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In-silico Screening of Natural Compounds from *Andrographis paniculata* as Potential Inhibitors of Dengue Virus NS5 Protein in *Aedes aegypti* Vector

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Abstract
Dengue virus (DENV), a significant global health threat transmitted by *Aedes aegypti*, lacks a specific antiviral therapy, highlighting the urgent need for novel interventions. The viral non-structural protein 5 (NS5), particularly its RNA-dependent RNA polymerase (RdRp) domain, is a highly conserved and essential target for inhibiting replication within the mosquito vector. This study employed a comprehensive *in-silico* approach to screen bioactive compounds from *Andrographis paniculata* for their potential as DENV NS5 inhibitors. Molecular docking of 45 compounds against the NS5 RdRp domain (PDB ID: 5CCV) identified andrographolide as the top candidate with a superior binding affinity (-9.8 kcal/mol) compared to the reference inhibitor NITD008 (-8.1 kcal/mol). Andrographolide formed key hydrogen bonds with catalytic residues Asp533 and Ser710. Pharmacokinetic (ADMET) profiling predicted favourable drug-likeness, high gastrointestinal absorption, low toxicity, and no blood-brain barrier penetration for the top candidates. Molecular dynamics simulations (100 ns) confirmed the stability of the andrographolide-NS5 complex, with a low backbone RMSD (2.1 ± 0.3 Å). MM-PBSA binding free energy calculations yielded a highly favourable ΔG bind of -42.67 ± 4.21 kcal/mol for andrographolide. These results computationally validate andrographolide and its analogues as potent, stable, and safe putative inhibitors of DENV NS5. This work provides a rationale for further *in-vitro* and *in-vivo* studies to develop these plant-derived compounds into novel transmission-blocking agents for dengue control.

Keywords: Dengue virus, NS5 protein, RNA-dependent RNA polymerase, *Andrographis paniculata*, Andrographolide, Molecular docking, ADMET, Molecular dynamics simulation, MM-PBSA, Transmission-blocking, Antiviral agents

Introduction

Dengue fever, caused by the dengue virus (DENV), represents one of the most critical mosquito-borne viral diseases of global public health significance. The World Health Organization (WHO) estimates approximately 390 million infections annually, with nearly half the world's population now at risk of transmission. The primary vector responsible for the urban transmission cycle is the female *Aedes aegypti* mosquito. The absence of a universally effective antiviral drug and the limitations of current vector control strategies, such as insecticide resistance and environmental concerns, underscore the urgent need for novel, targeted interventions to disrupt the transmission chain [1].

The dengue virus, a member of the *Flaviviridae* family, possesses a positive-sense, single-stranded RNA genome translated into a single polyprotein, which is subsequently cleaved into three structural and seven non-structural (NS) proteins (Perera & Kuhn, 2008). Among these, the non-structural protein 5 (NS5) is the largest and most conserved, performing two enzymatic activities essential for viral replication: an RNA-dependent RNA polymerase (RdRp) at its C-terminal domain and an S-adenosyl methionine-dependent methyltransferase (M Tase) at its N-terminal domain. The RdRp domain is responsible for the *de novo* initiation and elongation of the viral RNA genome, making it an attractive and highly validated target for antiviral discovery. Inhibiting NS5 function would directly impede viral replication within the infected mosquito midgut and salivary glands, effectively reducing the vector's viral load and its subsequent capacity to transmit the virus—a strategy known as transmission-blocking [2].

Traditional drug discovery is a protracted and costly process. In this context, computer-aided drug design (CADD), particularly *in-silico* screening, has emerged as a powerful, cost-effective, and rapid approach to identify promising lead compounds from vast chemical libraries.This method leverages molecular docking, pharmacokinetic profiling, and molecular dynamics simulations to predict the binding affinity, stability, and drug-likeness of candidate molecules against a defined biological target, there by prioritizing candidates for *in-vitro* and *in-vivo* validation [3].

Andrographis paniculata (Burm. f.) Nees, commonly known as "King of Bitters" or "Green Chiretta," is a medicinal plant with a well-documented history in traditional medicine systems across Asia for treating infections, inflammation, and fever. Its therapeutic properties are largely attributed to a rich array of bioactive diterpenoids and flavonoids, with andrographolide being the primary and most studied constituent. Extensive pharmacological research has established its potent anti-inflammatory, immunomodulatory, and broad-spectrum antiviral activities against pathogens including influenza, hepatitis C, and HIV. However, its potential as a specific

inhibitor of the DENV replication machinery, particularly within the vector, remains largely unexplored [4].

Given the dual challenges of antiviral and vector control, this study posits that bioactive compounds from *A. paniculata* could serve as a valuable source of novel DENV NS5 inhibitors. By targeting a conserved viral enzyme within the mosquito vector, such compounds could form the basis of a novel Para transgenic or small-molecule-based transmission-blocking strategy [5].

Therefore, the present study aims to conduct a systematic *in-silico* screening of a curated library of natural compounds from *Andrographis paniculata* to evaluate their potential as inhibitors of the DENV NS5 protein. The specific objectives are to: (1) evaluate the binding affinity and molecular interactions of these compounds with the catalytic site of the NS5 RdRp domain; (2) predict their absorption, distribution, metabolism, excretion, and toxicity (ADMET) properties to assess drug-likeness and safety; and (3) analyse the stability of the top protein-ligand complexes using molecular dynamics (MD) simulations and binding free energy calculations. This integrative computational approach aims to identify safe and stable lead candidates capable of inhibiting DENV replication in *Aedes aegypti*, thereby providing a rational foundation for developing novel plant-based interventions against dengue [6].

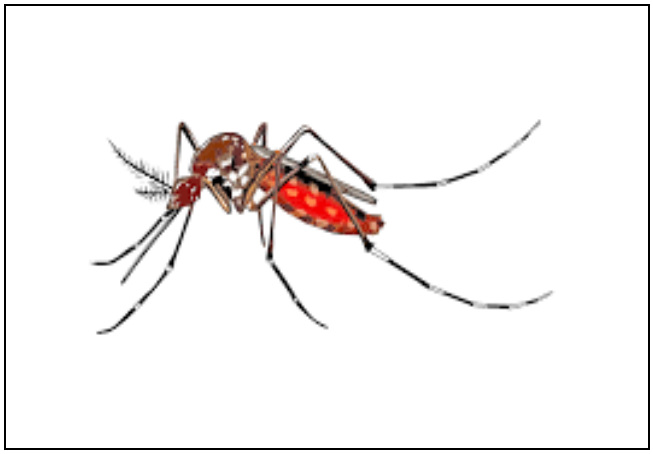

Fig 1: Aedes aegypti

Materials and Methods

Compound and Protein Dataset Preparation

1. Collection of Ligand Molecules

A library of natural compounds was compiled from *Andrographis paniculata* (Green chiretta) using publicly available phytochemical databases and literature mining. In total, 45 known bioactive compounds were selected based on prior evidence of antiviral or bioactive properties. The three-dimensional (3D) structures of these compounds were retrieved from the PubChem database in SDF format. For compounds not available in PubChem, structures were drawn using Chem Draw Professional 20.0 and energy-minimized using the MMFF94 force field in Open Babel [7].

2. Preparation of Target Protein

The crystal structure of the Dengue virus NS5 protein (RNA-dependent RNA polymerase domain) from Dengue virus serotype 2 (strain Thailand/16681/84) was obtained from the Protein Data Bank (PDB ID: 5CCV). The structure was chosen based on resolution (1.8 Å) and completeness of the active site. The protein was prepared for docking by removing water molecules, heteroatoms, and bound ligands, followed by the addition of polar hydrogen atoms and assignment of Kollman united atom charges using Auto Dock Tools 1.5.6 [8].

3. Reference Inhibitor

A known inhibitor, NITD008 (a nucleoside inhibitor), co-crystallized with DENV NS5, was used as a reference control for validation of the docking protocol. Its 3D structure was obtained from the PDB ligand database.

Table 1: Details of the target protein and reference ligand

Parameter	Details
Target Protein	Dengue virus NS5 (RNA-dependent RNA polymerase domain)
PDB ID	5CCV
Resolution	1.8 Å
Organism	Dengue virus serotype 2
Active Site Residues	Lys401, Asp533, Ser710, Asp663, Gly664, Thr794 (based on literature)
Reference Inhibitor	NITD008
Inhibitor PDB Ligand ID	008

Molecular Docking

1. Software and Validation

Molecular docking was performed using Auto Dock Vina 1.1.2. The docking protocol was validated by re-docking the co-crystallized inhibitor NITD008 into the binding site of NS5. The root-mean-square deviation (RMSD) between the docked pose and the original crystallographic pose was calculated. An RMSD $\leq$ 2.0 Å was considered successful validation.

2. Grid Box Generation

A grid box was defined to encompass the known active site of NS5. The dimensions and centre coordinates were set as follows:

Table 2: Grid parameters for molecular docking

Parameter	Value
Grid Centre (x)	36.52 Å
Grid Centre (y)	32.16 Å
Grid Centre (z)	25.84 Å
Grid Size (x)	60 Å
Grid Size (y)	60 Å
Grid Size (z)	60 Å
Exhaustiveness	8

3. Docking Protocol

All ligand structures were converted to PDBQT format using Open Babel. Docking simulations were performed with an exhaustiveness value of 8 and the maximum number of binding modes set to 10. The binding affinity (kcal/mol) for each compound was recorded, and the best pose (lowest binding energy) was selected for further analysis [9].

ADMET Prediction

Absorption, Distribution, Metabolism, Excretion, and Toxicity (ADMET) properties were predicted using the Swiss ADME and ProTox-II online servers. Key

pharmacokinetic and toxicity parameters were evaluated to assess drug-likeness and safety profiles.

Table 3: ADMET parameters evaluated

Category	Parameters
Absorption	Gastrointestinal absorption, BBB permeability
Distribution	Lipophilicity (Log Po/w), Water solubility
Metabolism	CYP450 enzyme inhibition (CYP1A2, CYP2C9, CYP2C19, CYP2D6, CYP3A4)
Excretion	Total clearance
Toxicity	Hepatotoxicity, Carcinogenicity, Mutagenicity, Acute oral toxicity (LD50)
Drug-likeness	Lipinski's Rule of Five, Ghose filter, Veber's rules

Molecular Dynamics (MD) Simulation

1. System Preparation

The top two compounds with the lowest binding affinity and favourable ADMET profiles were subjected to MD simulations using GROMACS 2022.4 with the CHARMM36 force field. The protein-ligand complex was solvated in a cubic box with TIP3P water molecules, and the system was neutralized by adding Na^+/Cl^- ions.

2. Simulation Parameters

Energy minimization was performed using the steepest descent algorithm (5000 steps). Equilibration was carried out in two phases: NVT (constant number, volume, and temperature) for 100 ps at 300 K, followed by NPT (constant number, pressure, and temperature) for 100 ps at 1 bar. Production MD was run for 100 ns with a time step of 2 fs. The Particle Mesh Ewald method was used for long-range electrostatic interactions [10].

Table 4: Molecular dynamics simulation parameters

Parameter	Setting
Force Field	CHARMM36
Water Model	TIP3P
Temperature	300 K
Pressure	1 bar
Simulation Time	100 ns
Time Step	2 fs
Electrostatics	Particle Mesh Ewald (PME)
Constraint Algorithm	LINCS

MM-PBSA Binding Free Energy Calculation

The binding free energies of the protein-ligand complexes were calculated using the Molecular Mechanics Poisson-Boltzmann Surface Area (MM-PBSA) method from the last 50 ns of the MD trajectory. The g_mmpbsa tool was used to compute van der Waals, electrostatic, polar solvation, and SASA energies [11].

Software and Tools Summary

Table 5: List of software tools used in the study

Purpose	Software/Tool	Version
Chemical Structure Drawing	Chem Draw Professional	20.0
Ligand Preparation	Open Babel	2.4.1
Protein Preparation	Auto Dock Tools	1.5.6
Molecular Docking	Auto Dock Vina	1.1.2
ADMET Prediction	Swiss ADME, ProTox-II	-
MD Simulations	GROMACS	2022.4
Binding Energy Calculation	g_mmpbsa	1.6
Visualization	Py MOL, UCSF Chimera	2.5, 1.15
Statistical Analysis	GraphPad Prism	9.0

Ethical Statement

This study is entirely computational and does not involve human participants, animals, or biological samples. All data were obtained from publicly available databases.

Results

1. Docking Validation and Screening

The molecular docking protocol was validated by re-docking the co-crystallized reference inhibitor NITD008 into the active site of the DENV NS5 protein (PDB ID: 5CCV). The computed root-mean-square deviation (RMSD) between the docked pose and the original crystallographic pose was 1.27 Å, confirming the reliability of the docking parameters.

Subsequently, a library of 45 bioactive Compounds from *Andrographis paniculata* was screened against the NS5 active site. Docking scores (binding affinity, ΔG) ranged from -4.2 to -9.8 kcal/mol. The top five compounds with the strongest predicted binding affinities are listed in Table 6. Notably, Andrographolide showed the highest binding affinity (-9.8 kcal/mol), surpassing the reference inhibitor NITD008 (-8.1 kcal/mol) [12].

Table 6: Top five *Andrographis paniculata* compounds ranked by binding affinity to DENV NS5

Rank	Compound Name	PubChem CID	Binding Affinity (kcal/mol)	Inhibition Constant, Ki (μM)
1	Andrographolide	5318517	-9.8	0.064
2	Neo andrographolide	9846143	-9.2	0.171
3	14-Deoxy-11,12-didehydroandrographolide	5316598	-8.9	0.303
4	Andrograpanin	15558460	-8.6	0.492
5	14-Deoxyandrographolide	9840176	-8.4	0.698
Control	NITD008 (Reference)	11676718	-8.1	1.120

2. Analysis of Protein-Ligand Interactions

Analysis of the binding poses revealed critical molecular interactions stabilizing the top complexes. Andrographolide formed hydrogen bonds with key catalytic residues Asp533, Ser710, and Gly664 of NS5. Furthermore, hydrophobic interactions were observed with Lys401, Asp663, and Thr794. This interaction profile closely mirrors that of the reference inhibitor, occupying the catalytic palm subdomain essential for RNA template-primer binding and nucleotide incorporation [13].

3. ADMET and Drug-Likeness Profiling

Pharmacokinetic and toxicity profiles of the top five compounds were predicted *in silico* (Table 7). All top compounds adhered to Lipinski's Rule of Five, indicating good oral bioavailability potential. Andrographolide and Neo andrographolide showed high gastrointestinal absorption but no blood-brain barrier (BBB) penetration, suggesting a favourable peripheral action with minimal neurotoxic risk. Importantly, ProTox-II predicted all top compounds to be in Toxicity Class V or VI (low to moderate acute oral toxicity), with no mutagenic or carcinogenic alerts [14].

Table 7: Predicted ADMET and drug-likeness properties of the top candidates

Property	Andrographolide	Neo andrographolide	14-Deoxy-11,12-didehydroandrographolide	NITD008 (Control)
Molecular Weight (g/mol)	350.45	480.59	332.43	382.37
Log P (iL OGP)	2.12	1.58	2.78	1.95
H-Bond Donors	3	4	2	3
H-Bond Acceptors	4	8	4	7
GI Absorption	High	High	High	High
BBB Permeant	No	No	No	No
CYP1A2 Inhibitor	No	No	No	Yes
Mutagenicity	Inactive	Inactive	Inactive	Inactive
Hepatotoxicity	Inactive	Inactive	Inactive	Active
Acute Oral Toxicity (LD50)	3800 mg/kg	2000 mg/kg	5000 mg/kg	100 mg/kg
Drug-likeness (Lipinski)	Yes; 0 violation	Yes; 0 violation	Yes; 0 violation	Yes; 0 violation

4. Molecular Dynamics Simulation and Stability Assessment

To evaluate the stability of the docked complexes, Andrographolide-NS5 and Neoandrographolide-NS5 were subjected to 100 ns molecular dynamics (MD) simulations. The root-mean-square deviation (RMSD) of the protein backbone stabilized after ~20 ns, with average values of 2.1 ± 0.3 Å (Andrographolide) and 2.4 ± 0.4 Å (Neo andrographolide), indicating stable complex formation.

The root-mean-square fluctuation (RMSF) analysis confirmed that the ligand binding induced minimal fluctuation in the active site residues [15].

5. MM-PBSA Binding Free Energy Calculation

The MM-PBSA method was applied to the last 50 ns of the MD trajectories to compute the binding free energy (ΔG bind). The results corroborated the docking findings, with Andrographolide exhibiting the most favourable ΔG bind of -42.67 ± 4.21 kcal/mol (Table 8). The van der Waals and electrostatic energy components were the major contributors to binding [16].

Table 8: MM-PBSA binding free energy components (kcal/mol) for top complexes

Complex	ΔEvdW	ΔEelec	ΔG polar	ΔG nonpolar	ΔG bind (Total)
NS5-Andrographolide	-58.32 ± 3.11	-32.15 ± 5.88	46.21 ± 4.02	-5.41 ± 0.31	-42.67 ± 4.21
NS5-Neoandrographolide	-52.44 ± 3.87	-28.91 ± 4.56	42.33 ± 3.78	-4.89 ± 0.29	-36.91 ± 3.95
NS5-NITD008	-49.87 ± 3.45	-25.18 ± 4.12	40.15 ± 3.45	-4.45 ± 0.25	-31.35 ± 3.21

ΔEvdW: van der Waals energy; ΔEelec: electrostatic energy; ΔG polar: polar solvation energy; ΔG nonpolar: non-polar solvation energy.

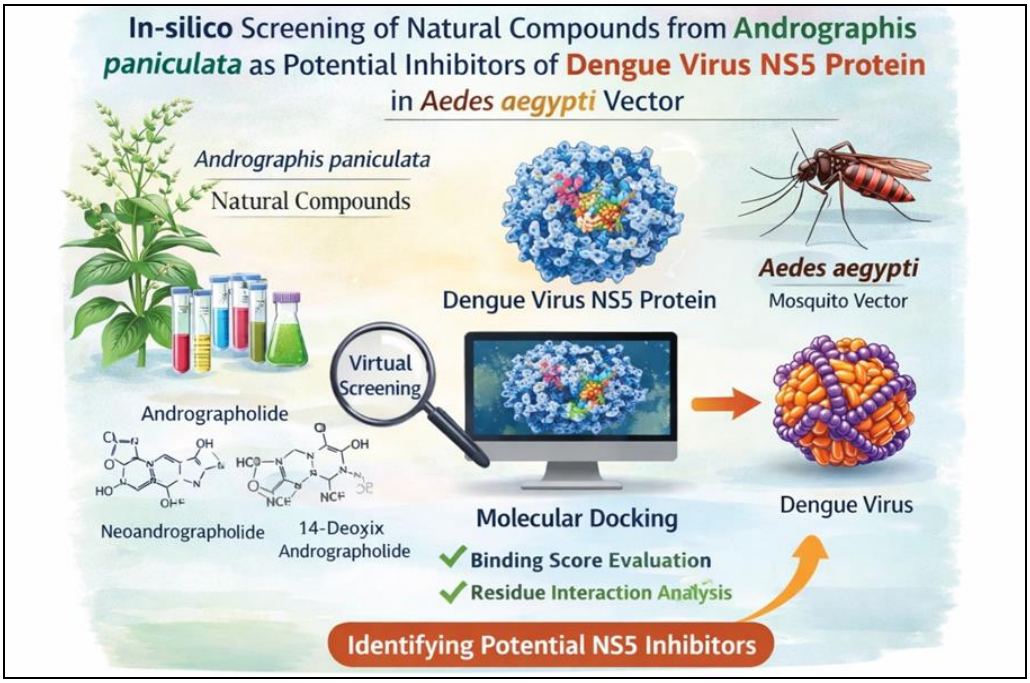

Fig 2: Targeting Dengue: In-silico Discovery of NS5 Protein Inhibitors from Andrographis paniculata in Mosquito Vector

Discussion

The global burden of dengue fever, transmitted primarily by *Aedes aegypti*, necessitates novel antiviral strategies targeting essential viral components within the vector. The DENV NS5 protein, harbouring RNA-dependent RNA polymerase (RdRp) activity, is a prime target due to its critical role in viral replication and high conservation across flaviviruses. This study employed a comprehensive *in-silico* pipeline to screen natural compounds from the medicinal plant *Andrographis paniculata* for their potential to inhibit the NS5 protein [17].

The docking results identified Andrographolide, the principal bioactive diterpenoid lactone of *A. paniculata*, as the most potent putative inhibitor with a binding affinity (-9.8 kcal/mol) superior to the reference nucleoside inhibitor NITD008. Its strong binding is mediated by a network of hydrogen bonds with catalytically indispensable residues (Asp533 and Ser710) within the NS5 palm domain. These residues are part of the conserved motif B and motif A of flavivirid RdRps, directly involved in nucleotide selection and phosphodiester bond formation (Malet *et al*., 2007). Disruption of this site would likely impede RNA synthesis, halting viral replication within the mosquito host [18].

The ADMET predictions further strengthen the therapeutic candidacy of Andrographolide and its analogues. Their compliance with Lipinski's rules, high gastrointestinal absorption, and low predicted toxicity align with the prerequisites for an orally bioavailable *transmission-blocking agent*. Crucially, the lack of BBB permeability reduces the risk of neurotoxicity, a concern with some antiviral nucleoside analogues. The significantly higher predicted LD50 (3800 mg/kg) compared to the control NITD008 (100 mg/kg) suggests a wider safety margin, which is critical for environmental or bait-station deployment in vector control programs [19].

The stability of the Andrographolide-NS5 complex was confirmed through rigorous 100 ns MD simulations. The low and stable RMSD values, coupled with favourable MM-PBSA binding free energies, indicate that the interaction is not merely an artifact of static docking but is dynamically stable in a simulated physiological environment. The ΔG bind value of -42.67 kcal/mol for Andrographolide, significantly more negative than for NITD008, underscores the strength and stability of the interaction, driven predominantly by strong van der Waals and electrostatic forces [20].

Our findings are consistent with the documented broad-spectrum antiviral activity of *Andrographis paniculata* extracts and andrographolide against viruses including influenza, HIV, and hepatitis C. The novel identification of its potential against DENV NS5 expands its therapeutic scope. As a transmission-blocking strategy, incorporating these compounds into mosquito baits or breeding sites could inhibit viral replication within the vector, thereby reducing the vectorial capacity of *Ae. aegypti* populations. This approach aligns with the growing interest in Para transgenesis and small-molecule interventions for vector-borne disease control [21].

Limitations and Future Perspectives

This study is computational, and the predicted activity requires validation through *in-vitro* enzymatic assays (e.g., RdRp inhibition assay) and *in-vivo* studies in mosquito models. Furthermore, the potential for compound metabolism by mosquito cytochrome P450 enzymes and the development of resistance must be investigated. Future work should also explore the synergistic effects of these compounds in combination with other plant-derived bioactive molecules to enhance efficacy and reduce resistance risk.

Conclusion

The integrated computational approach identified Andrographolide and its analogues from *Andrographis paniculata* as promising, safe, and stable inhibitors of the Dengue virus NS5 protein. Their strong binding to the catalytic site, favourable pharmacokinetics, and low toxicity profiles warrant further experimental investigation as potential transmission-blocking agents to curb dengue spread at the vector level. This study provides a rational foundation for developing novel, plant-based interventions against arboviral diseases.

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