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Dual Mechanisms of Apigenin and Luteolin Against Dengue Virus Serotype 2: Allosteric Inhibition of NS2B/NS3 Protease and Interference with Host C-Type Lectin Receptor-Mediated Entry

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Abstract:

Objective: This study aimed to perform a computational analysis of apigenin and luteolin, focusing on their biological activities, pharmacokinetic properties, and interactions with dengue virus serotype 2 (DENV-2) proteins and host cell receptors associated with viral entry.

Material and Methods: The biological activities of the compounds were first predicted using PASS Online to evaluate their potential pharmacological profiles. Pharmacokinetic properties, including gastrointestinal absorption, skin permeation, blood–brain barrier penetration, cytochrome P450 interactions, bioavailability score, and overall drug-likeness, were assessed using SwissADME. In addition, toxicity parameters, such as LD_{50} values and organ-specific toxicities, were predicted using ProTox 3.0. Subsequently, molecular docking simulations were performed using the SeamDock web server to evaluate the binding affinities of the compounds with dengue viral proteins and host receptor targets.

Results: Both compounds exhibited favorable pharmacokinetic characteristics and low predicted toxicity, alongside antioxidant and anti-inflammatory activities. Docking simulations revealed that luteolin had a slightly stronger affinity (–7.74 kcal/mol) than apigenin (–7.67 kcal/mol) toward the NS2B-NS3 protease, particularly at allosteric sites.

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In addition, both flavonoids displayed interactions with host C-type lectin receptors, suggesting potential interference with viral attachment and entry mechanisms.

Conclusion: These computational findings highlight apigenin and luteolin as promising anti-dengue candidates with dual mechanisms of action through viral protease inhibition and host receptor interactions, supported by favorable pharmacokinetic and safety profiles.

Keywords: anti-dengue, apigenin, dengue serotype-2, dengue virus, flavonoids, luteolin

Introduction

Dengue virus (DENV) infection remains a significant global public health concern, particularly in Asia, which accounts for approximately 70% of all reported cases[1]. DENV is transmitted to humans primarily through the bites of female *Aedes aegypti* and *Aedes albopictus* mosquitoes. The virus comprises four distinct serotypes, DENV-1, DENV-2, DENV-3, and DENV-4[2,3]. According to the World Health Organization Regional Office for Southeast Asia (WHO SEARO), 10,992 cases and 10 deaths were reported during the first four months of 2025[4].

Among the four dengue virus serotypes, DENV-2 was selected as the target serotype because several epidemiological reports consistently indicated that DENV-2 is associated with more severe clinical symptoms[5]. DENV-2 is often linked to secondary infections and has been reported to cause dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS)[6], which occurs at a higher rate than other serotypes. Additionally, DENV-2 is the main serotype found in several major outbreaks across Southeast Asia, including Thailand, Vietnam, Laos, and Cambodia, which underscores its significant role in the regional outbreak patterns[7]. Due to its higher clinical severity and epidemiological importance, DENV-2 is considered the most clinically significant serotype and is suitable for selection as a target for the development of new antiviral drugs[8,9].

Despite ongoing efforts to control the disease, no specific antiviral therapy is currently approved, and the available vaccines present limitations in terms of serotype coverage and age-related efficacy. Therefore, the development of novel antiviral agents with broad efficacy and favorable safety profiles remains a critical priority[10,11].

In recent years, natural compounds have attracted increasing attention as potential sources of bioactive molecules, particularly flavonoids, which are abundantly present in various plants, vegetables, and fruits. Flavonoids have emerged as promising natural compounds due to their diverse pharmacological properties[12,13]. Among them, apigenin and luteolin, which are widely distributed in fruits, vegetables, and medicinal plants, have garnered increasing attention for their potent anti-inflammatory, antioxidant, and anticancer properties. Recent research reports that apigenin and luteolin can inhibit the replication of DENV-2 through mechanisms involving the activation of STAT2, particularly luteolin, with luteolin showing the strongest stimulation of STAT2 phosphorylation at Tyr689 position[14]. These findings suggest that apigenin and luteolin may represent promising natural candidates for anti-dengue drug development. However, further investigation is required to clarify their specific binding interactions with viral proteins and their pharmacokinetic and toxicity profiles, which remain poorly understood.

In addition to their previously reported anti-dengue and immunomodulatory effects, apigenin and luteolin bind stably to key catalytic residues of the NS2B–NS3 protease, contributing to a stable enzyme–ligand

complex. Both compounds also share the conserved flavone pharmacophore, comprising a C2=C3 double bond and multiple hydroxyl groups that strengthen hydrogen bonding and π–π stacking interactions within viral serine protease pockets[15]. Furthermore, both flavonoids exhibit favorable drug-likeness and pharmacokinetic profiles[16,17]. These properties provide strong support for selecting apigenin and luteolin for further *in silico* evaluation against DENV proteins.

Despite the broad pharmacological relevance of flavonoids, their antiviral mechanisms against dengue virus (DENV) remain insufficiently defined. Among these, apigenin and luteolin are two structurally related flavones that provide a valuable framework for dissecting structure–activity relationships within this subclass. Although differing by only a single hydroxyl group on the B-ring, this modification in luteolin is known to influence molecular interactions, antiviral potency, and modulation of host cellular pathways compared with apigenin[12,14]. Both compounds have shown preliminary inhibitory effects on DENV replication and viral enzyme activity; however, their specific mechanisms, particularly the distinction between direct viral inhibition and modulation of host factors required for viral propagation, remain poorly understood[14]. Given the urgent need for effective antiviral agents, this study specifically focused on apigenin and luteolin to evaluate their binding affinity to both structural and non-structural proteins of dengue virus serotype 2 (DENV-2), as well as to selected host receptors involved in dengue infection, to determine their target specificity through in silico analysis. Furthermore, their pharmacokinetic properties and toxicity profiles were assessed to explore their suitability as potential lead compounds for antiviral drug development.

Material and Methods

Dengue dengue virus proteins retrieval and evaluation

The three-dimensional (3D) structures of dengue virus proteins, including Capsid (PDB ID: 6VG5)[18], Envelope (PDB ID: 1OKE)[19], Envelope Domain III (PDB ID: 6FLC)[20], NS1 (PDB ID: 7BSC) [21], NS2B-NS3 protease (PDB ID: 2FOM) [22], NS3 helicase (PDB ID: 2BMF) [23], NS5 methyltransferase (MTase, PDB ID: 2P41)[24], and NS5 RNA-dependent RNA polymerase (RdRp, PDB ID: 5K5M)[25], along with host receptors DC-SIGN (PDB ID: 1SL4) [26] and C-type lectin (PDB ID: 2YHF)[27], were retrieved in PDB format from the RCSB Protein Data Bank (https://www.rcsb.org).

Ligand preparation

The chemical structures of apigenin and luteolin were obtained from the PubChem database (https://pubchem.ncbi.nlm.nih.gov) with compound IDs (CID) 5280443 and 5280445, respectively, in the structure data file (SDF) format (Figure 1). The structures were converted to PDB format using Discovery Studio. The prepared ligands were then uploaded to the SeamDock web server for docking simulations, where energy minimization and hydrogen addition were performed automatically by the platform.

Biological activities using PASS online

The biological activities of apigenin and luteolin were predicted using the PASS Online webserver (https://www.way2drug.com/PassOnline/), a tool based on the principle of structure–activity relationship to assess the probability of specific activity of compounds. The structures of the two compounds were entered into SMILES format in the system. The prediction results were expressed in terms of Pa (probability of activity) and Pi (probability of inactivity), considering only activities with Pa values higher than Pi as potential bioactivity and suitable for further analysis.

Pharmacokinetic property using SWISSADME

The pharmacokinetic properties of apigenin and luteolin were predicted using the SwissADME web server (http://www.swissadme.ch), based on their SMILES representations. Key absorption, distribution, metabolism,

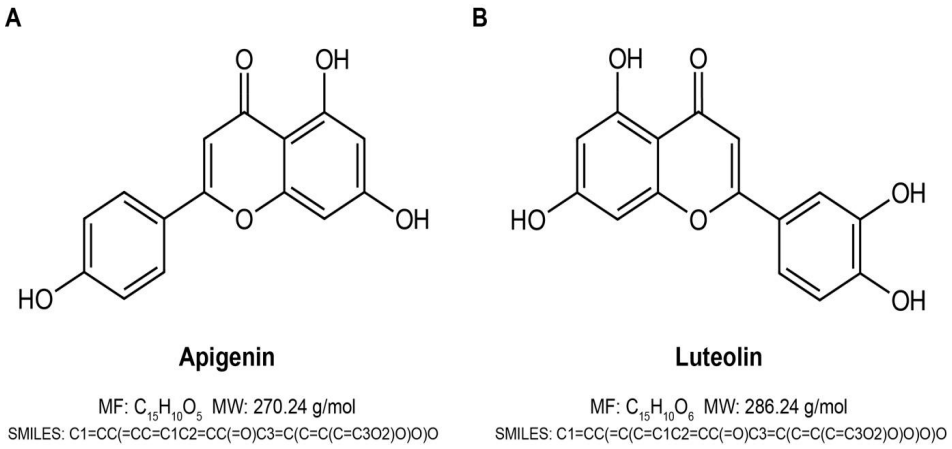

Figure 1 The chemical structures of apigenin (A) and luteolin (B)

and excretion (ADME) parameters were evaluated, with particular focus on their compliance with Lipinski's Rule of Five. These results were used to assess the oral bioavailability potential and overall pharmacokinetic suitability of both compounds.

Toxicity prediction using ProTox 3.0

The toxicity profiles of apigenin and luteolin were assessed using the ProTox 3.0 web server (https://tox.charite.de/protox3/). The SMILES structures of each compound were submitted to the system to evaluate organ-specific toxicities (including hepatotoxicity, neurotoxicity, nephrotoxicity, respiratory toxicity, and cardiotoxicity), LD_{50} values, and toxicity classification. The results were used to support the safety assessment of both compounds as model drugs for drug development.

Molecular docking analysis

Molecular docking studies were conducted using the SeamDock web server (https://bioserv.rpbs.univ-paris-diderot.fr/services/SeamDock/), which integrates the AutoDock Vina docking engine for structure-based ligand–receptor interaction analysis. The three-dimensional (3D) structures of dengue virus proteins and host receptors were input as PDB IDs, while the ligands apigenin and luteolin were uploaded as PDB files. All water molecules and heteroatoms were removed prior to docking, polar hydrogens were added, and Gasteiger charges were assigned automatically using the built-in AutoDockTools module. The docking grid box was defined to encompass the relevant binding region of each protein, with default grid dimensions (30×30×30 $Å^3$). Binding affinities were expressed as predicted free energies of binding (ΔG, kcal/mol), and the best-ranked docking poses were analyzed to identify key molecular interactions, including hydrogen bonds, hydrophobic contacts, and π–π stacking with active site residues.

Molecular simulation study (normal mode analysis)

Normal mode analysis (NMA) of the Luteolin/Apigenin-NS2B/NS3 complex was performed using the iMODS web server (http://imods.iqfr.csic.es/)[28]. The protein structure in PDB format was uploaded directly without modification. Default settings were applied for the elastic network model calculations. Outputs including

residue deformation energies, normal mode vectors, and cross-correlation matrices were generated and analyzed. Visualization of motions was performed using PyMOL and ChimeraX (version 1.9).

Results

Prediction of antiviral biological activities

The bioactivity of apigenin and luteolin was predicted using the PASS Online tool. The results for apigenin revealed a high probability of exhibiting antioxidant activity (Pa = 0.732), free radical scavenging (Pa=0.719), and anti-inflammatory properties. Notably, apigenin showed a strong potential to inhibit drug-metabolizing enzymes, including CYP1A2, and CYP1A1. Furthermore, it was predicted to induce the expression of HMOX1 (Pa=0.899), a gene associated with anti-inflammatory responses, and to inhibit HIF1A expression (Pa=0.911), which may contribute to its overall anti-inflammatory effect (Table 1).

Similarly, luteolin demonstrated a high probability of antioxidant (Pa=0.798) and free radical scavenging activities (Pa=0.755), suggesting its potential to mitigate oxidative stress-related cellular damage. It also exhibited strong anti-inflammatory potential by inducing HMOX1 expression (Pa=0.935) and inhibiting HIF1A expression (Pa=0.964). Like apigenin, luteolin was also predicted to inhibit the major cytochrome P450 enzymes involved in drug metabolism, including CYP1A1, and CYP1A2 (Table 1).

Pharmacokinetic property prediction

Apigenin and luteolin both exhibit favorable properties for oral drug development. They show high gastrointestinal absorption, lack predicted blood-brain barrier (BBB) penetration, and are not substrates of P-glycoprotein (P-gp). Both compounds are predicted to inhibit key cytochrome P450 enzymes, including CYP1A2, CYP2D6, and CYP3A4, which may influence drug-drug interaction potential. In terms of drug-likeness, apigenin and luteolin fully comply with Lipinski's Rule of Five, suggesting good oral bioavailability. Additionally, both compounds are considered synthetically accessible, with apigenin rated as having ease to moderate difficulty and luteolin as having moderate to easy biosynthetic potential. However, caution is advised for luteolin due to structural alerts associated with its catechol moiety, which may affect its metabolic stability or reactivity (Table 2).

Table 1 PASS online-Based prediction of the biological properties of apigenin and luteolin

Predicted Activity	Apigenin		Luteolin	
	Pa[a]	Pi[b]	Pa[a]	Pi[b]
1. HIF1A expression inhibitor	0.911	0.005	0.964	0.003
2. HMOX1 expression enhancer	0.899	0.003	0.935	0.002
3. Antihemorrhagic	0.837	0.002	0.849	0.002
4. CYP1A1 inhibitor	0.807	0.002	0.833	0.006
5. CYP1A2 inhibitor	0.800	0.004	0.820	0.002
6. JAK2 expression inhibitor	0.796	0.008	0.816	0.004
7. Antioxidant	0.732	0.004	0.798	0.002
8. NOS2 expression inhibitor	0.732	0.002	0.775	0.004
9. Free radical scavenger	0.719	0.004	0.755	0.003

[a]=probability of activity (Pa), [b]=probability of inactivity (Pi)

Table 2 Pharmacokinetic properties and drug-likeness of apigenin and luteolin predicted using SwissADME

Pharmacokinetic parameter	Properties	Apigenin	Luteolin
Absorption	Gastrointestinal absorption	High	High
	P-glycoprotein substrate	No	No
	Water solubility	Soluble	Soluble
Distribution	Blood–Brain Barrier (BBB) permeation	No	No
Metabolism	CYP1A2 inhibitor	Yes	Yes
	CYP2C19 inhibitor	No	No
	CYP2C9 inhibitor	No	No
	CYP2D6 inhibitor	Yes	Yes
	CYP3A4 inhibitor	Yes	Yes
Drug-likeness	Lipinski's Rule of Five	Yes	Yes
	Violations	0	0
Excretion & Bioavailability	Bioavailability Score	0.55	0.55
	Synthetic Accessibility	2.96	3.02

Toxicity and safety profile assessment

The toxicity assessment of apigenin and luteolin was performed using ProTox 3.0. Apigenin was predicted to have an LD_{50} value of 2,500 mg/kg body weight, while luteolin had a higher LD_{50} value of 3,919 mg/kg body weight. Both compounds were classified in Toxicity Class 5, indicating relatively low acute toxicity. Neither compound exhibited significant risks of hepatotoxicity, neurotoxicity, or cardiotoxicity. However, both apigenin and luteolin were predicted to pose potential risks of nephrotoxicity and respiratory toxicity, which may warrant further investigation (Table 3).

Molecular docking analysis of apigenin and luteolin against dengue viral proteins

Apigenin and luteolin demonstrated favorable binding affinities toward several dengue virus proteins, with both compounds showing the strongest interaction with the NS2B-NS3 protease, a critical enzyme in viral replication. Apigenin exhibited the highest binding affinity for NS2B-NS3 protease (–7.67 kcal/mol), followed by C-type lectin (–7.36 kcal/mol), capsid protein (–6.94 kcal/mol), and NS1 (–6.85 kcal/mol) (Figure 2A-D), as summarized in Table 4. Luteolin showed slightly stronger binding overall, with the highest affinity for NS2B-NS3 protease (–7.74 kcal/mol), followed by NS1 (–7.67 kcal/mol), C-type lectin (–7.19 kcal/mol), and NS5 MTase (–7.08 kcal/mol) (Figure 3A-D), as shown in Table 5.

Binding and allosteric interaction sites of apigenin and luteolin

Docking analysis showed that apigenin and luteolin bind stably to a potential allosteric site on the NS2B–NS3 protease (Supplementary Figure 1A), located in a surface groove (orange region) distinct from the active site at the NS2B/NS3 interface (green region). Both ligands adopted similar orientations with minor differences in contact residues. The ribbon representation reveals that both ligands are situated in regions of relatively low flexibility, as indicated by the blue-colored structure, primarily encompassing loops and surface-exposed helices (Supplementary Figure 4B). In contrast, regions with higher B-factor values, shown in orange, were observed exclusively at the C-terminal end of chain A (NS2B) within the NS2B/NS3 complex, reflecting localized increases in molecular flexibility.

Table 3 Predicted toxicity profile of apigenin and luteolin using ProTox 3.0

Parameter	Apigenin	Luteolin
Predicted LD_{50}	2,500 mg/kg body weight	3,919 mg/kg body weight
Toxicity Class	5	5
Hepatotoxicity	Inactive	Inactive
Neurotoxicity	Inactive	Inactive
Nephrotoxicity	Active	Active
Respiratory toxicity	Active	Active
Cardiotoxicity	Inactive	Inactive

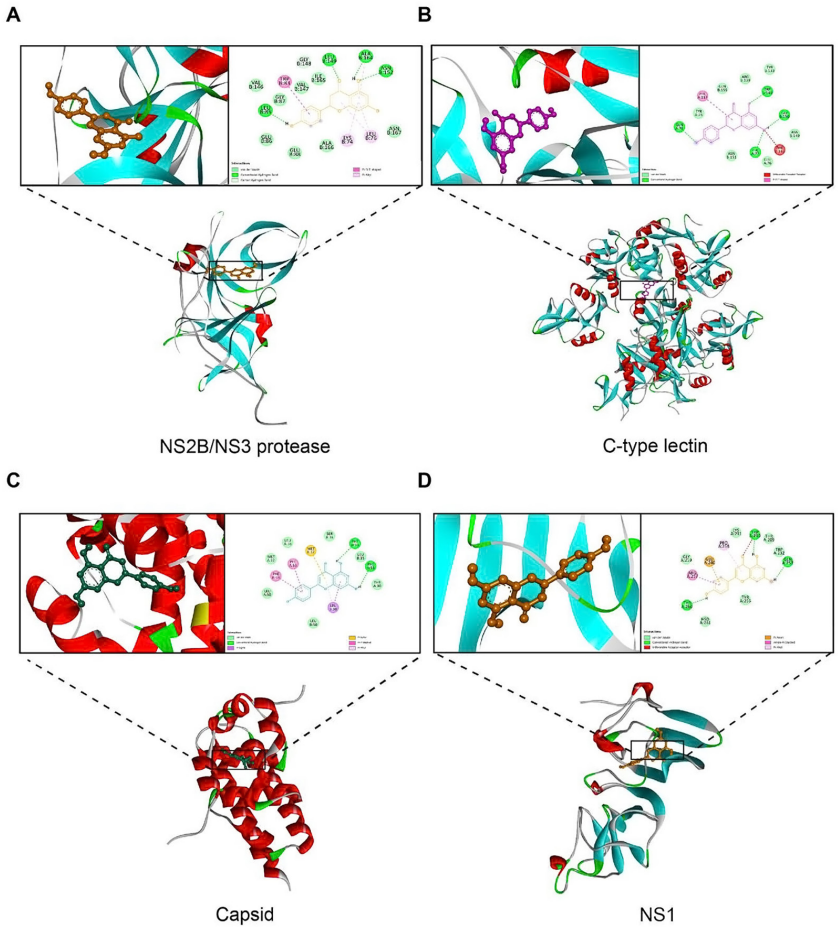

Figure 2 The interaction between apigenin and its target proteins is illustrated for each complex using two focused panels. The left panel displays the three-dimensional (3D) binding orientation of apigenin within the protein structure, while the right panel highlights the two-dimensional (2D) interaction map, detailing the key amino acid residues involved. The analyzed target proteins include: (A) NS2B–NS3 protease (PDB: 2FOM), (B) C-type lectin (PDB: 2YHF), (C) Capsid protein (PDB: 6VG5), and (D) NS1 protein (PDB: 7BSC)

Table 4 The binding affinity of apigenin using the SeamDock web server

Rank	Receptor	Apigenin–protein complex		Binding affinity (kcal/mol)
		Interaction type	Protein residue	
1	NS2B–NS3 protease	Hydrogen bond	LEU85, LEU149, ALA164 and ASN152	–7.67
		Pi–Pi T–shaped	TRP83	
		Pi–Alkyl	LYS74, ALA164 and LEU76	
		Van der Waals	GLY148	
2	C–type lectin	Hydrogen bond	GLN79, TRP140, GLY150 and PHE77	–7.36
		Pi–Pi T–shaped	PHE117	
3	Capsid	Hydrogen bond	PHE33	–6.94
		Pi–Pi T–shaped	PHE53	
		Pi–sigma	LEU38	
		Pi–Alkyl	LEU38	
		Pi–sulfur	MET37	
4	NS1	Hydrogen bond	TRP210, GLN253 and TYR260	–6.85
		Amide–Pi stacked	ARG257	
		Pi–Alkyl	PRO258	
		Pi–anion	GLU240	
5	NS5 MTase	Hydrogen bond	ASP131 and ASP146	–6.76
		Carbon hydrogen bond	GLY81	
		Pi–sigma	ILE147 and LYS105	
		Pi–Alkyl	VAL132	
6	NS5 RdRp	Hydrogen bond	GLN742, ASN406 and THR793	–6.74
		Pi–Donor hydrogen bond	TRP795	
		Van der Waals	TRP746, SER791, LEU754, SER741, ILE740, TYR758, ARG737 and THR794	
		Pi–Alkyl	ARG792	
7	Envelope	Hydrogen bond	ASP215, TRP212 and GLN256	–6.32
		Pi–Pi T–shaped	TRP212	
		Carbon hydrogen bond	PRO217	
		Pi–Alkyl	PRO217, ALA263, LEU216, LEU218 and ALA259	
		Pi–sulfur	MET260	
8	DC–SIGN	Hydrogen bond	GLN306, ARG312 and SER271	–6.25
		Carbon hydrogen bond	ARG31	
		Pi–Alkyl	MET270, ARG312 and SER310	
9	NS3 helicase	Hydrogen bond	ASP496, VAL516 and ARG513	–6.14
		Pi–sigma	ARG513	
		Pi–Alkyl	LEU495, ARG51 and LYS492	
		Pi–Donor hydrogen bond	MET508	
10	Envelope D III	Hydrogen bond	AlA49, THR58 and PHE84	–5.64
		Pi–Pi stacked	PHE84	
		Pi–cation	THR58	
		Pi–Alkyl	ALA52 and AlA49	

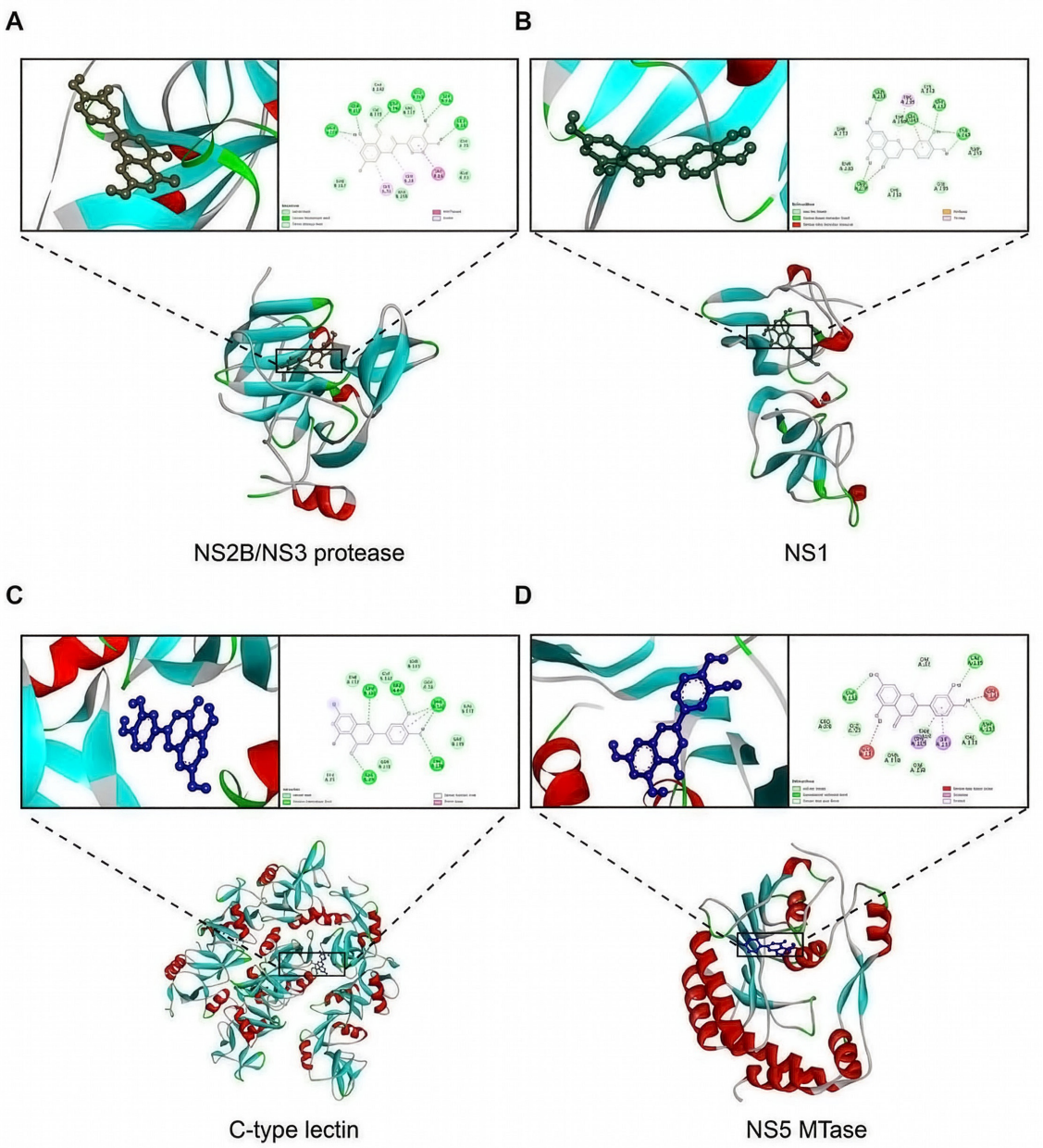

Figure 3 The interaction between luteolin and its target proteins is illustrated for each complex using two focused panels. The left panel displays the three-dimensional (3D) binding orientation of apigenin within the protein structure, while the right panel highlights the two-dimensional (2D) interaction map, detailing the key amino acid residues involved. The analyzed target proteins include: (A) NS2B–NS3 protease (PDB: 2FOM), (B) NS1 protein (PDB: 7BSC), (C) C-type lectin (PDB: 2YHF), and (D) NS5 MTase (PDB: 2P41)

Table 5 The binding affinity of luteolin using the SeamDock web server

Rank	Receptor	Luteolin–protein complex		Binding affinity (kcal/mol)
		Interaction type	Protein residues	
1	NS2B–NS3 protease	Hydrogen bond	ALA164, ASN152, LEU149, VAL146, GLY87 and LEU85	−7.74
		Pi–Pi T–shaped	TRP83	
		Pi–Alkyl	LYS74 and LEU76	
		Carbon hydrogen bond	ILE165 and GLY148,	
2	NS1	Hydrogen bond	GLN253, GLU240, ARG257, TYR260 and TRP210	−7.67
		Pi–Alkyl	PRO258	
		Pi–anion	GLU240	
3	C–type lectin	Hydrogen bond	GLN79, GLN155, PHE77, PHE148 and TRP140	−7.19
		Carbon hydrogen bond	TYR78	
		Pi–Pi T–shaped	PHE148	
4	NS5 MTase	Hydrogen bond	ASP146, VAL130 and ASP131	−7.08
		Carbon hydrogen bond	GLY81	
		Pi–Alkyl	LYS105	
		Pi–sigma	LYS105 and ILE147	
5	Capsid	Hydrogen bond	PHE33	−6.72
		Carbon hydrogen bond	LEU50	
		Pi–Alkyl	LEU38	
		Pi–sigma	LEU38	
		Pi–sulfur	MET37	
		Pi–Pi stacked	PHE53	
6	Envelope D III	Hydrogen bond	PHE33	−6.72
		Carbon hydrogen bond	LEU50	
		Pi–Alkyl	LEU38	
		Pi–sigma	LEU38	
		Pi–sulfur	MET37	
		Pi–Pi stacked	PHE53	
7	NS5 RdRp	Hydrogen bond	SER661, ASP664, ASP663, ILE797, ASP539 and GLY600	−6.67
		Carbon hydrogen bond	SER601	
		Pi–Alkyl	ILE797	
		Pi–Pi stacked	TYR607	
8	NS3 helicase	Hydrogen bond	LEU238, GLY237, GLU232 and THR252	−6.52
		Pi–anion	GLU255	
9	DC–SIGN	Hydrogen bond	SER271, ARG312 and GLN306	−5.97
		Carbon hydrogen bond	ARG312	
		Pi–Alkyl	MET270 and ARG309	
10	Envelope	Hydrogen bond	MET201, LYS204, ASP203 and GLU62	−5.88
		Pi–Alkyl	LYS202 and VAL252	
		Pi–anion	GLU257	

Normal mode analysis and dynamic correlations

Normal mode analysis (NMA) using the iMODS server was employed to investigate the flexibility and dynamic behavior of the NS2B-NS3 complex upon ligand binding. The deformability plot showed localized flexibility, particularly at the N-terminal and mid-regions, suggesting potential hinge-like motions (Supplementary Figure 1C, top left). The B-factor profile indicated overall structural rigidity, with slightly elevated fluctuations at the terminal regions (Supplementary Figure 1C, top right). A low eigenvalue for the first mode (4.002076e-04) reflects high structural adaptability with minimal energy input (Supplementary Figure 1C, bottom left). The covariance matrix revealed correlated and anti-correlated residue movements, indicating coordinated domain motions are likely important for enzymatic function and allosteric regulation (Supplementary Figure 1C, bottom right).

Discussion

This study found that apigenin and luteolin have many bioactivities related to reducing inflammation and oxidative stress, including their ability to act as antioxidants and free radical scavengers. This result is consistent with the reports by Panda and Kar (2007) and Liu et al. (2016), who found that apigenin and luteolin reduced ROS activity and restored the intracellular antioxidant system[29]. In addition, apigenin and luteolin can inhibit NOS2 (iNOS), which plays a role in nitric oxide (NO) production in macrophages stimulated by viruses or cytokines. Reducing iNOS reduces the amount of NO, which is consistent with the research of Liu et al. (2016) and Salehi et al. (2019), who showed significant inhibition of iNOS and NO[30,31]. Both compounds also inhibit the expression of JAK2, which is involved in inflammatory signaling via the JAK-STAT pathway[9,29,30], and tend to inhibit HIF1A, which induces the production of pro-inflammatory cytokines and increases vascular permeability, especially in severe dengue fever. Therefore, the inhibition of HIF1A may reduce vascular leakage[31–33]. According to Miron et al. (2017), apigenin and luteolin significantly inhibit CYP1A2 in human microsomes[34]. The results from PASS online and SwissADME in our study also suggest that apigenin and luteolin may inhibit both CYP1A1 and CYP1A2, especially CYP1A2, which plays a role in the metabolism of theophylline, caffeine, and other drugs. Kimura et al. (2009) reported that apigenin and luteolin can significantly inhibit the activity of CYP3A4, which is the major enzyme responsible for the metabolism of more than 50% of clinical drugs[32].

The evaluation of the effects of these compounds on cytochrome P450 enzyme inhibition should be further studied. The pharmacokinetic properties of apigenin and luteolin using the SwissADME tool from Daina et al. (2017) also provided important data supporting the potential of both compounds for development as oral antiviral agents, indicating that apigenin and luteolin have high gastrointestinal absorption, dissolve in water, and don't interact with P-gp substrates, which could potentially maintain levels in target cells for longer periods. Both compounds pass the Lipinski criteria, don't cross the blood-brain barrier, reduce the risk of neurologic effects, have a moderate bioavailability score, and have low synthetic accessibility, all of which support their potential for development as oral antivirals[33]. ProTox-II reported that apigenin and luteolin are in toxicity class 5, indicating low acute toxicity. Neither compound presents a risk of hepatotoxicity, neurotoxicity, or cardiotoxicity; however, both have the potential for nephrotoxicity and respiratory toxicity, warranting further study[35].

Molecular docking analysis revealed that both apigenin and luteolin exhibit strong binding affinities to multiple dengue virus and host receptors, with notable differences in ranking and interaction profiles. Luteolin demonstrated the strongest binding to the NS2B-NS3 protease (−7.74 kcal/mol), slightly higher than apigenin's binding (−7.67 kcal/mol) to the same target, indicating potent inhibition potential. Both compounds interacted

with key catalytic and structural residues such as LEU85, LEU149, ALA164, and TRP83 via hydrogen bonding and π-interactions, suggesting stable complex formation at the protease allosteric site. Notably, Peng et al. (2018) reported that luteolin exhibited weak in vitro inhibition of the human furin enzyme, with a Ki of approximately 140 μM, which may indirectly contribute to reducing dengue virus replication. Consistently, our docking analysis demonstrated multi-target interactions of luteolin with both viral and host proteins, including NS2B-NS3 protease, NS1, the capsid protein, and the host receptor DC-SIGN, indicating possible synergistic effects in inhibiting viral replication and entry. In addition, the docking results provided molecular insights into key amino acid residues and binding interactions, offering valuable guidance for the rational design of luteolin derivatives with improved structural features and enhanced antiviral activity. Moreover, previous studies have demonstrated apigenin's antiviral potential against flaviviruses, including dengue virus, by inhibiting viral replication and interfering with host pathways involved in viral entry[36,37]. NS1 was the second-ranked target for luteolin (–7.67 kcal/mol) and fourth for apigenin (–6.85 kcal/mol), where both ligands formed multiple stabilizing interactions, including hydrogen bonds with GLN253, TYR260, and GLU240. Apigenin showed a stronger affinity for the C-type lectin receptor (–7.36 kcal/mol) compared to luteolin (–7.19 kcal/mol), while luteolin exhibited stronger binding to NS5 MTase (–7.08 kcal/mol vs. apigenin's –6.76 kcal/mol). Interestingly, both flavonoids formed consistent interactions with conserved aromatic residues, such as PHE77, TRP140, and PHE148, within the lectin binding site, suggesting potential interference with host-mediated viral entry. Capsid protein ranked fifth for both compounds, with similar binding modes involving π–π stacking and sulfur interactions with MET37 and PHE53. Although apigenin exhibited a stronger interaction with the DC-SIGN receptor (–6.25 kcal/mol) compared to luteolin (–5.97 kcal/mol), luteolin displayed superior affinity across more viral non-structural proteins, including NS5 RdRp and NS3 helicase. These findings highlight both compounds as multi-target inhibitors with overlapping but distinct binding profiles, with luteolin slightly outperforming apigenin in terms of overall binding strength and coverage of essential viral enzymes. Although no in vitro data from the present study are available to validate these findings, previous work by Mukhtar et al. (2022) reported that apigenin exhibited a binding energy of –8.3 kcal/mol[37], lending additional support to its structural potential as a dengue antiviral agent. Nonetheless, comprehensive experimental validation, including both in vitro and in vivo studies, is essential to confirm these computational predictions and fully assess the pharmacological promise of apigenin and related flavonoids[36,38].

Notably, molecular docking revealed that both apigenin and luteolin interact with the DENV NS2B-NS3 protease at an allosteric site, an underexplored target in dengue but previously noted in Zika and West Nile viruse[22,39]. The NS2B-NS3 protease is essential for viral polyprotein processing, making it a key antiviral target[40]. Binding at the allosteric site may enhance selectivity and reduce resistance risk. In addition, normal mode analysis and B-factor coloring showed that these ligands interact with flexible regions, suggesting an induced-fit mechanism. Cross-correlation analysis indicated that ligand binding alters dynamic motions across the protease, including regions near the NS2B cofactor, which is critical for enzyme activation[22]. These findings support the possibility of modulating protease function through allosteric inhibition and underscore the potential of apigenin and luteolin as anti-dengue, broad-spectrum flavivirus inhibitors.

Conclusion

Luteolin exhibited higher predicted biological activity, lower toxicity, and stronger binding affinity compared to apigenin, suggesting its greater potential as an anti-

dengue agent. Both flavonoids demonstrated favorable pharmacokinetic properties, low predicted toxicity, and strong binding affinities toward the DENV-2 NS2B-NS3 protease (allosteric site) and the host C-type lectin receptor, indicating dual mechanisms targeting both viral and host receptors. However, this study is primarily based on computational analyses without experimental validation. Therefore, further in vitro and in vivo investigations are required to confirm their efficacy and safety under physiological conditions.

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Conflict of interest

There are no potential conflicts of interest to declare.

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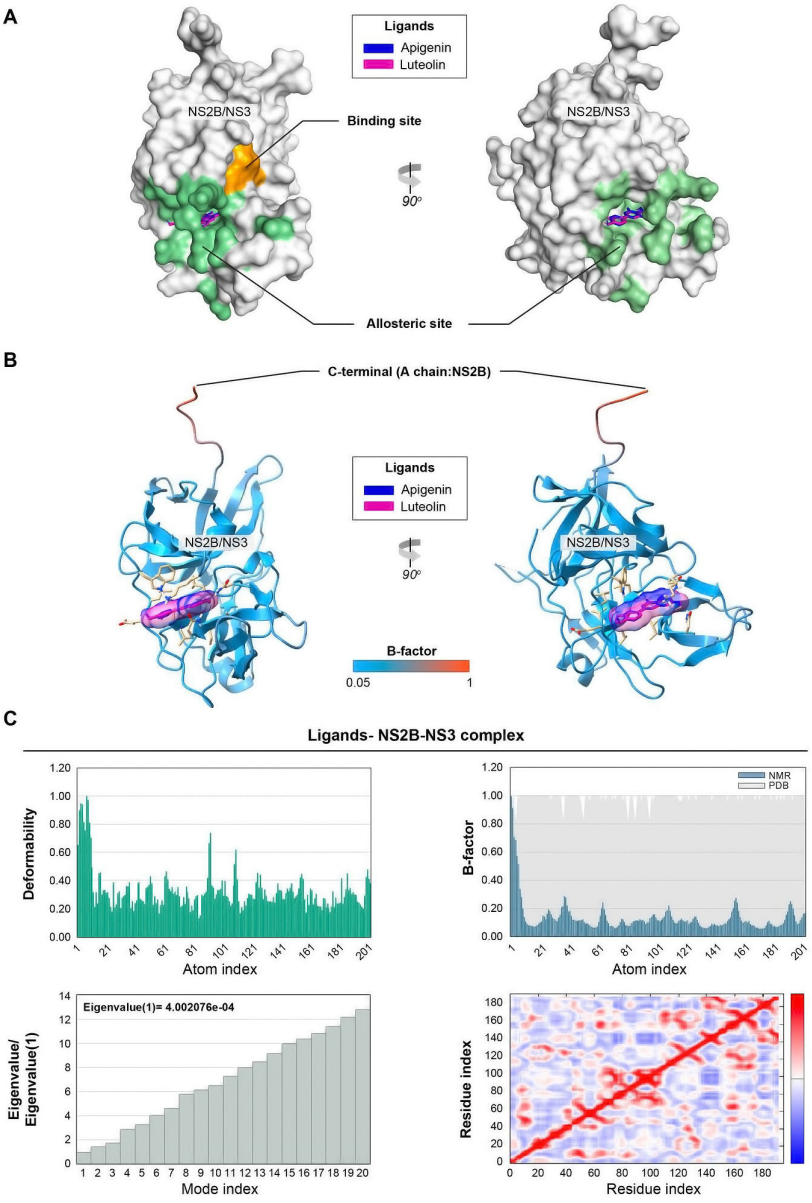

Supplementary Figure 1 Molecular simulation and structural flexibility analysis of apigenin and luteolin bound to the NS2B–NS3 protease of dengue virus, (A) Binding site visualization highlighting the canonical active site (orange) and a predicted allosteric site (green). (B) B-factor mapping based on structural dynamics shows that both ligands occupy relatively flexible regions of the protein. (C) Normal Mode Analysis (NMA) results, including deformability per residue (top left), predicted B-factors (top right), eigenvalue distribution indicating the energy required for structural deformation (bottom left), and a covariance matrix showing correlated and anti-correlated residue motions (bottom right)