Hesperidin	Kaempferol	Quercetin	Acyclovir (Control)
Molecular weight (g/mol)	170.12	610.56	286.24	302.24	225.20
Rotatable bonds	1	7	1	1	4
H-Bond acceptors	5	15	6	7	5
H-Bond donors	4	8	4	5	3
GI absorption	High	Low	High	High	High
BBB permeability	No	No	No	No	No
CYP1A2 inhibition	No	No	Yes	Yes	No
CYP2C19 inhibition	No	No	No	No	No
CYP2C9 inhibition	No	No	No	No	No
CYP2D6 inhibition	No	No	Yes	Yes	No
CYP3A4 inhibition	Yes	No	Yes	Yes	No
Lipinski compliance	Yes	No	Yes	Yes	Yes
Toxicity	Non-toxic	Toxic	Non-toxic	Non-toxic	Non-toxic

From a plant protection perspective, these ADMET predictions also provide valuable preliminary insights into the potential environmental safety and practical applicability of the investigated compounds. Phytochemicals exhibiting favorable drug-likeness properties, such as gallic acid, kaempferol, and quercetin, may also be associated with lower environmental persistence and reduced phytotoxic risk, supporting their suitability for sustainable agricultural applications. Conversely, compounds with less favorable pharmacokinetic profiles may display limited bioavailability under field conditions, although their environmental behavior requires further investigation. These observations may further assist in guiding the future formulation strategies for the development of safe and effective plant-based antiviral agents. From the metabolic interaction standpoint, the inhibitory potential of kaempferol and quercetin towards CYP1A2 and CYP3A4 enzymes raises considerations for drug-drug interactions that should be developed further. This is consistent with previous studies indicating that flavonoids such as kaempferol may interfere with viral life cycles partly *via* modulation of host metabolic or signaling pathways (Castro e Silva *et al*., 2022; Periferakis *et al*., 2023; Elshaer *et al*., 2025). For example, kaempferol has been shown to inhibit reactivation of the Epstein–Barr virus (EBV) by down-regulating Sp1 promoter activity, and accordingly, the viral lytic gene expression (Wu *et al*., 2022). Similarly, gallic acid has demonstrated virucidal and replication-blocking activities against a range of DNA and RNA viruses *in vitro* (Uozaki *et al*., 2007; Choi *et al*., 2010; Megahed *et al*., 2025). Moreover, quercetin has been reported to exert antiviral effects by modulating host immune responses, such as induction of type I interferon signaling in fish infected with iridovirus (Huang *et al*., 2022). These findings support the notion that natural phenolic compounds may exert multifaceted antiviral mechanisms, including direct viral inhibition, interference with viral protein synthesis, and modulation of host defenses (Nasr-Eldin *et al*., 2019; Abozaid *et al*., 2025), which align with our molecular docking rationale study targeting viral proteins of TMV. The predicted safety profiles further supported the potential of the tested compounds, with all compounds except hesperidin, shown expected low toxicity and favorable drug-likeness characteristics.

Hence, gallic acid, kaempferol, and quercetin emerge as promising leads worthy of further experimental interrogation in the context of viral infections (You *et al*., 2018; Periferakis *et al*., 2023). Hesperidin, in contrast, may require formulation improvements (e.g., pro-drug approach, nanocarrier delivery) or structural modification to overcome its absorption and toxicity limitations. Finally, the comparative performance of acyclovir in this study serves as a valuable benchmark, attributed to its full compliance with Lipinski parameters, high predicted oral bioavailability, and minimal Cytochrome P450 (CYP) inhibition, reflecting its well-established pharmacological reliability. The fact that the *H. sabdariffa* -derived compounds have shown similar or near-similar drug-like profiles are encouraging for their future development.

In conclusion, our ADMET/pharmacokinetic profiling supports the advancement of *H. sabdariffa*-derived compounds, particularly gallic acid, kaempferol, and quercetin, as prospective antiviral agents, deserving further *in vivo* validation, formulation optimization, and toxicological studies. Hesperidin may still hold potential but requires more careful optimization before use.

Molecular docking of H. sabdariffa bioactive compounds against TMV proteins

The current molecular docking of *H. sabdariffa* -derived bioactive compounds, mainly gallic acid, hesperidin, kaempferol, and quercetin-against key TMV proteins (*i.e*., coat protein, movement protein, and replicase) revealed distinct differences in their binding affinity and potential antiviral activity (Table 3). The polar surface area (PSA) of these compounds ranged from 97.99 Å² (gallic acid) to 234 Å² (hesperidin), reflecting differences in size and polarity that can influence both binding interactions and bioavailability. Hesperidin displayed the strongest binding affinity across all the tested TMV proteins, particularly with replicase (BA = -9.1 kcal/mol; DS = -9.5), suggesting its potential inhibitory effect on viral RNA replication. Quercetin and kaempferol also demonstrated notable binding affinities, especially toward replicase (BA= -8.5 and -7.7 kcal/mol, respectively), indicating their potential to interfere with viral replication and systemic spread. Gallic acid showed moderate interactions (BA= -6.5 kcal/mol with replicase), whereas the reference antiviral acyclovir exhibited lower binding affinities across all the tested proteins, underscoring the promising potential of the *H. sabdariffa* bioactive compounds. RMSD values were 0 for all ligand-

Table 3: *Molecular docking of H. sabdariffa bioactive compounds against TMV proteins (i.e., coat, movement, and replicase).*

Ligands	Polar surface area (Å)	Virtual screening and docking score							
		Coat-protein		Movement		Replicase		RMSD/ub	RMSD/lb
		BA	DS	BA	DS	BA	DS		
Gallic-acid	97.99	-5.0	X	-5.1	X	-6.5	-6.5	0	0
Hesperidin	234	-7.8	-7.9	-6.9	-7.0	-9.1	-9.5	0	0
Kaempferol	111.13	-7.0	-6.7	-6.2	-6.2	-7.7	-8.5	0	0
Quercetin	127	-7.1	-6.8	-6.3	-6.3	-8.5	-8.6	0	0
Acyclovir	119	-5.0	-5.2	-5.2	-4.8	-5.4	-6.4	0	0

BA: Binding affinity, DS: Docking score, RMSD/ub: Root mean square deviation/ Upper bound, RMSD/lb: Root mean square deviation/ Lower bound.

protein interactions, indicating stable and consistent docking conformations. These obtained results suggest that strong binding to replicase, coat, and movement proteins could disrupt multiple stages of TMV infection, including viral RNA synthesis, virion assembly, and cell-to-cell movement. While hesperidin's high PSA (234 Å^2), which refers to polar surface area and was associated with reduced passive membrane permeability, may limit its ability to cross biological membranes; however, its strong binding interactions suggested its potential efficacy if suitable delivery methods are employed. In contrast, gallic acid, with a lower PSA, may exhibit better bioavailability but relatively weaker antiviral interactions. The observed docking patterns align with a previous study demonstrating the antiviral potential of *H. sabdariffa* bioactive compounds against several viral pathogens (Hassan *et al*., 2017). Kaempferol has been reported to inhibit Epstein–Barr virus reactivation and other viral infections by modulating viral replication and host signaling pathways (Lu *et al*. 2024; Hassan, 2025). Quercetin has demonstrated antiviral efficacy against grouper iridovirus through activation of host defense mechanisms (Huang *et al*., 2025), while gallic acid exhibits broad-spectrum antiviral potency, including inhibition of human viruses (Naithani *et al*., 2008; Choi *et al*., 2010; Kim *et al*., 2020). Collectively, these findings indicate that hesperidin, quercetin, and kaempferol possess favorable binding characteristics toward TMV proteins, potentially outperforming acyclovir in this context. This supports their development as natural antiviral agents for plant protection, although in planta validation and formulation optimization are recommended to confirm their efficacy and bioavailability.

Amino acid interactions of H. sabdariffa compounds with TMV coat protein

Table 4 and Figures 1 and 2 illustrate the amino acid interactions and bonding patterns of selected *H. sabdariffa* bioactive compounds (*i.e*., hesperidin, kaempferol, and quercetin) along with the control drug acyclovir with the TMV-CP. The diversity and nature of these interactions such as hydrogen bonds, hydrophobic interactions, and electrostatic forces- provide valuable insights into the stability and specificity of the ligand–protein binding, which are crucial for the antiviral activity (Ferreira *et al*., 2015). Among the tested *H. sabdariffa* compounds, hesperidin formed the highest number of interactions with the TMV-CP, including multiple *conventional hydrogen bonds* (with ARG:A42, GLN:A39, GLN:A37, ASN:A92, and THR:A112) and *alkyl interactions* (ILE:A95, PRO:A103, VAL:A97), along with a *π-anion interaction* with GLU:A96. This extensive bonding network indicates strong stabilization of the ligand within the active pocket of the viral coat protein (Du *et al*., 2016), suggesting potential interference with the capsid assembly or stability. The presence of multiple hydrogen bonds may also contribute to the hesperidin's high docking affinity observed earlier (BA= –9.1 kcal/mol), supporting its role as a potent inhibitor candidate. Kaempferol and quercetin, both are flavonoids, also exhibited remarkable binding interactions.

Table 4: *Amino acid interactions and bonding patterns of H. sabdariffa bioactive compounds and acyclovir with TMV-CP.*

Amino acids	Sites	Type of bonds
Hesperidin		
ARG	A:42	Conventional Hydrogen Bond
GLN	A:39	Conventional Hydrogen Bond, Pi- Donor Hydrogen Bond
GLN	A:37	Conventional Hydrogen Bond
ASN	A:92	Conventional Hydrogen Bond, Unfavorable Donor-Donor
THR	A:112	Conventional Hydrogen Bond
GLU	A:96	Pi-Anion
ILE	A:95	Alkyl
PRO	A:103	Alkyl
VAL	A:97	Alkyl
Kaempferol		
GLU	A:96	Attractive Charge, Pi-Anion
ASN	A:92	Conventional Hydrogen Bond
GLN	A:39	Conventional Hydrogen Bond, Unfavorable Positive-Positive, Unfavorable Donor-Donor
ARG	A:42	Conventional Hydrogen Bond, Unfavorable Positive-Positive, Unfavorable Donor-Donor
Quercetin		
GLU	A:96	Attractive Charge, Pi-Anion
ARG	A:42	Conventional Hydrogen Bond
ASN	A:92	Conventional Hydrogen Bond, Unfavorable Donor-Donor
ARG	A:91	Conventional Hydrogen Bond
GLN	A:39	Unfavorable Donor-Donor, Pi-Door Hydrogen bound
Acyclovir		
GLU	A:96	Attractive charge, Pi-Anion
GLN	A:39	Conventional Hydrogen Bond
ASN	A:92	Conventional Hydrogen Bond
THR	A:38	Carbon Hydrogen Bond
ARG	A:91	Unfavorable Positive-Positive

ARG: Arginine, ASN: Asparagine, GLN: Glutamine, GLU: Glutamic Acid, ILE: Isoleucine, PRO: Proline, THR: Threonine, VAL: Valine.

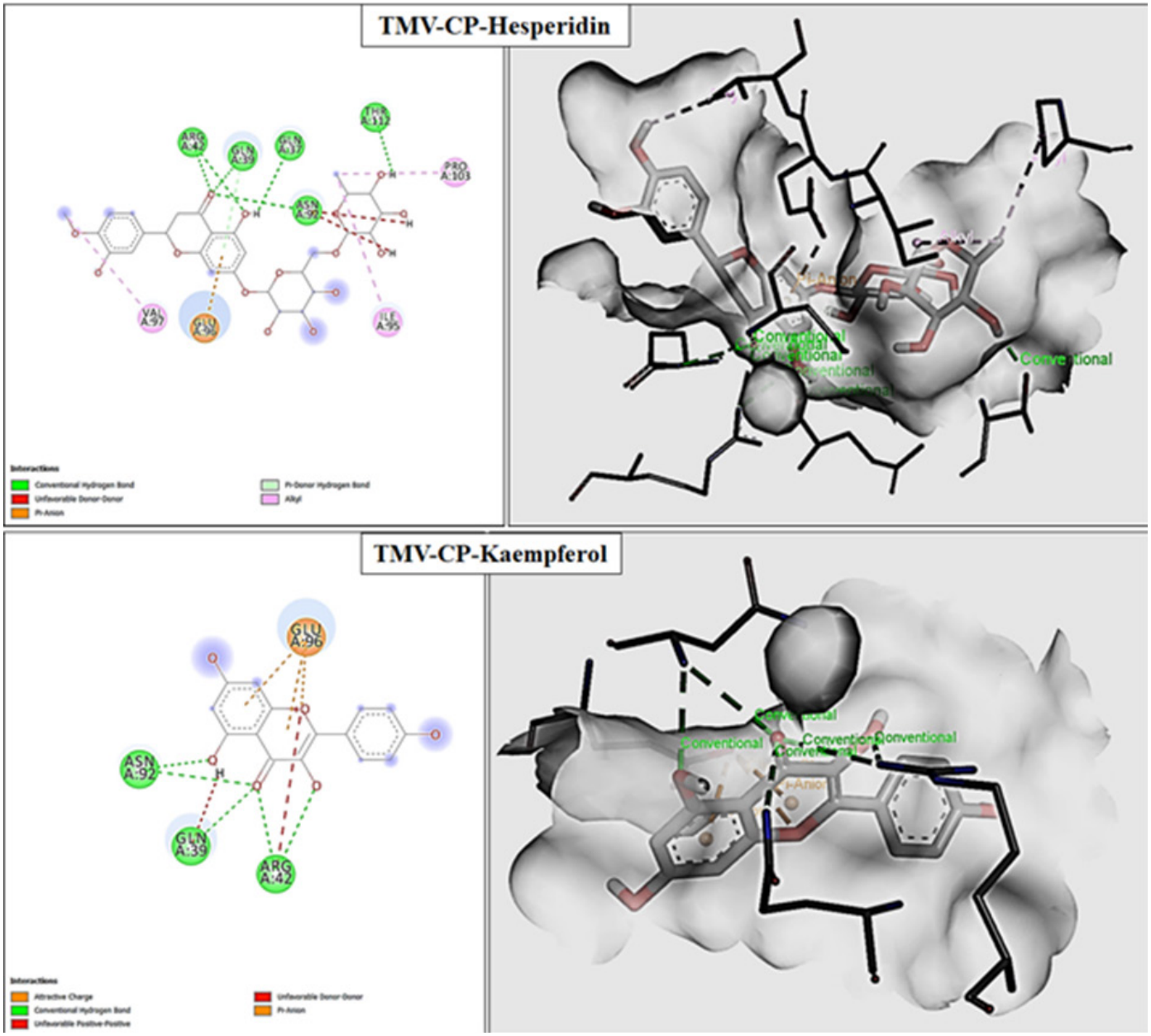

Figure 1: *Molecular docking interactions of TMV coat protein with hesperidin and kaempferol. The 2d interaction diagrams (left) highlight key residues involved in hydrogen bonding, π-interactions, and electrostatic contacts. The 3D representations (right) show the spatial orientation of ligands within the TMV-CP binding pocket, emphasizing conventional hydrogen bonds and unfavorable donor-donor interactions. These results suggest that both compounds exhibit strong binding affinity through multiple stabilizing interactions, indicating their potential as antiviral agents targeting TMV.*

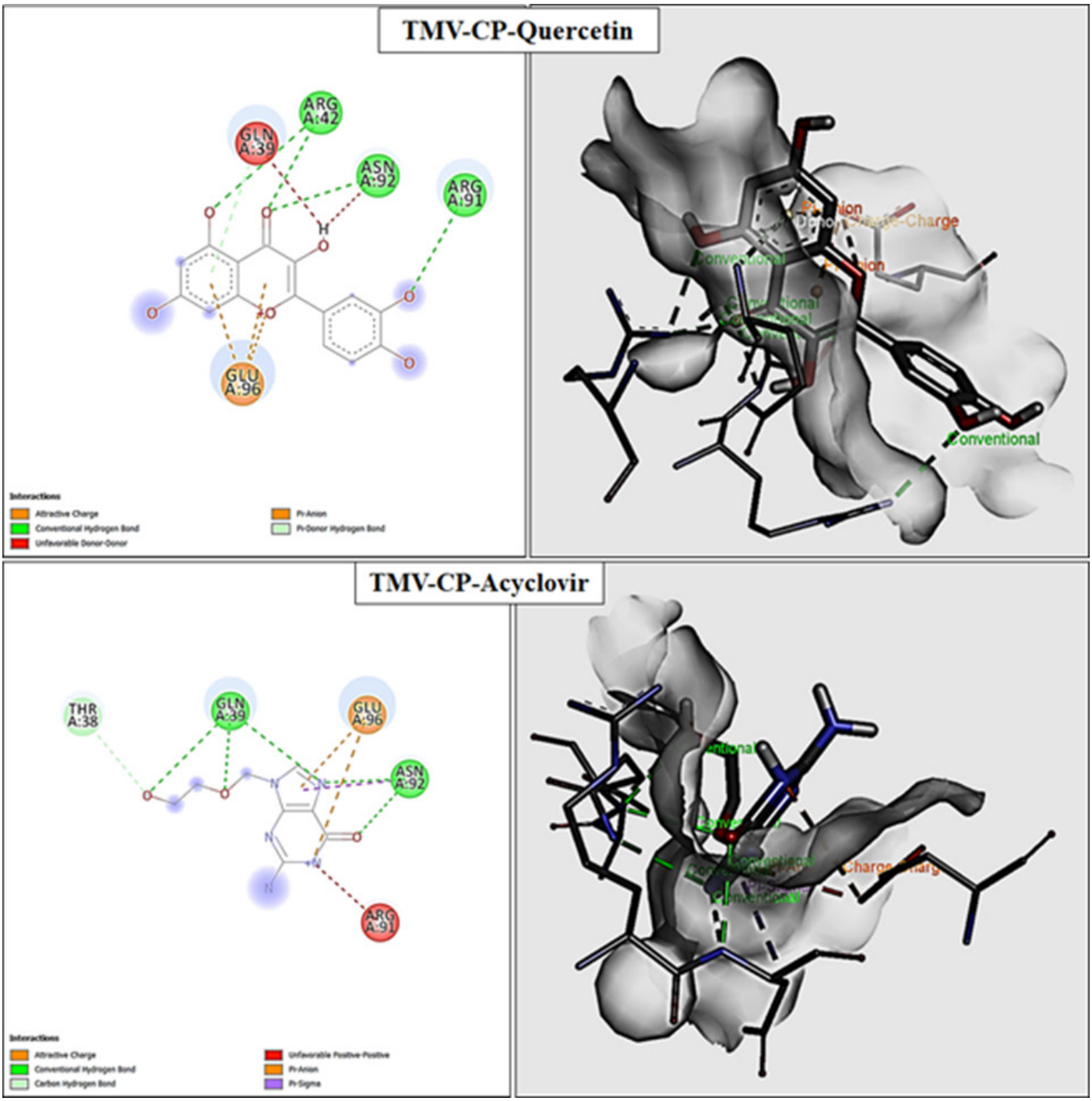

Figure 2: *Molecular docking interactions of TMV coat protein with quercetin and acyclovir. The 2D interaction diagrams (left) show the key amino acid residues involved in binding, highlighting hydrogen bonds, π-interactions, and electrostatic contacts. Quercetin forms multiple conventional hydrogen bonds and π-anion interactions, suggesting strong stabilization within the binding pocket. The 3D representations (right) illustrate the spatial orientation of each ligand inside the TMV-CP active site, emphasizing favorable and unfavorable interactions. Acyclovir exhibits hydrogen bonding and electrostatic interactions, indicating moderate binding affinity compared to quercetin.*

Kaempferol formed key hydrogen bonds with GLN:A39, ASN:A92, and ARG:A42, as well as π-anion, and attractive charge interactions with GLU:A96. Similarly, quercetin showed strong hydrogen bonding with ARG:A42, ARG:A91, and ASN:A92, alongside an electrostatic (π-anion) interaction with GLU:A96. These results indicate that both flavonoids stabilize through polar and charged interactions, which may inhibit the coat protein-mediated viral assembly. Comparable binding residues among these compounds suggest a shared binding site, possibly at the active or the allosteric region of the CP. The control compound, acyclovir, showed fewer interactions, forming conventional

hydrogen bonds with GLN:A39 and ASN:A92 and a carbon hydrogen bond with THR:A38. Although acyclovir had also interacted electrostatically with GLU:A96, its overall interaction network was less complex, consistent with its lower binding affinity values (BA = –5.4 kcal/mol). This contrast underscores the stronger potential of *H. sabdariffa* -derived phytochemicals as natural antiviral agents against TMV. The present findings are consistent with earlier studies that highlighted the antiviral potential of several plant-derived phytochemicals (Di Petrillo *et al*., 2022; Alhemaly and Sofy, 2024; Kowalczyk, 2024; Choi *et al*., 2025). Kaempferol, for example, has been documented to suppress Epstein–Barr virus reactivation and other viral infections by influencing viral gene regulation and strengthening host immune defense mechanisms (Wu *et al*., 2022). Likewise, quercetin manifests a wide antiviral spectrum, disrupting viral replication in influenza and irido viruses through its affinity for viral proteins and ability to enhance antioxidant enzyme activity in the host cells (Mehrbod *et al*., 2020). Hesperidin has also demonstrated a strong tendency to bind with viral polymerases and structural components, thereby impairing the replication process of several RNA viruses (Agrawal *et al*., 2021). Although gallic acid is not included in the present interaction analysis, earlier reports confirmed its efficacy against both DNA and RNA viruses, mainly through mechanisms associated with redox balance and oxidative stress regulation (Choi *et al*., 2010; Govea-Salas *et al*., 2016; Wu *et al*., 2017).

Overall, the interaction patterns observed for hesperidin, kaempferol, and quercetin with TMV-CP residues suggest the formation of stable, multi-type bonds, primarily hydrogen and those electrostatic in nature, which could interfere with virion assembly or stability. These molecular behaviors emphasize the potential of *H. sabdariffa* derived compounds as promising natural antiviral candidates that might exhibit superior protein-binding efficiency compared to conventional synthetic antivirals such as acyclovir.

Interaction analysis of H. sabdariffa bioactive compounds with TMV movement protein

Molecular docking analysis of *H. sabdariffa*-derived bioactive compounds with the TMV movement protein revealed diverse binding interactions involving hydrogen bonds, π-interactions, and hydrophobic contacts (Table 5 and, Figures 3 and 4). Among the tested ligands, hesperidin displayed the strongest binding affinity, characterized by multiple conventional hydrogen bonds and hydrophobic interactions with residues TYR(A:109), ALA(A:111), and CYS(A:84). The presence of π–sulfur and π–π stacking interactions with PHE(A:117) and CYS(A:84) further enhances the structural stability of the hesperidin protein complex (Salentin *et al*., 2015). These multifaceted interactions suggest that hesperidin may effectively block or destabilize the movement protein's functional conformation; thereby, impeding viral cell to cell translocation. Kaempferol and quercetin also formed stable interactions with key amino acid residues in the movement protein's binding pocket. Kaempferol displayed hydrogen bonding with ARG(A:116) and GLY(A:87) and π-interactions with aromatic residues TRP(A:78) and LYS(A:114), suggesting strong affinity and potential to interfere with the viral RNA binding or the transport functions. Quercetin showed a similar interaction profile, forming hydrogen and π–π interactions with residues THR(A:110), SER(A:89), and TRP(A:78), implying that its binding may inhibit the protein's structural flexibility required for efficient viral movement. In contrast, acyclovir, the standard antiviral control, exhibited a comparatively weaker binding, involving a few hydrogen bonds with LYS(A:95), ASP(A:94), and VAL(A:93), accompanied by unfavorable donor–donor and electrostatic repulsions with GLU(A:98) and MET(A:97). This observation supports the hypothesis that the plant-derived flavonoids and polyphenols may express stronger, more stable molecular interactions with the TMV proteins than the traditional antiviral compounds. These results corroborate earlier reports that flavonoids such as hesperidin, kaempferol, and quercetin can exert antiviral effects by binding to key viral proteins and obstructing their replication or movement mechanisms (Khazdair et al., 2021; Di Petrillo et al., 2022; Shahrajabian et al., 2022; Hu et al., 2025).

Furthermore, the involvement of aromatic residues and π-interactions aligns with previous docking and biochemical studies highlighting the role of these interactions in stabilizing the protein–ligand complexes of the viral targets (Castro e Silva et al., 2022; Periferakis et al., 2023). Overall, these findings suggest that *H. sabdariffa* derived phytochemicals, particularly hesperidin and kaempferol, possess promising antiviral potential through strong, multi-type interactions with the the TMV movement

Table 5: *Amino acid interactions and bonding patterns of H. sabdariffa bioactive compounds and acyclovir with TMV movement protein.*

Amino acids	Sites	Type of bonds
Hesperidin		
TYR	A:109	Conventional Hydrogen Bond, Alkyl
CYS	A:84	Pi-Sulfur
PHE	A:117	Pi-Pi Stacked
ALA	A:111	Alkyl
Kaempferol		
GLY	A:87	Conventional Hydrogen Bond
ARG	A:116	Conventional Hydrogen Bond
LYS	A:114	Pi-Alkyl
TRP	A:78	Pi-Donor Hydrogen Bond, Pi-Pi T-Shaped
SER	A:89	Pi-Donor Hydrogen Bond
Quercetin		
THR	A:110	Conventional Hydrogen Bond
SER	A:89	Pi-Donor Hydrogen Bond
TRP	A:78	Pi-Pi T-Shaped
Acyclovir		
LYS	A:95	Conventional Hydrogen Bond, Unfavorable Positive-Positive
ASP	A:94	Conventional Hydrogen Bond
VAL	A:93	Conventional Hydrogen Bond
GLU	A:98	Unfavorable Positive-Positive, Unfavorable Donor-Donor
MET	A:97	Unfavorable Positive-Positive, Unfavorable Donor-Donor

ALA: Alanine, ARG: Arginine, ASP: Aspartic Acid, CYS: Cysteine, GLU: Glutamic Acid, GLY: Glycine, LYS: Lysine, MET: Methionine, PHE: Phenylalanine, SER: Serine, THR: Threonine, TRP: Tryptophan, TYR: Tyrosine, VAL: Valine.

protein. Such interactions may inhibit the intercellular spread of the virus, providing a molecular basis for their use as natural antiviral agents in plant protection controls.

Molecular interactions of H. sabdariffa compounds with TMV replicase protein: Insights potential

Molecular docking of the *H. sabdariffa* derived bioactive compounds (*i.e.*, gallic acid, hesperidin, kaempferol, and quercetin) with the TMV replicase protein (Table 6 and Figures 5 and 6) revealed diverse interaction networks, primarily dominated by hydrogen bonding, π–cation, and hydrophobic interactions. These bonds are essential for stabilizing the ligand–protein complexes (Du *et al.*, 2016) and indicate strong binding potential at catalytically relevant residues of the viral replicase. Hesperidin exhibited the most extensive binding network among all the tested compounds, forming multiple conventional hydrogen bonds with residues such as GLN936, GLU1045, THR1046, and ARG868, along with π–cation interactions with LYS839 and ARG968. These findings suggest that hesperidin interacts at multiple functional domains of the replicase, possibly disrupting the enzyme's catalytic activity and viral RNA synthesis. The compound's strong electrostatic and hydrogen bonding profile supports its high binding affinity (–9.1 kcal/mol), observed earlier in docking simulations. Kaempferol and quercetin also demonstrated robust interactions with key amino acid residues, including ARG868, ARG968, THR840, and CYS837, through hydrogen and π–sulfur bonds. These interactions likely interfere with the active site configuration, reducing the efficiency of the viral polymerase complex. Both of these flavonoids are known to possess antiviral activity by targeting viral replication enzymes or modulating the host defense mechanisms (Wu *et al.*, 2022; Periferakis *et al.*, 2023).

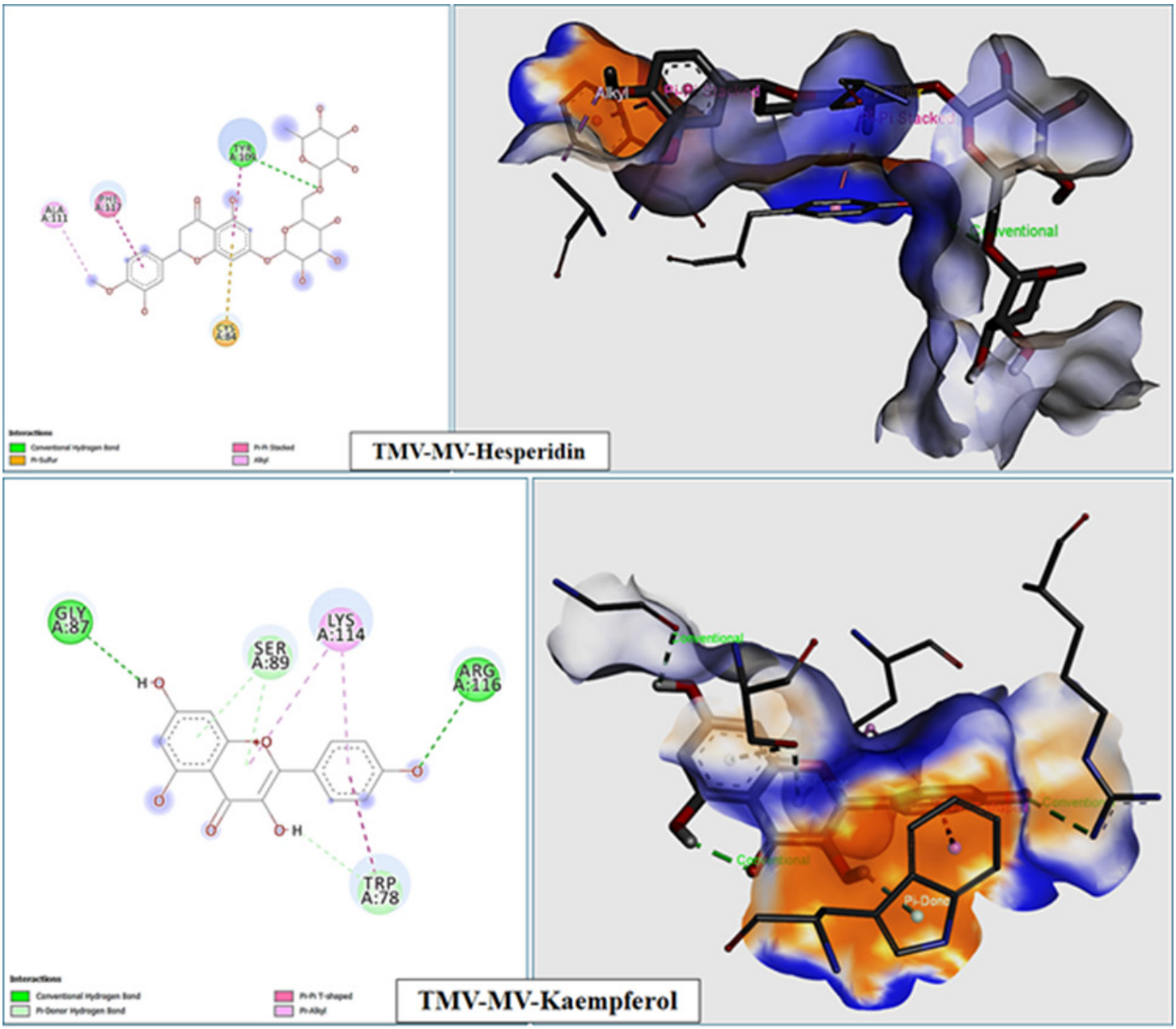

Figure 3: *Molecular docking interactions of TMV movement protein with hesperidin and kaempferol. The 2D interaction diagrams (left) display key amino acid residues involved in ligand binding, highlighting conventional hydrogen bonds and π-interactions. Hesperidin forms multiple hydrogen bonds and hydrophobic contacts, suggesting strong stabilization within the binding pocket. The 3D representations (right) show the spatial orientation of each ligand inside the TMV-MV active site, emphasizing hydrophobic regions and favorable interactions. Kaempferol exhibits hydrogen bonding with residues such as Gly, Ser, Lys, and Arg, along with π-π stacking, indicating a stable binding conformation. Overall, these docking results suggest that both hesperidin and kaempferol interact effectively with TMV-MV through hydrogen bonding and hydrophobic interactions, supporting their potential as antiviral agents targeting TMV movement protein.*

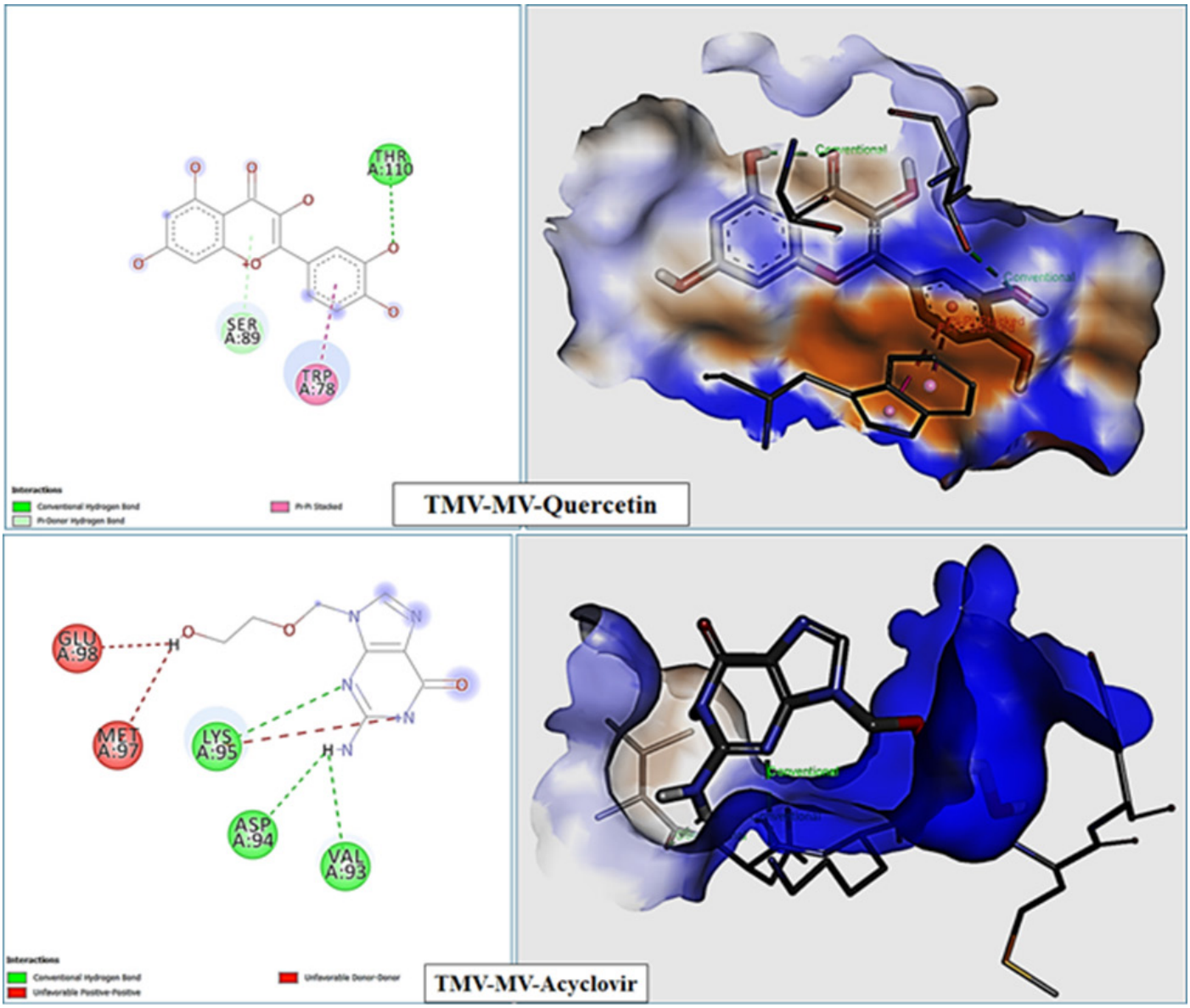

Figure 4: *Molecular docking interactions of TMV movement protein with quercetin and acyclovir. The 2D interaction diagrams (left) highlight the amino acid residues involved in ligand binding. Quercetin forms multiple conventional hydrogen bonds with residues such as Thr110, Ser89, and Trp78, along with π-stacking interactions, indicating strong stabilization within the binding pocket. The 3D representations (right) illustrate the spatial orientation of each ligand inside the TMV-MV active site. Quercetin shows favorable interactions in hydrophobic regions, while acyclovir not only interacts through hydrogen bonds with residues like Glu398, Lys95, and Asp94, but also exhibits some unfavorable donor-donor interactions, suggesting comparatively weaker binding affinity. Overall, these results indicate that quercetin demonstrates stronger and more diverse interactions with TMV-MV than acyclovir, supporting its potential as an effective antiviral candidate.*

Gallic acid, though smaller in structure, engaged in π–alkyl and π–π T-shaped interactions with hydrophobic residues such as PHE888 and CYS898, along with multiple hydrogen bonds (e.g., GLY893, ARG897). These interactions reveal a moderate but stable affinity, consistent with its antioxidant and antiviral potential previously reported against several RNA and DNA viruses (Uozaki *et al.*, 2007; Choi *et al.*, 2010). In comparison, acyclovir formed fewer and less diverse bonds, primarily hydrogen bonds with GLN936, GLY836, and ARG868, along with limited π–alkyl interactions at LYS839. Although acyclovir maintains a known antiviral mechanism through viral DNA polymerase inhibition, its relatively lower docking score and interaction diversity indicate that *H. sabdariffa* flavonoids may provide stronger or complementary inhibitory effects on the TMV replicase activity. Overall, these results highlight the potential of hesperidin, kaempferol, and quercetin as potent natural inhibitors of the TMV replicase protein.

Their multifunctional binding behaviors spanning hydrogen bonding, hydrophobic, and electrostatic interactions underscore their ability to disrupt the viral replication pathways. These findings align with previous reports confirming the broad-spectrum antiviral actions of flavonoids *via* interference with viral enzymatic functions, inhibition of genome replication, and induction of host defense responses (Castro e Silva *et al.*, 2022; Huang *et al.*, 2022; Di Petrillo *et al.*, 2022).

Table 6: *Amino acid interactions and bonding patterns of H. sabdariffa bioactive compounds and acyclovir with TMV replicase protein.*

Amino acids	Sites	Type of bonds
Gallic-acid		
CYS	A:898	Conventional Hydrogen Bond, Pi-Alkyl
GLY	A:893	Conventional Hydrogen Bond, Unfavorable Donor-Donor
PHE	A:888	Pi-Pi T-Shaped
LYS	A:883	Unfavorable Donor-Donor
ARG	A:897	Carbon Hydrogen Bond
Hesperidin		
GLN	A:936	Conventional Hydrogen Bond
ARG	A:968	Conventional Hydrogen Bond, Pi- Cation
THR	A:1046	Conventional Hydrogen Bond
GLU	A:1045	Conventional Hydrogen Bond
GLU	A:907	Conventional Hydrogen Bond
THR	A:840	Conventional Hydrogen Bond
ARG	A:868	Conventional Hydrogen Bond, Unfavorable Donor-Donor
LYS	A:839	Conventional Hydrogen Bond, Pi- Cation, Pi-Alkyl
GLY	A:833	Carbon Hydrogen Bond
GLY	A:836	Carbon Hydrogen Bond
PRO	A:835	Pi-Donor Hydrogen Bond
CYS	A:837	Pi-Sulfur
ILE	A:865	Alkyl
ALA	A:861	Alkyl
VAL	A:856	Alkyl
Kaempferol		
ARG	A:968	Conventional Hydrogen Bond, Unfavorable Positive-Positive
ARG	A:868	Conventional Hydrogen Bond
THR	A:840	Conventional Hydrogen Bond, Unfavorable Donor-Donor
LYS	A:839	Unfavorable Donor-Donor, Pi-Cation
CYS	A:837	Pi-Sulfur
GLY	A:836	Carbon Hydrogen Bond, Pi-Donor Hydrogen Bond, Pi-Alkyl
Quercetin		
LYS	A:841	Conventional Hydrogen Bond
ARG	A:868	Conventional Hydrogen Bond
THR	A:840	Conventional Hydrogen Bond
LYS	A:839	Conventional Hydrogen Bond, Pi-Cation
GLN	A:936	Conventional Hydrogen Bond
VAL	A:834	Conventional Hydrogen Bond
ARG	A:968	Conventional Hydrogen Bond, Pi-Cation, Unfavorable Positive-Positive
GLY	A:836	Carbon Hydrogen Bond, Pi-Donor Hydrogen Bond
CYS	A:837	Pi-Sulfur
Acyclovir		
ARG	A:1076	Conventional Hydrogen Bond
GLN	A:936	Conventional Hydrogen Bond
GLY	A:836	Conventional Hydrogen Bond
ARG	A:968	Conventional Hydrogen Bond, Unfavorable Positive-Positive, Pi-Cation
ARG	A:868	Conventional Hydrogen Bond, Unfavorable Positive-Positive
LYS	A:839	Pi-Alkyl

ALA: Alanine, ARG: Arginine, CYS: Cysteine, GLN: Glutamine, GLU: Glutamic Acid, GLY: Glycine, ILE: Isoleucine, LYS: Lysine, PHE: Phenylalanine, PRO: Proline, THR: Threonine, VAL: Valine.

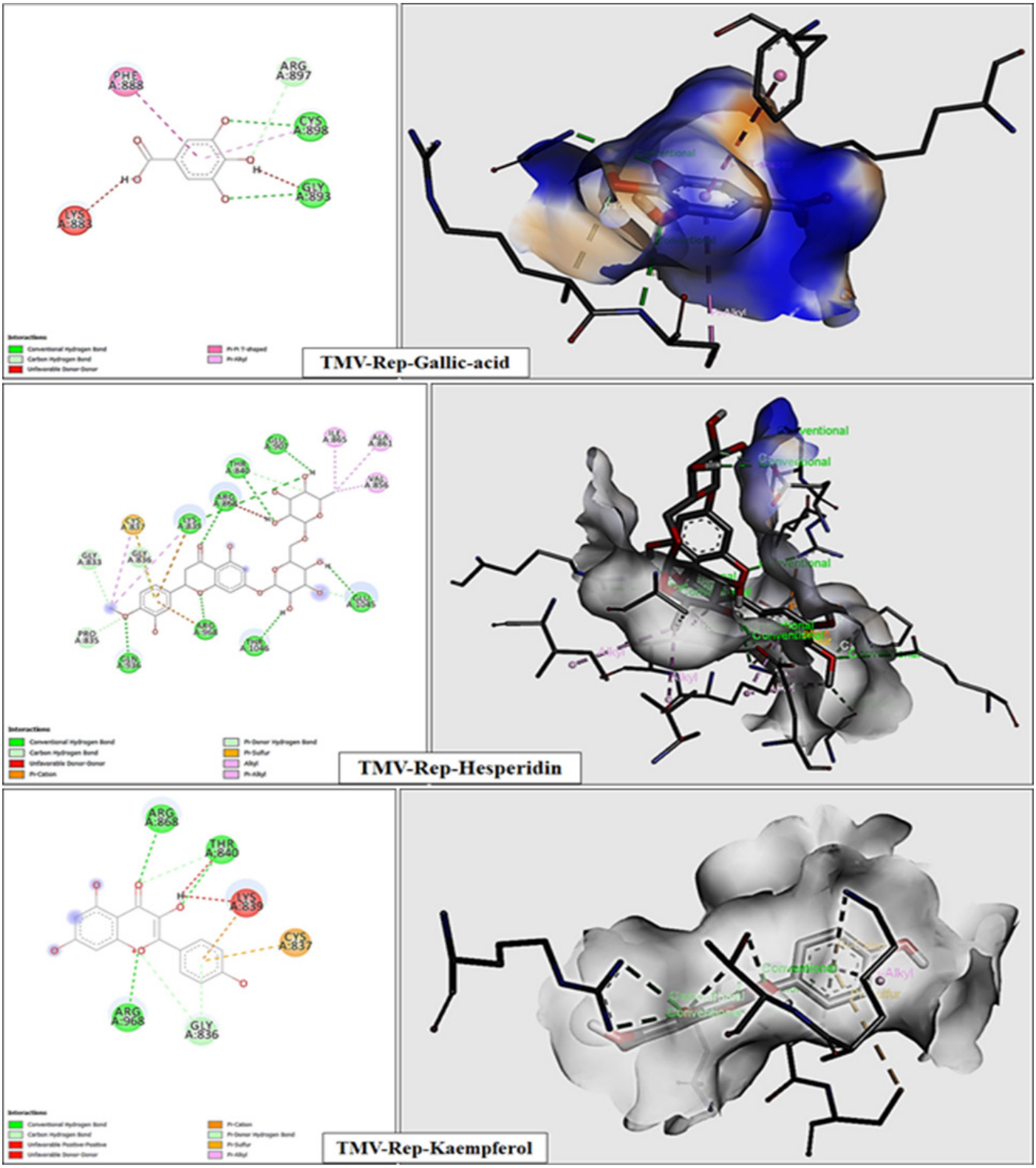

Figure 5: *Molecular docking of TMV replicase with gallic acid, hesperidin, and kaempferol. gallic acid shows moderate stabilization via hydrogen bonds and π-interactions. Hesperidin forms extensive hydrogen bonds and hydrophobic contacts, indicating the strongest binding affinity. Kaempferol exhibits stable docking through hydrogen bonds and π-interactions. Overall, hesperidin demonstrates the most robust interaction network, followed by kaempferol and gallic acid, supporting their potential as TMV inhibitors. Representative 2D interaction diagrams are shown on the left, while the corresponding 3D binding conformations are presented on the right.*

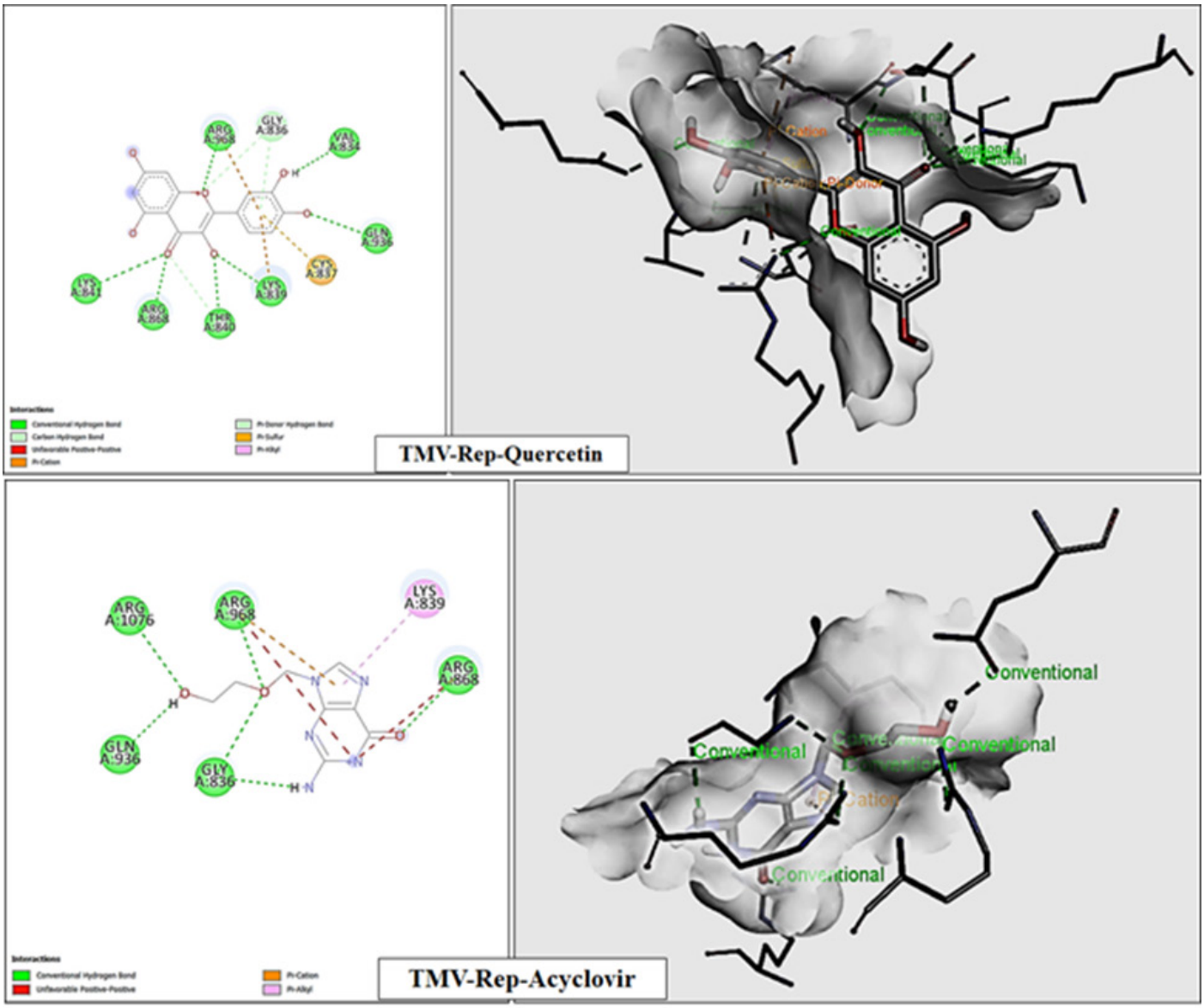

Figure 6: *Molecular docking interactions of TMV replicase protein with quercetin and acyclovir. 2D interaction map (left): Quercetin forms multiple conventional hydrogen bonds (green dashed lines) with amino acid residues such as GLY, ASN, and SER. There are also π-π stacking interactions (orange lines) and hydrophobic contacts. 3D View (right): Quercetin fits snugly into the binding pocket, stabilized by hydrogen bonds and aromatic interactions. The visualization suggests strong binding affinity due to multiple interaction points. Quercetin shows a richer interaction profile, suggesting it could be a more effective inhibitor of TMV-Rep compared to acyclovir. The difference in interaction types (π-π stacking for Quercetin vs. mainly hydrogen bonds for acyclovir) may influence their antiviral potential.*

In vitro anti-TMV activity of H. sabdariffa ethanolic extract

The findings presented in Table 7 and Figure 7 indicate that the ethanolic extract of *H. sabdariffa* exhibited substantial *in vitro* antiviral activity against TMV, as demonstrated by a marked reduction in the number of necrotic local lesions (NLL) with increasing the extract concentration. This dose-dependent antiviral effect is consistent with previous observations that plant extracts rich in bioactive compounds can inhibit plant virus infection by interfering with viral replication or entry mechanisms (Uozaki *et al.*, 2007; Choi *et al.*, 2010; Joseph *et al.*, 2023; Ahmed *et al.*, 2024). For example, aqueous and ethanolic extracts from other medicinal plants, such as licorice and green tea, considerably reduced the TMV lesion formation in a concentration-dependent manner, suggesting that the phenolic and flavonoid constituents may play a key role in antiviral activity against TMV and similar plant viruses (Joseph *et al.*, 2023; Ahmed *et al.*, 2024). Although specific studies on *Hibiscus* activity against TMV are limited, a previous study conducted on *Hibiscus* extracts against a range of human and animal viruses supports the general antiviral potential

of this species (Shaheen *et al*., 2023). *H. sabdariffa* extracts have shown inhibitory effects against influenza A virus, feline calicivirus, murine norovirus, and hepatitis A virus, likely due to its high content of organic acids and phenolic compounds, including protocatechuic acid and anthocyanins, which can disrupt viral particles or interfere with the infection processes (Joshi *et al*., 2015; Mohammed *et al*., 2023). Additionally, fractionated *Hibiscus* components were found to reduce viral titers and inhibit replication of H5 avian influenza strains *in vitro*, indicating that multiple bioactive constituents may contribute to the observed antiviral effects (Baatartsogt *et al*., 2016). The antiviral mechanisms of such plant extracts are thought to involve multiple pathways, including direct inactivation of viral particles, inhibition of viral binding to host cell receptors, suppression of viral replication, and modulation of host defense responses (Musarra-Pizzo *et al*., 2021). For instance, the phenolic compounds and flavonoids can bind to viral proteins, destabilize viral envelopes, or modulate oxidative states unfavorable for viral proliferation (Chojnacka *et al*., 2021). Although the precise mechanism of *H. sabdariffa* extract activity against TMV was not elucidated in the present study, the observed reduction in lesions number and high inhibition percentage at higher concentrations support the hypothesis that *H. sabdariffa* bioactive compounds disrupt TMV infectivity or early stages of infection. The high coefficient of determination (R^2 = 0.9601) currently obtained from probit analysis further underscores the robustness of the relationship between extract concentration and antiviral impact, reinforcing the potential of *H. sabdariffa* extracts to act as natural antiviral agents in plant pathology research. Collectively, these obtained results align with a growing body of evidence suggesting that phenolic-rich plant extracts have broad-spectrum antiviral properties, and they warrant further investigations into specific active compounds, mechanisms of action, and potential field applications against TMV and other plant viruses (Denaro *et al*., 2020; Davidova *et al*., 2024).

Table 7: *In vitro* effect of different dilutions of *H. sabdariffa* ethanolic extract on the number of local necrotic lesions and percentage inhibition of TMV on *Datura metel* leaves.

Dilutions	Concentration (%)	Log con.	Inhibition (%)	Probit	ED_{50} %	ED_{90} %	Y = a + bX	R^2
1/4	0.25	-0.60	82.67	4.05	1.57	2.82	y = 1.0275x + 3.3861	R^2 = 0.9601
1/8	0.13	-0.90	78.67	4.19				
1/16	0.06	-1.20	65.33	4.61				
1/32	0.03	-1.51	52.00	4.95				
1/64	0.02	-1.66	38.67	5.28				
1/128	0.01	-2.11	32.67	5.44				
Control			00.00					

Y: Probit values of the mean inhibition percentage (%), X: Log-transformed values of the mean dilutions of the aqueous extracts tested, ED_{50}: Half-maximal effective dilution, ED_{90}: Effective dilution at 90% inhibition, Coeff. of determination (r^2): Indicates the regression model's goodness of fit.

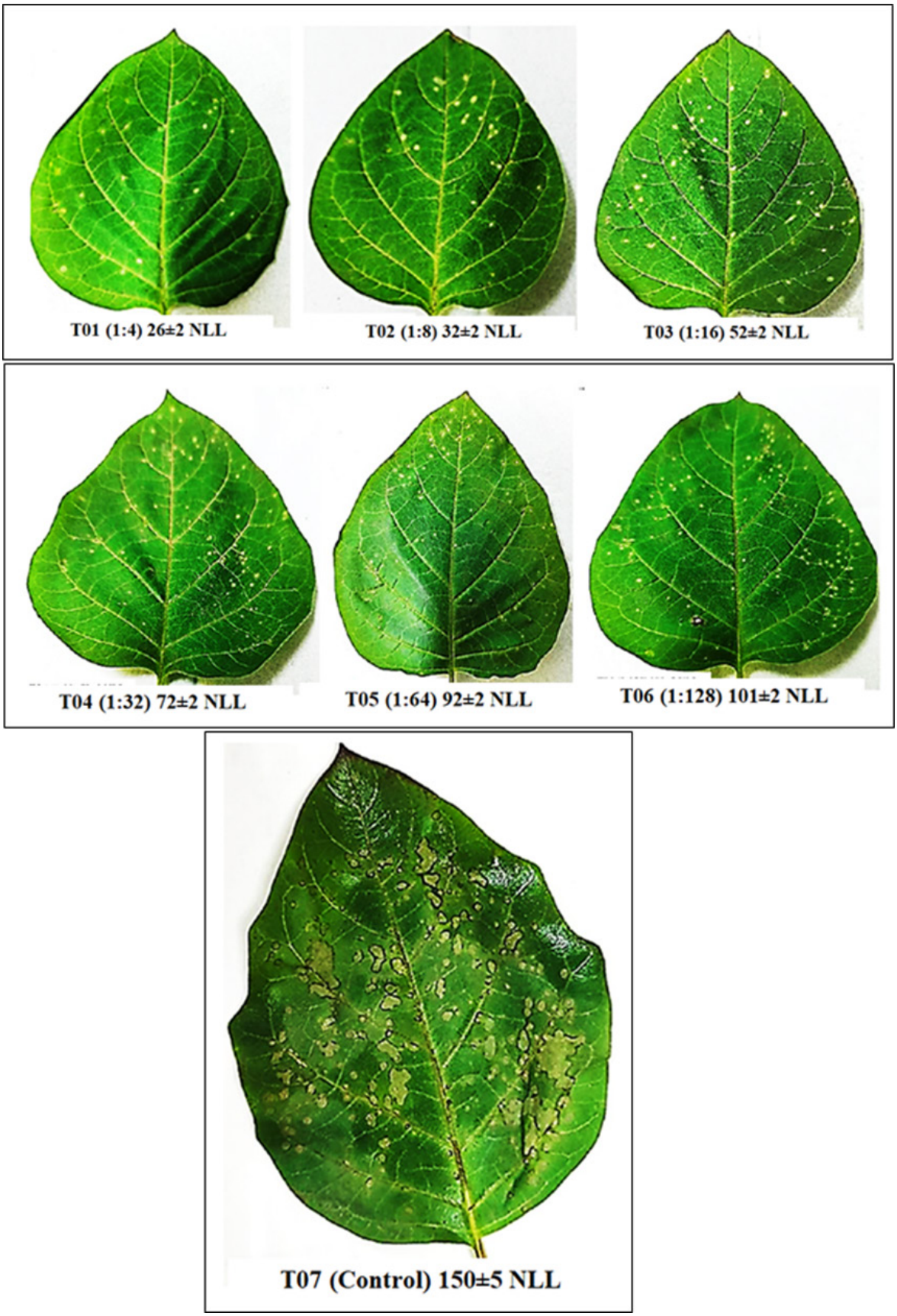

Figure 7: *In vitro evaluation of the anti-TMV activity of bioactive compounds from H. sabdariffa, based on the mean number of necrotic local lesions (NLL) on Datura metel leaves recorded 7 days after inoculation with different treatments consisting of a mixture of serial dilutions of an ethanolic extract prepared from H. sabdariffa leaves and infectious sap from tobacco plants containing TMV. The figures demonstrate the variation in necrotic local lesion numbers as a measure of antiviral activity. Increasing the dilution resulted in a reduced concentration of bioactive compounds, which in turn led to an increased number of lesions due to the diminished inhibitory effect against the virus.*

Overall, the molecular docking findings of this study should be interpreted as predictive computational insights rather than definitive evidence of antiviral mechanisms. The observed binding affinities and interaction profiles provide a theoretical indication of the potential affinity of *H. sabdariffa*-derived phytochemicals toward TMV proteins. However, these results do not confirm inhibition of viral replication, assembly, or movement, but rather suggest possible molecular interactions that require further experimental validation. The integration of docking with ADMET analysis offers a useful preliminary framework for prioritizing the plant's bioactive compounds for future manipulation in *in vitro* and *in vivo* studies aiming to plant virus management.

Conclusions and Recommendations

This study highlights that *H. sabdariffa* (Roselle)-derived phytochemicals-particularly hesperidin, kaempferol, and quercetin exhibit a promising antiviral activity against TMV through multi-target interactions with key viral proteins, potentially disrupting RNA synthesis, virion assembly, and cell-to-cell movement. Molecular docking showed strong and diverse binding patterns, while ADMET analysis supported the drug-likeness of kaempferol and quercetin. Although hesperidin demonstrated high binding affinity, its limitations suggest the need for optimized formulations. Notably, these compounds outperformed acyclovir in docking performance, indicating their potential as natural antiviral agents. Further future studies are recommended to validate these findings through *in vivo* experiments, optimizing compound formulations, investigating the mechanisms of action, and ensuring environmental safety for sustainable agricultural use.

Acknowledgment

We extend our heartfelt thanks to Miss El-Shymaa Tarek Abdel-Aziz for her exceptional support in conducting the molecular docking analysis. We are truly grateful for her insightful guidance throughout the study.

Novelty Statement

To the best of our knowledge, this study is the first to systematically evaluate the potential of *H. sabdariffa*-derived phytochemicals, mainly hesperidin, kaempferol, and quercetin against multiple functional proteins of TMV, including coat and movement proteins, and replicase, using an integrated molecular docking and ADMET/pharmacokinetic approach. Unlike the previous studies that focused primarily on general antiviral properties of flavonoids, this study provides detailed insights into multi-target binding mechanisms, interaction networks with catalytically relevant residues, and comparative efficacy relative to a standard antiviral agent (acyclovir). The current findings highlight hesperidin, kaempferol, and quercetin as promising natural inhibitors with superior docking performance and favorable drug-likeness profiles, offering a novel plant-based strategy for TMV management and sustainable crop protection.

Authors' Contributions

AMA: Methodology, data collection, analysis, and drafting the manuscript.
AIA: Study design.
ASS: supervision, review, and validation.
AAM: Data analysis, validation of results, and revision of the manuscript.
SKA: Supervision, and review. All authors contributed to the final review and approved of the manuscript.

Ethical approval
As this research did not include human or animal subjects, formal ethical approval was not required. All experimental work adhered to established institutional policies and internationally recognized guidelines for safe laboratory practice and scientific integrity.

Funding source
This research was conducted without any specific financial support from public, commercial, or non-profit funding bodies.

Generative AI and AI assisted technology statement
The authors declare that no generative AI and AI assisted technology was used in the creation of this manuscript.

Conflict of interests
The authors declare that they have no known competing interests that could have influenced the work reported in this study.

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