Ind. J. Pharm. Edu. Res., 2025; 59(3s):s930-s944.
https://www.ijper.org

Original Article

Exploring Phytocompounds' Interaction with Multi-Serotype Dengue Virus NS5-MTase: Insights into Binding Affinity and Activity

Afaf Salim Alwabli[1,*], Mohammed Balubaid[2], Mohammed Tarique[3]

[1]Department of Biological Sciences, College of Science & Arts, Rabigh Campus, King Abdulaziz University, Jeddah, SAUDI ARABIA.
[2]Department of Industrial Engineering, Faculty of Engineering, King Abdulaziz University, Jeddah, SAUDI ARABIA.
[3]Center for Interdisciplinary Research in Basic Science, Jamia Millia Islamia, Jamia Nagar, New Delhi, INDIA.

ABSTRACT

Background: Dengue Virus (DENV) infection poses a formidable global health threat, spanning from mild breakbone fever to severe conditions such as dengue hemorrhagic fever and dengue shock syndrome. The NS5-MTase enzyme assumes a pivotal role in DENV replication by catalyzing methylation at guanine N7 and ribose 2'-OH during viral cap structure formation. **Materials and Methods:** Targeting the MTase presents a promising strategy for combating flavivirus infections. In recent years, plant-derived metabolites have garnered attention for their antiviral properties, particularly flavones and flavonoids, which have shown efficacy against the DENV MTase protein. **Results:** This study seeks to identify phytocompound capable of interacting with multiple DENV serotypes. Employing a combination of docking, molecular dynamics, and QSAR techniques, we identified potent inhibitors of the NS5-MTase protein. Our investigation identified a flavonoid exhibiting high binding affinity towards the MTase protein across major DENV serotypes. **Conclusion:** Furthermore, molecular dynamics simulations and QSAR analysis were employed to assess the stability of molecular interactions and predicted activity, respectively; shedding light on the potential of the identified phytocompound with calculated IC_{50} of 76.009 nM as a therapeutic agent against MTase of DENV infection.

Keywords: Baicalin, Dengue, Methyltransferase, Molecular Dynamics simulations, QSAR, Virtual screening.

Correspondence:
Dr. Afaf Salim Alwabli
Department of Biological Sciences, Rabigh College of Science and Arts, King Abdulaziz University, Jeddah, SAUDI ARABIA.
Email: aalwabli@kau.edu.sa

Received: 15-11-2024;
Revised: 28-01-2025;
Accepted: 08-05-2025.

INTRODUCTION

Dengue, a viral infection transmitted by mosquitoes such as Aedes aegypti or Ae. Albopictus, poses a significant threat to public health in tropical and subtropical regions, particularly amid the ongoing COVID-19 pandemic due to its overlapping clinical symptoms with COVID-19.[1] Reported dengue cases have surged substantially, increasing from 505,430 cases in 2000 to 2.4 million in 2010 and 5.2 million in 2019, with Southeast Asia and the Western Pacific regions contributing 75% of the global dengue burden.

Despite extensive efforts to develop vaccines and antiviral treatments, there are currently no FDA-approved drugs targeting the DENV NS5-MTase enzyme. Most previous studies have focused on single-serotype inhibitors, limiting their applicability across multiple viral strains.

In this study, we present a novel multi-serotype screening approach that integrates molecular docking, Molecular Dynamics (MD) simulations, and QSAR modeling to identify phytocompounds with broad-spectrum inhibition potential. Unlike previous studies that focused on single-serotype interactions, our approach systematically evaluates inhibitors against multiple DENV serotypes, providing a comprehensive understanding of cross-serotype binding efficiency. This strategy enhances the potential for developing antiviral agents capable of inhibiting diverse DENV variants, a critical aspect in dengue drug discovery.

Furthermore, we employ an advanced QSAR framework that incorporates external validation and independent test sets to ensure the robustness and predictive accuracy of our model, addressing a common limitation in computational drug discovery studies.

Our findings demonstrate that Baicalin exhibits the highest binding affinity across multiple DENV serotypes, making it a promising candidate for further preclinical evaluation.

DOI: 10.5530/ijper.20251313

India, in particular, stands as the global epicenter of dengue, accounting for about 34% of clinical infections worldwide, with the disease becoming endemic in almost all states since 1990.[2] Climate changes contribute to the proliferation of vector mosquitoes, amplifying DENV transmission.[3] Dengue Viral (DENV) infection manifests in various ways, ranging from mild dengue to severe forms characterized by bleeding, plasma leakage, and shock. Many infections remain asymptomatic, fuelling virus transmission.[4]

DENV comprises four main serotypes, namely Dengue Virus type 1 (DENV 1), Dengue Virus type 2 (DENV 2), Dengue Virus type 3 (DENV 3), and dengue virus type 4 (DENV 4). Despite sharing similar antigenic properties, these serotypes lack cross-immunity. In 2013, Malaysia identified a fifth serotype, Dengue Virus type 5 (DENV 5), which emerged possibly due to genetic recombination, natural selection, and genetic bottlenecks, representing a crossover from sylvatic DENV in wildlife to human transmission.[5] However, in this study, only DENV1, DENV2, and DENV3 were considered due to the unavailability of high-resolution crystal structures for DENV4 and DENV5 NS5-MTase. Since molecular docking and MD simulations heavily rely on accurate structural data, including unreliable or low-resolution structures may compromise the validity of the computational predictions. Future studies may address this limitation by employing homology modeling to predict the structural conformations of DENV4 and DENV5 NS5-MTase, enabling a more comprehensive analysis of their interactions with potential inhibitors. DENV is a positive-sense RNA virus. The viral genome encodes three structural proteins: the Capsid (C), Envelope (E), and Membrane (M) protein, and seven non-structural proteins such as NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5. The non-structural proteins are involved in viral replication and assembly. NS5, the largest (104 kDa) and most conserved non-structural protein in the DENV serotypes and the Flavivirus genus, harbors crucial enzymatic functions in two distinct domains: the N-terminal methyltransferase domain (NS5-MTase, residues 1-263, 30 kDa) and the C-terminal RdRp domain (NS5-Pol, residues 272–900, 74 kDa). These domains are linked by a flexible linker. The NS5-MTase domain facilitates RNA cap methylation at both the N7 position of the cap guanosine and the 2′O position of the initial nucleotide of the newly synthesized positive-strand RNA. The NS5-MTase domain plays a role in RNA cap formation, although the specific timing of this process remains unknown.[6] As a component of NS5, the NS5-MTase domain potentially influences various stages of RNA synthesis facilitated by the NS5-Pol domain, making it a potent and ideal target for drug development. Currently, despite worldwide initiatives aimed at vaccine development, there are no cross-protective vaccines or approved antiviral therapies available for DENV infections. The current scenario presents an urgent need to develop potent antiviral drugs to combat DENV infection. Researchers around the globe have reported the protective potential of natural compounds derived from plants against viral infections. In recent decades, screening compounds through various methods has led to the discovery of natural products with activity against different serotypes of the dengue virus.

Targeting the viral methyltransferase enzyme represents a promising strategy for developing therapeutics against Dengue fever. By disrupting viral RNA capping and inhibiting viral replication, methyltransferase inhibitors have the potential to combat Dengue infections and mitigate the global burden of this disease. Multi-serotype screening lies the exploration of stable molecular interactions between small molecules and pathogen targets. By employing a repertoire of advanced techniques such as high-throughput screening assays, molecular docking simulations, and structural biology approaches, researchers can delve deep into the molecular mechanisms underlying the interactions between small molecules and pathogen-specific biomolecules. This deep understanding of stable molecular interactions not only elucidates the mode of action of promising compounds but also facilitates the rational design of next-generation antimicrobial agents with enhanced efficacy and reduced propensity for resistance development. In this study, Quantitative Structure-Activity Relationship (QSAR) analysis is employed to identify the essential descriptors of NS5-MTase. The QSAR model is used to predict the inhibitory activity of phytocompounds against the NS5-MTase protein. A virtual screening strategy is applied to determine highly potent phytocompounds targeting the NS5-MTase protein. Molecular dynamics and simulation analysis are conducted to comprehend the dynamics of the NS5-MTase-phytocompound complex in relation to DENV serotypes.

MATERIALS AND METHODS

Sero-types & Phytocompound used for virtual screening

The NS5-MTase protein structures of DENV1 (5IKM), DENV2 (1R6A) and DENV3 (4R8S) were obtained from PDB database.[6,7] Despite the availability of genomic sequences for DENV4 and DENV5, high-resolution crystallographic or cryo-EM structures of their NS5-MTase proteins are currently unavailable in public structural databases. This limitation necessitated the exclusion of these serotypes from the study, as reliable docking and molecular simulation results require well-resolved atomic structures. The structures of the phytocompounds were drawn using Chemical Sketch Tool provided by RCSB PDB. All the structures were cleaned and geometry optimized using AM1 forcefield using ArgusLab.[8]

Library of NS5-MTase inhibitors

An extensive survey was conducted to identify NS5-MTase inhibitors from various databases and literature.[9-14] The DenvInD

Database of inhibitors of Dengue virus, a curated database of Dengue virus inhibitors contained 115 inhibitors against DENV NS5-MTase protein. The database listed inhibitors, associated PubChem's Compound ID (CID), Substance ID (SID), Bioassay ID (AID), SMILE notation, IC_{50}, Ki, EC_{50}, CC_{50} and PubMed ID (PMID). Out of these 115 inhibitors, compounds with unknown potency were removed. In addition, phytocompounds which have proven activity against DENV were also collected from various literature resources.[15-21] The SMILES notation of the inhibitors were retrieved from PubChem database.[22] The SMILES were converted to 2D SDF files using Data warrior.

Molecular interaction studies with Docking

Molecular docking was performed using the program Autodock Vina.[23] To reproduce ligand binding, a grid box extending 60 Å in each direction with its centre located at the ligand binding site, and an exhaustiveness parameter of thirty-two was set. These parameters were used to dock the entire subset phytocompound library to the NS5-MTase protein. The docked structures were analysed using PyMol (Schrodinger, LLC. 2010. The PyMOL Molecular Graphics System, Version 2.5.5) and PLIP webserver.[24]

Molecular Dynamic-Interaction stability evaluation

Molecular Dynamics Simulations (MDS) were conducted using GROMACS v2020.6 to investigate dynamics of DENV serotypes protein-ligand complexes.[25] The Gromos 54a7 force field was employed for proteins and ligands. Ligand topology files were prepared and obtained from the ATB server. A dodecahedron water box was generated, and subsequently, TIP3P (Transferable Intermolecular Potential 3-Point) water molecules and counter ions were added to solvate the box. The DENV protein-ligand complexes were positioned at the centre of the dodecahedron box, maintaining a distance of 1.0 nm from the box edges. The initial energy minimization of the complex systems was performed using the steepest descent minimization method. First equilibration was carried out with the NVT ensemble utilizing a V-rescale thermostat for 1 ns, followed by the second equilibration with an NPT ensemble using the Parrinello-Rahman barostat for another 1 ns. During the equilibration phase, position restraints were exclusively applied to the ligand atoms along the x, y, and z axes, with force constants set to 1000 kJ mol^{-1} nm^{-2} for each axis. The production simulations were conducted for 100 ns with a time step of 0.2 ps, maintaining a constant temperature and pressure through the implementation of the leapfrog algorithm. To ensure bond constraints during equilibration, the LINCS algorithm was employed, and long-range ionic interactions were approximated using the particle-mesh Ewald algorithm. Trajectory data were recorded at 0.2 ps intervals throughout the Molecular Dynamics (MD) simulation for subsequent post-dynamic analysis.

Activity (IC_{50}) prediction with QSAR modelling

To predict the possible activity of identified phytocompound, Quantitative Structure Activity Relationship (QSAR) models established between various molecular descriptors and anti-dengue activity. Various 1D and 2D descriptors of the compounds were calculated using PaDEL Descriptor software.[26] Data pre-processed and independent feature selection was performed. Finally initial dataset contained 46 samples containing high correlation with activity vector. For development of regression model, since the descriptors were not significantly independent. Therefore, individual descriptors were used for model building, 03 different models were developed using Linear modeling with WEKA.[27] Activity of the phytocompound was calculated from more than one models, and average activity was considered for further interpretation.

RESULTS

Multi-Serotype based Screening provides deep inside into stable molecular interaction

Virtual screening was conducted to identify compounds exhibiting strong affinity for the NS5-MTase protein across all DENV serotypes considered in the study (Table 1). The binding energies of the phytocompounds with DENV1 NS5-MTase protein ranged from -12.5 to -6.5 kcal/mol (Table 1). Baicalin displayed the highest preference, forming hydrogen bonds with Ser56, Gly86, Trp87, His110, Glu111, and Asp146, and engaging in hydrophobic interactions with Val132 and Ile147 (Figure1A (a&b)). In the case of DENV2 NS5-MTase protein, the binding energies of ligands ranged from -10.7 to -6.3 kcal/mol. Baicalin again exhibited the highest binding energy, forming hydrogen bonds with Ser56, Gly58, Asp79, Gly85, Gly86, Trp87, His110, and Glu111, and showed hydrophobic interactions with Thr104, Lys105, Val132, Phe133, and Ile147 (Figure1B (a&b)). For DENV3 NS5-MTase protein, binding energies of the ligand ranged between -10.5 and -6.3 kcal/mol. The highest affinity, forming hydrogen bonds with His110, Glu111, Asp131, and Val132, and engaging in hydrophobic interactions with Lys105, Val132, Ile147, and Glu149. Baicalin also exhibited high binding energy (-10.4 kcal/mol) for DENV3 NS5-MTase, forming hydrogen bonds with Ser56, Arg84, Gly85, Gly86, Trp87, and Asp146, and interacting hydrophobically with Lys105, Val132, and Ile147. Additionally, it formed a π-Cation interaction with Lys180 (Figure 1C (a&b)). The overall analysis revealed that Baicalin consistently demonstrated strong affinity for the NS5-MTase protein across all DENV serotypes. The compound bound to the SAM binding site with high energy, favouring consistent hydrogen bonding and hydrophobic interactions with the same residues in S-Adenosyl-L-Methionine (SAM) binding site across all three serotypes (Table 1).

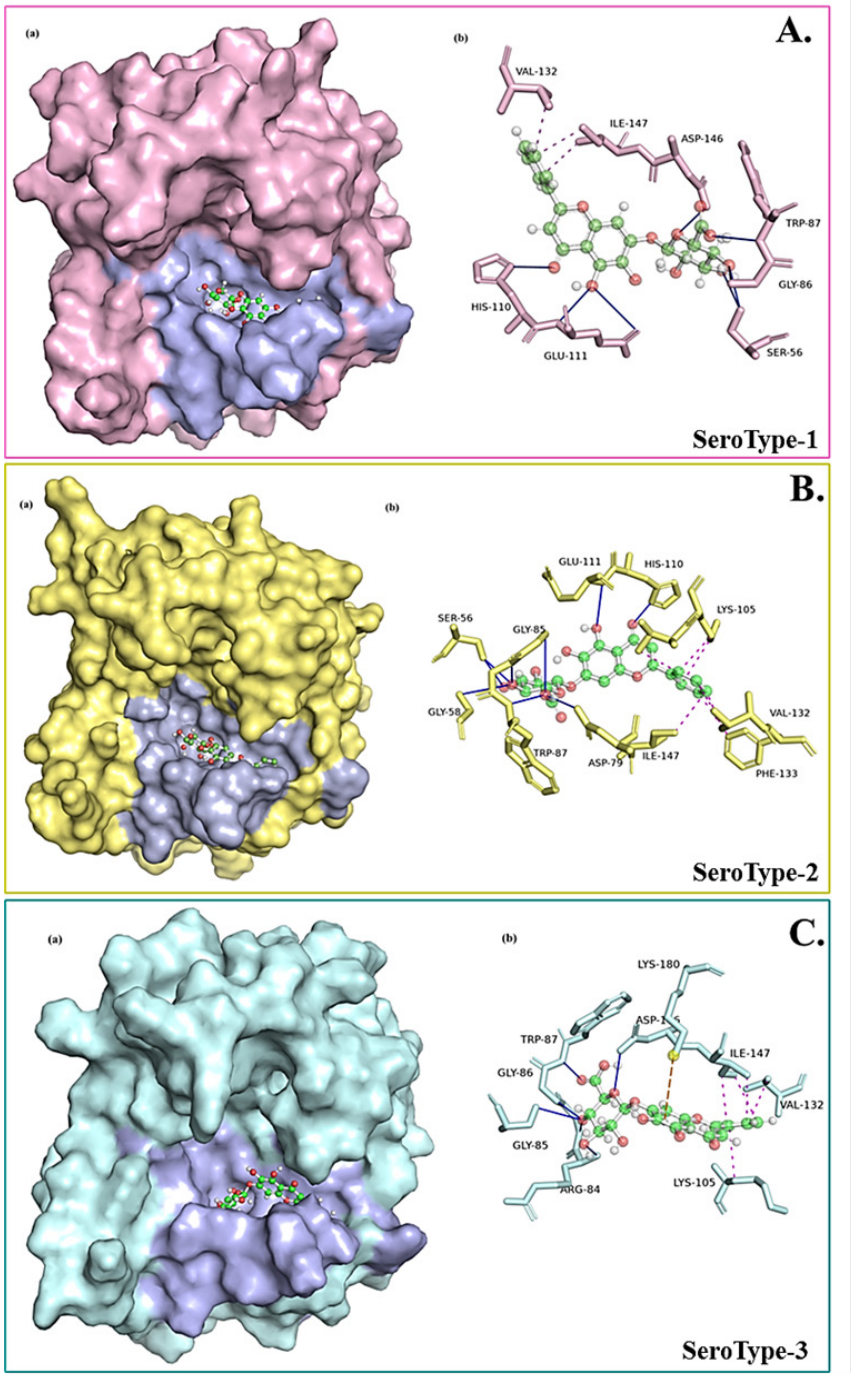

Figure 1: Molecular interaction of Baicalin with MTase. (A) SeroType-1(B) SeroType-2 (SeroType-3).

Baicalin as possible stable phytocompound against Dengue

Consistency in molecular interaction binding affinity as well as residual interaction suggest the Baicalin as the possible most significant binder of the MTase. To evaluate the consistency in molecular interaction, dynamics behaviour was evaluated through Molecular Dynamics Study. The NS5-MTase-Baicalin complexes were subjected to molecular dynamics simulation to analyse the flexibility and stability of the protein-ligand complexes. The changes in the complex structure and conformation were assessed for a simulation time frame of 100 ns through MD simulations. Different parameters were determined to understand the stability of the molecular trajectory, flexibility, ligand-receptor affinity and the extent of compactness and folding behaviour.

Root Mean Square deviation and Radius of gyration

RMSD is commonly employed to evaluate the dynamic stability of systems, serving as a comprehensive indicator of protein

fluctuations. In order to gauge the stability of Molecular Dynamics (MD) simulations of the DENV serotype protein-ligand complexes, the RMSD of backbone atoms was computed over time relative to the corresponding native structure. As evident from Figure 2A (a-c) the MD simulations of the protein-ligand systems have reached equilibrium after 50 ns in DENV1 and DENV3 complexes and around 75 ns in DENV2 complex, respectively. The overall RMSD values of the DENV serotype protein-ligand complexes fluctuated between an average of 0.11 nm to 0.33 nm. The ligand's Root Mean Square Deviation (RMSD) was computed throughout the simulation, providing insights into the stability of the ligand concerning both the protein and its binding pocket. The RMSD of the ligand converged and remained stable after 50 ns, however only minor drifts were seen before 50 ns in all the three complexes which was evident from Figure 2A (d) revealing the stability of the ligand binding within the binding pocket of the protein.

The radius of gyration (Rg) evaluates alterations in the compactness of a protein–ligand complex throughout the simulation period. The decline in compactness shows decrease in the stability of the complex, as it introduces weaker intermolecular bonds. The compactness of the DENV serotypes protein-ligand complexes were determined from their Rg calculated as a function of time during the simulations time period. The Rg plots Figure 2B (a-c) of the DENV serotypes protein-ligand complexes remained stable maintaining the fluctuations between 1.85 - 1.9 nm indicating the stable nature of the protein upon Baicalin binding. The Rg plot suggested that tight packing of the protein in all the DENV serotypes complex systems. During the simulation, the complex systems showed similar pattern Rg values, indicating there were no major changes in the overall structure and folding of the protein after ligand binding.

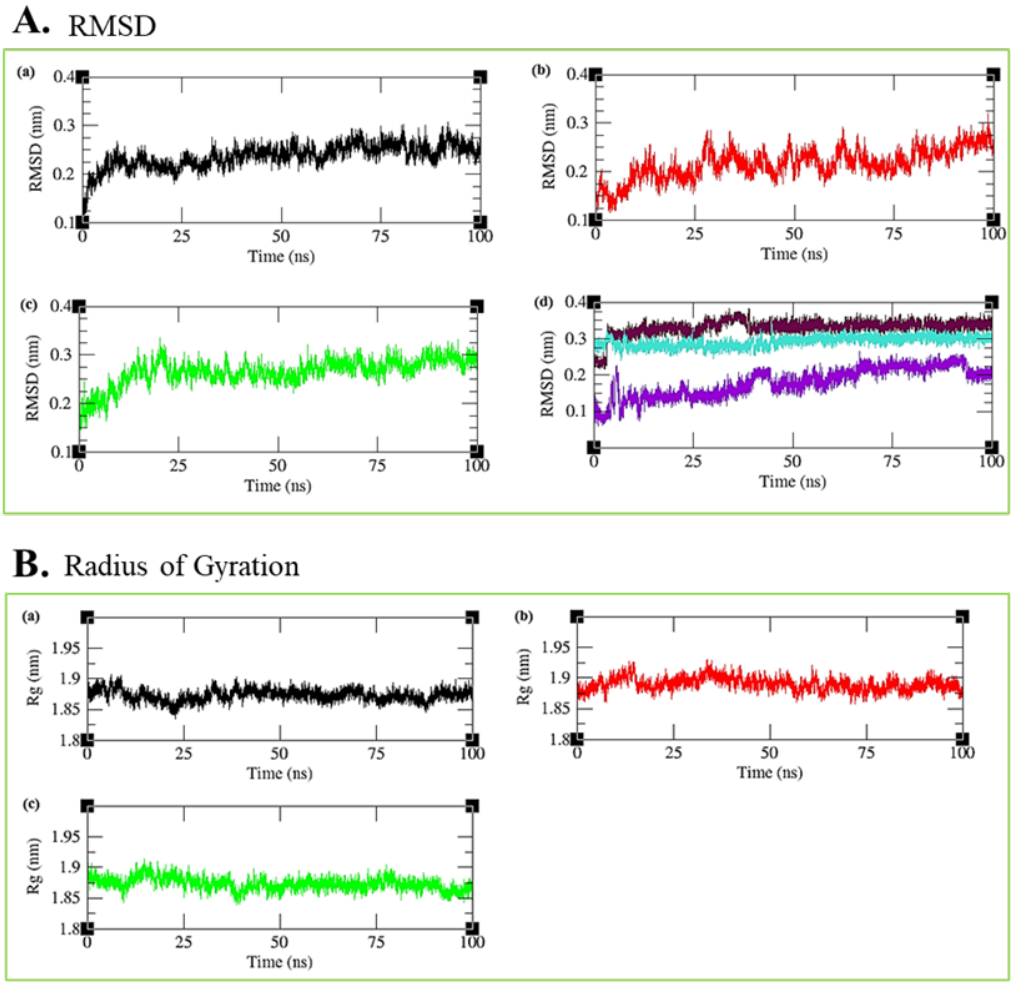

Figure 2: (A) RMSD, revealing the stability of the ligand binding within the binding pocket of the protein (a)-(c) protein RMSD and (d) Ligand RMSD. (a) DENV1 MTase protein (b) DENV2 MTase protein (c) DENV3 MTase protein (d) Violet - DENV1 complex, Maroon – DENV2 complex and Turquoise -DENV3 complex. (B) Radius of Gyration showing DENV serotypes protein-ligand complexes remained stable maintaining the fluctuations between 1.85 -1.9 nm indicating the stable nature of the protein upon Baicalin binding. (a) DENV1 (b) DENV2 (c) DENV3.

Table 1: Results of virtual screening of phytocompounds against NS5-MTase.

Phytocompounds	DENV1*	DENV2*	DENV3*
Quercitrin	-11.8	-8.9	-9.6
Myricetin	-10.5	-8.2	-9.1
Luteolin	-10.5	-8.3	-8.9
7-methoxyfavone	-10.0	-8.2	-7.8
Mycophenolic acid	-8.7	-7.1	-7.1
Betulinic acid	-9.7	-8.9	-8.1
Bigelovin	-10.1	-7.9	-8.1
Ineupatorolide A	-8.9	-8.0	-8.0
Lupeol	-9.9	-9.0	-9.1
Nimbin	-9.6	-8.3	-8.4
Ursolic acid	-10.0	-9.3	-9.3
Flinderole	-10.4	-9.4	-9.4
Hirsutine	-8.7	-7.5	-8.1
Isoborreverine	-10.0	-8.6	-8.4
Pungiolide	-11.2	-10.2	-9.8
Reserpic acid	-10.4	-8.8	-8.7
Tubulosine	-10.4	-8.8	-10.0
Avicularin	-9.9	-8.4	-8.9
Baicalin	-12.5	-10.7	-10.4
Catechin	-9.7	-7.8	-8.5
Quercetin	-10.6	-8.4	-9.2
Fucoidan	-6.5	-6.3	-6.3
Glabranine	-10.1	-8.2	-9.0
Hyperoside	-10.7	-8.6	-9.1
4-hydroxypanduratin A	-8.7	-8.2	-7.8
Panduratin A	-9.0	-8.3	-7.7
Zosteric acid	-7.4	-6.4	-6.3
2',3,4,4',6'-pentamethoxychalcone	-8.3	-7.2	-7.5
3,3',4,5'-Tetrahydroxy-5-prenylbibenzyl	-9.9	-8.5	-8.7
3,3',5'-Trihydroxy-4-methoxy-5-prenylbibenzyl	-9.9	-8.0	-8.8
Lignan	-7.5	-6.4	-6.8
Warfarin	-10.1	-8.0	-8.5
Silandrin	-11.7	-9.7	-10.2
Silibinin	-12.0	-9.4	-10.5
Terrestriamide	-9.2	-8.0	-8.1
Rhinacanthin K	-10.4	-8.7	-9.1
Macarangaflavanone B	-11.8	-9.3	-10.2
Kanzonol Y	-10.1	-7.8	-8.8
Isosilybin B	-11.8	-9.6	-9.6
Hispaglabridin A	-11.6	-9.1	-9.7
Furost-20(22)-ene-2,3,26-triol	-10.1	-9.3	-9.3

Phytocompounds	DENV1*	DENV2*	DENV3*
Diplacone	-10.8	-9.3	-9.5
Curcumin	-9.9	-7.8	-8.4
Chandalone	-12.0	-10.2	-10.7

*kcal/mol.

Table 2: MM-PBSA contributing energies and binding energy.

Complex	van der Waal energy (kJ/mol)	Electrostatic energy (kJ/mol)	Polar solvation energy (kJ/mol)	SASA energy (kJ/mol)	Binding energy (kJ/mol)
DENV1-Baicalin	-101.467 ± 35.567	-338.125 ± 19.842	409.280 ± 23.790	-21.337 ± 0.905	-51.649 ± 31.001
DENV2-Baicalin	-85.519 ± 23.773	-264.800 ± 41.747	333.883 ± 59.319	-18.142 ± 1.539	-34.578 ± 30.280
DENV3-Baicalin	-121.198 ± 17.717	-331.391 ± 36.112	405.744 ± 18.527	-18.828 ± 0.787	-65.673 ± 13.793

Root Mean Square Fluctuation and Essential dynamics

The Root Mean Square Fluctuations (RMSF) of Cα atoms offer direct insights into the structural variability and flexibility of proteins. The residues involved in the hydrogen bonding and hydrophobic interactions showed very less fluctuations. Two residues Gly48 (0.26 nm, 0.30 nm and 0.35 nm) and Gly109 (0.35 nm, 0.5 nm and 0.32) located closer to the interacting residues reported highest residual fluctuation in all the three DENV serotype protein-ligand complexes, respectively. In addition, the residues located in the loop region also reported high fluctuations in all the three protein-ligand complexes (Figure 3A. a-c).

For a more comprehensive understanding and deeper insights into the directional flexibilities of residue, Principal Component Analysis (PCA) was carried out. This analytical approach delineates the overall motions of the Cα atoms within the protein, as indicated by the eigenvectors derived from the covariance matrix. The primary advantage of focusing on the first principal components lies in their ability to capture a substantial portion of the variance present in the data, thereby facilitating the observation of dominant motions within the protein-ligand system. In comparison to the DENV3 protein-ligand complex, the protein-ligand complexes involving DENV1 and DENV2 demonstrated a more extensive exploration of conformational space. The overall analysis underscored that the DENV1 and DENV2 complex systems displayed increased conformational variability and notable motional changes, suggesting a diminished stability in the presence of Baicalin binding (Figure 3B. a-c).

DCCM, SASA and Intermolecular Hydrogen Bonding analysis

To further explore the impact of ligand binding on conformational dynamics, Dynamic Cross-Correlation Matrix (DCCM) analysis was employed to investigate Cα atom fluctuations over the course of a 100-ns Molecular Dynamics (MD) simulation. The analysis focused on examining the correlated motions within each DENV serotype protein-ligand system. Notably, the DENV2 protein-ligand complex exhibited more robust positive correlations in comparison to the other complexes. Conversely, the other two DENV serotype complex systems displayed a combination of both positive and negative correlations. Analysis of principal components and dynamic cross-correlation maps revealed that ligand binding induced significant alterations in the primary motions of the proteins. These findings underscore the notable impact of ligand binding on the conformational flexibility of proteins (Figure 4A. a-c).

The analysis of Solvent Accessible Surface Area (SASA) was employed to quantify the exposure of amino acid residues to the surrounding environment. A higher SASA value signifies a more diffuse protein structure, whereas a lower SASA value indicates a more compact structure. Variations in SASA values indicate alterations in the protein's structural conformation upon ligand binding. The SASA values of the DENV serotypes protein-ligand complexes were scrutinized to anticipate the impact of ligand binding on protein structure. The average SASA values were 135.482 nm^2, 138.625 nm^2, and 136.250 nm^2 for DENV1, DENV2, and DENV3 protein-ligand complexes, respectively. Examination of SASA plots across all systems revealed that the proteins maintained a consistently compact and composed structure, as evidenced by stable and convergent SASA plots (Figure 4B. a-c). Similar to the Radius of gyration (Rg) analysis, no significant differences were observed among the DENV serotypes protein-ligand complexes, indicating a consistent effect on SASA profiles.

The binding affinity of Baicalin with NS5-MTAse protein can be ascertained by hydrogen bond formation. DENV1 NS5-MTase-Baicalin complex maintained minimum of 7 and maximum of 13 hydrogen bonds. The DENV2 and DENV3 NS5-MTase-Baicalin

complex maintained minimum of 1 and maximum of 12 and 13 hydrogen bonds, respectively, throughout the simulation time (Figure 4C. a-c). The Hydrogen bond analysis indicated that the Baicalin remained within the binding site and maintained the interactions with the binding site residues.

MM-PBSA binding free energy

One of the widely accepted methods for estimation of binding free energy of small ligands with biological macromolecules is Molecular Mechanics Poisson Boltzmann Surface Area continuum solvation (MM-PBSA). The comparison of energy contributors revealed that electrostatic energy term contributed more, followed by van der Waals energy in the binding of Baicalin with the NS5-MTase protein. The contribution of SASA energy was smaller, indicating the compact nature of the protein-ligand complex. The MM-PBSA binding energy comparison indicated that DENV3 NS5-MTase Baicalin complex showed higher binding energy compared to other serotypes (Table 2).

Activity Prediction for Baicalin

The initial data curation identified a total of 46 anti-NS5-MTase inhibitors, collected from diverse databases and literature sources (Table 3). Molecular descriptors were calculated using PaDEL Descriptor software, resulting in 1444 descriptors. To enhance model reliability, we employed correlation analysis for feature selection, identifying five relevant descriptors: JGI4, SpMin4_Bhm, SpMin5_Bhi, SaaN, and n5Ring. However, due to inter-descriptor correlation issues, a subset of three independent

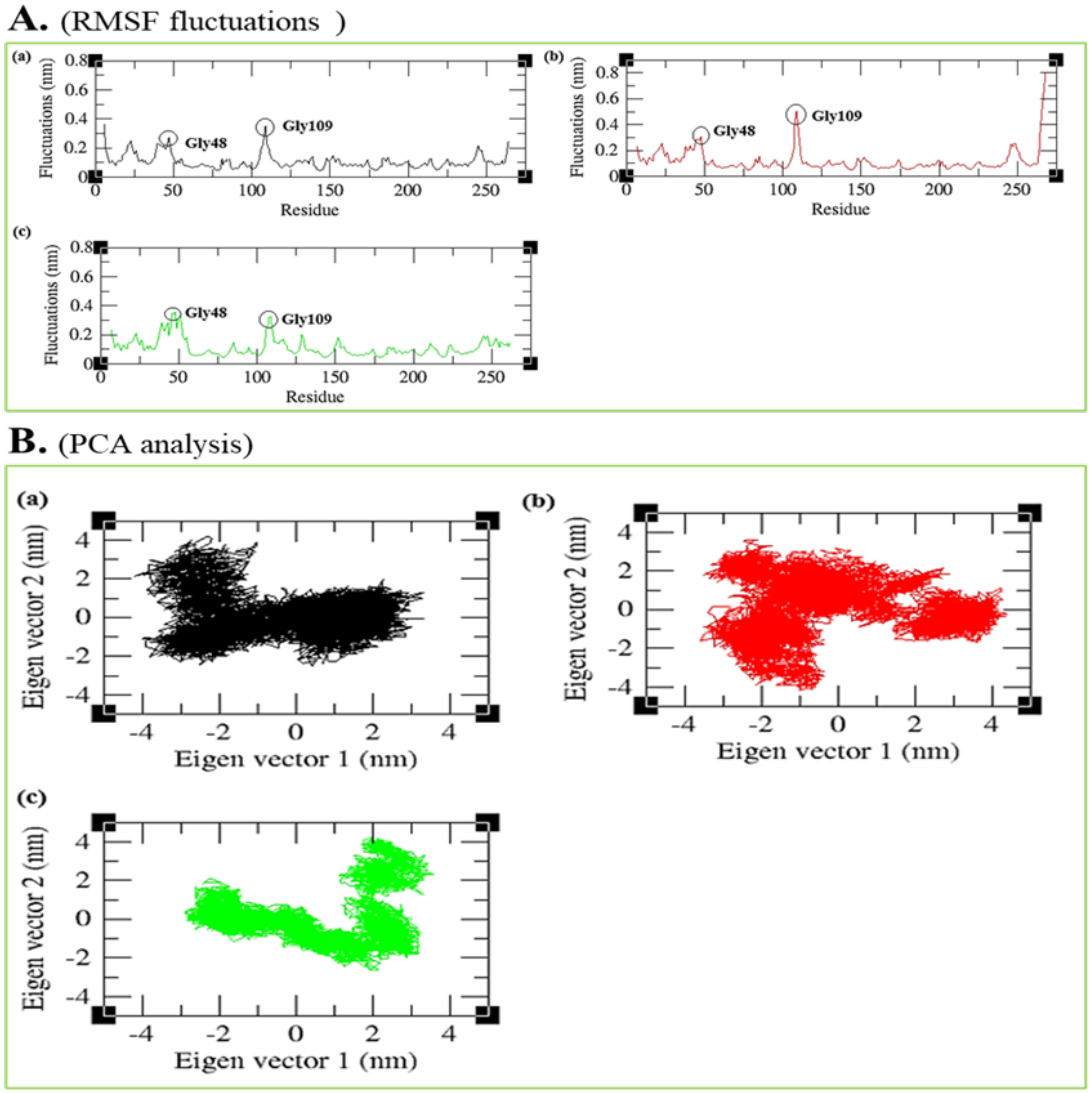

Figure 3: (A) RMSF fluctuations showing two residues Gly48 (0.26 nm, 0.30 nm and 0.35 nm) and Gly109 (0.35 nm, 0.5 nm and 0.32) located closer to the interacting residues reported highest residual fluctuation in all the three DENV serotype protein-ligand complexes. (a) DENV1 (b) DENV2 (c) DENV3. (B) PCA analysis showed that the DENV1 and DENV2 complex systems displayed increased conformational variability and notable motional changes, suggesting a diminished stability in the presence of Baicalin binding. (a) DENV1 (b) DENV2 (c) DENV3.

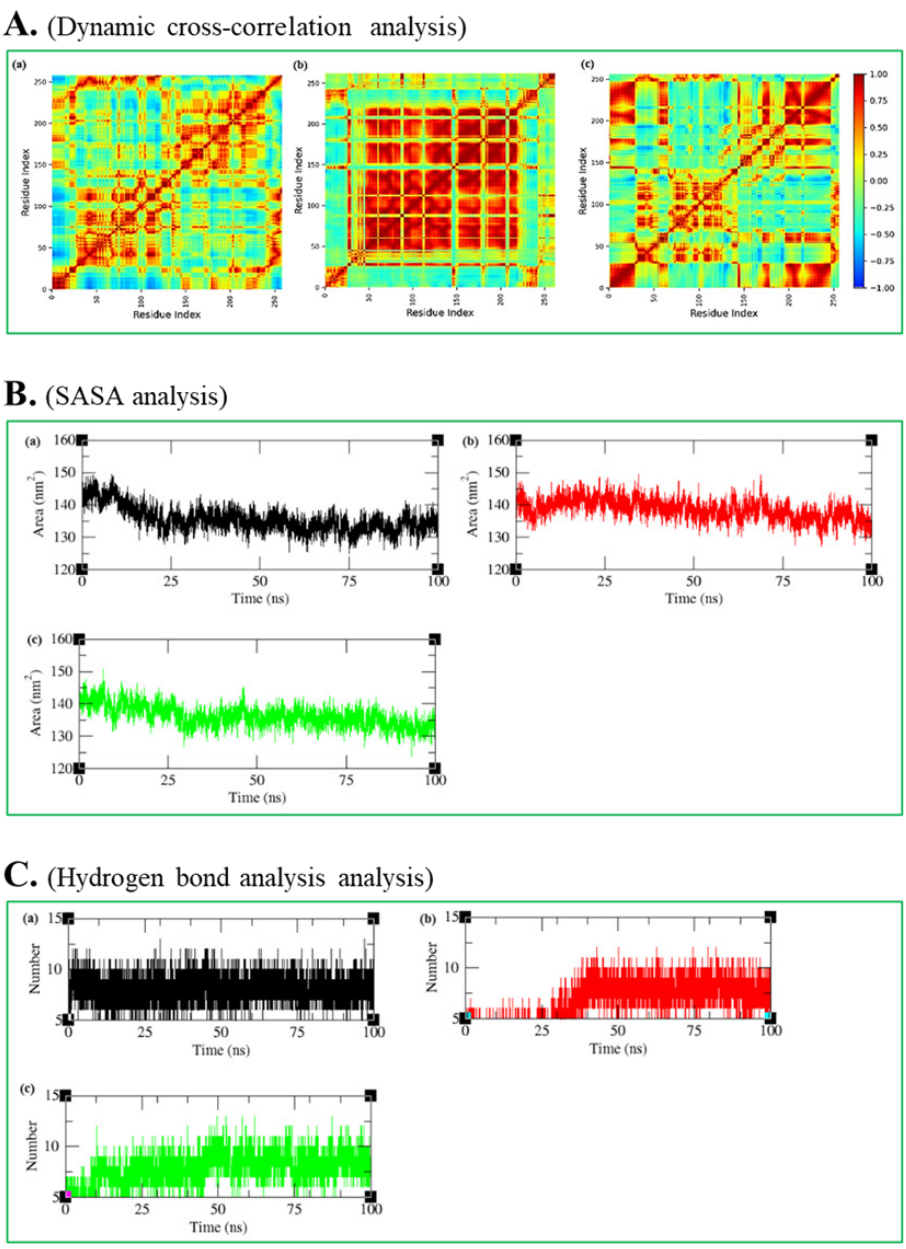

Figure 4: (A) Dynamic cross-correlation analysis of principal components revealed that ligand binding of Baicalin induced significant alterations in the primary motions of the proteins. (a) DENV1 (b) DENV2 (c) DENV3. (B) SASA showed that similar to the Radius of Gyration (Rg) analysis, no significant differences were observed among the DENV serotypes protein-ligand complexes, indicating a consistent effect on SASA profiles. (a) DENV1 (b) DENV2 (c) DENV3. (C) Hydrogen bond analysis indicated that the Baicalin remained within the binding site and maintained the interactions with the binding site residues. (a) DENV1 (b) DENV2 (c) DENV3.

Table 3: Correlation matrix of 05 descriptors along with dependent variable.

	*p*Value	JGI4	SpMin4_Bhm	SpMin5_Bhi	SaaN	n5Ring
pValue	1					
JGI4	-0.60261	1				
SpMin4_Bhm	0.617613	-0.55668	1			
SpMin5_Bhi	0.550097	-0.59202	0.850849	1		
SaaN	0.50301	-0.40245	0.224399	0.108577	1	
n5Ring	0.485893	-0.31665	0.436494	0.307249	0.588496	1

descriptors (JGI4, SpMin4_Bhm, and SpMin5_Bhi) was selected for model development.

To ensure model robustness, three different regression models were developed using Linear Regression in WEKA, each trained with a separate descriptor to avoid collinearity. The dataset was randomly split using an 80:20 ratio, with 80% of data used for training (24, 25, and 26 samples, respectively) and 20% reserved for independent validation (5 samples per model) (Figure 5).

The performance of each QSAR model was assessed using multiple statistical metrics:

- Coefficient of Determination (R^2) to evaluate goodness-of-fit.
- Mean Absolute Error (MAE) and Root Mean Squared Error (RMSE) to measure prediction accuracy.
- Leave-One-Out Cross-Validation (LOO-CV) for internal validation.

Table 4 presents a detailed comparison of the three models.

The model details are as follows:

- **Model 1:** *p*-Value = -73.3449 * JGI4 + 10.478
- **Model 2:** *p*-Value = 4.0315 * SpMin4_Bhm + 1.4651
- **Model 3:** *p*-Value = 2.2233 * SpMin5_Bhi + 4.7174

The predicted IC_{50} value of Baicalin was derived from the three QSAR models, yielding an average predicted activity of 76 nM (Table 5). The performance metrics of the models confirmed their predictive reliability, with the following key findings:

- **Model 1:** R^2 = 0.742, RMSE = 0.539, MAE = 0.462
- **Model 2:** R^2 = 0.989, RMSE = 0.381, MAE = 0.329
- **Model 3:** R^2 = 0.481, RMSE = 0.629, MAE = 0.548

The low RMSE and MAE values across all models suggest minimal prediction error, particularly in Model 2, which demonstrated the highest correlation (R^2 = 0.989). The external validation dataset exhibited consistent performance, reinforcing the model's ability to generalize to unseen compounds. Overall, these results validate the QSAR framework used to predict Baicalin's activity against NS5-MTase.

Target-specific molecular interaction studies and Quantitative Structure-Activity Relationship (QSAR) activity prediction are foundational for pre-clinical studies, therefore used for estimation of performance here. Overall, multi-serotype screening performed a comprehensive analysis to screening small molecule exhibited s for MTase inhibition activity, providing valuable insights into compound efficacy, resistance potential, and clinical relevance. This approach also maximized the likelihood of identifying promising drug candidates with broad-spectrum activity and therapeutic potential against infectious diseases. These studies allowed us to efficiently identify promising lead compounds by assessing their interactions with MTase and predicting their biological activity. This helped us to prioritize compounds with the highest likelihood of success for further pre-clinical evaluation. Molecular interaction studies elucidated how compounds interact with their intended targets at a molecular level. This understanding is crucial for determining the mechanism of action of a compound, which informs subsequent pre-clinical and clinical investigations. QSAR activity prediction enabled us to find out the structural feature responsible for activity against the MTase. By guiding compound selection, molecular interaction studies and QSAR activity prediction helped us to streamline the lead discovery process. In summary, target-specific molecular interaction studies and QSAR activity prediction served as essential tools for guiding compound selection, optimizing compound design, assessing safety, and expediting the drug discovery process, making them indispensable components of pre-clinical studies. Considering all these aspects, it can be concluded that Baicalin as possible natural

Table 4: Statistics of 03 different models with 03 different descriptors.

	Model1 with 'JGI4'			Model2 with 'SpMin4_Bhm'			Model3 with 'SpMin5_Bhi'		
	$R^2_{Training}$	R^2_{LOO}	R^2_{Test} (80:20)	$R^2_{Training}$	R^2_{LOO}	R^{-2}_{Test} (80:20)	$R^2_{Training}$	R^2_{LOO}	R^2_{Test} (80:20)
Regression Coefficient	0.675	0.623	0.742	0.860	0.835	0.989	0.752	0.726	0.481
Mean Absolute Error	0.378	0.419	0.426	0.291	0.315	0.072	0.398	0.423	0.239
Root Mean Squared Error	0.465	0.510	0.629	0.351	0.381	0.086	0.466	0.490	0.263
Relative Absolute Error	0.594	0.631	0.370	0.381	0.396	0.116	0.532	0.544	0.587
Root Relative squared error	0.570	0.598	0.443	0.375	0.391	0.115	0.498	0.503	0.579
Number of samples	24	24	5	25	25	5	26	26	5

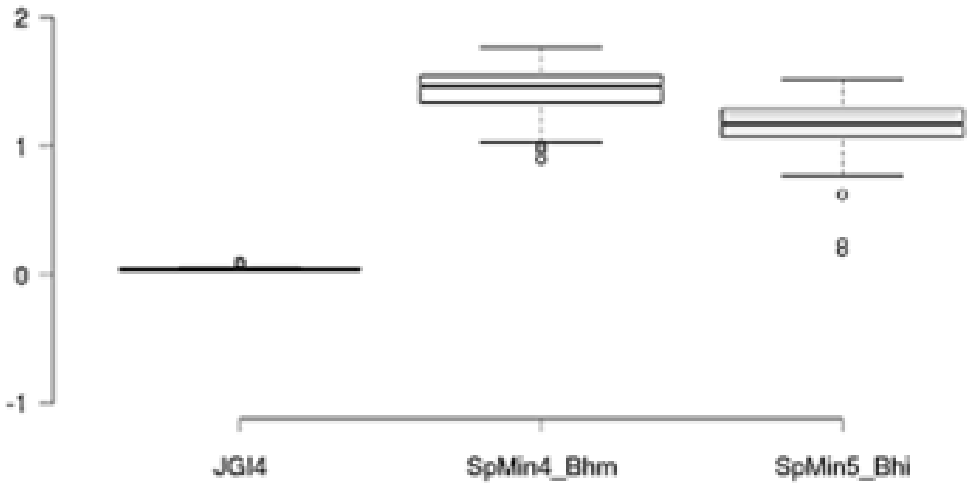

Figure 5: Box-plot showing data distribution of 03 descriptors used for model development.

molecule showing intense interaction at molecular level good activity of calculated IC_{50} of 76 nM against MTase. Calculated IC_{50} (nM) of Baicalin was found better than the known natural molecules Quercetin. Lead optimization is the subject for further research.

The results of this study suggest that Baicalin is the most promising lead compound due to its higher binding affinity, superior hydrogen bonding profile, and greater structural stability compared to other flavonoids. Baicalin displayed binding energies significantly lower (more negative) than Quercetin, Myricetin, and Luteolin, which are known inhibitors of viral methyltransferases.

Key factors contributing to Baicalin's superior inhibitory potential include:

- Stronger hydrogen bonding interactions: Baicalin forms multiple stable hydrogen bonds with the SAM binding site residues (His110, Glu111, Asp146, Val132, Ile147), whereas Quercetin and Myricetin showed fewer hydrogen bond interactions.
- Higher predicted inhibition potency: The QSAR-predicted IC_{50} value for Baicalin (76 nM) is lower than that of Quercetin (~110 nM), suggesting higher inhibition efficiency.
- Molecular Dynamics (MD) stability: RMSD and Radius of gyration (Rg) analyses indicate that Baicalin-bound complexes exhibit lower structural fluctuations, meaning it maintains stable binding to the NS5-MTase pocket.

Given these findings, Baicalin represents a strong candidate for further experimental validation, including *in vitro* enzyme inhibition assays and pharmacokinetic evaluations to confirm its antiviral potential.

Table 5: Predicted Activity of Baicalin.

Model	Predicted Activity *p*Value of 'Baicalin'
Model1 (JGI4)	6.655
Model2 (SpMin4_Bhm)	7.352
Model3 (SpMin5_Bhi)	7.351
Average	7.119
Calculated IC_{50} (nM) of 'Baicalin'	76.009

DISCUSSION

As a probable treatment selection for dengue fever, the interaction between phytocompounds & the multi-serotype Dengue Virus's (DENV) NS5 Methyltransferase (MTase) has attracted a lot of consideration. Through catalyzing the methylation of viral RNA, which is necessary for the synthesis, stability, & translation of viral caps, the NS5 MTase plays a key part in the replication. The binding affinity of phytocompounds to NS5 MTase is perilous in decisive their effectiveness as inhibitors. Molecular docking studies & *in silico* simulations have providing valuable perceptions into how numerous phytochemicals interact through the NS5 MTase. For illustration, flavonoids such as quercetin and kaempferol have demonstrated durable binding affinities to the MTase active site, often through hydrogen bonding, π-π stacking, & hydrophobic interactions. These interactions can lead to the inhibition of RNA methylation, effectively preventing viral replication.[28]

Moreover, approximately terpenoids, like betulinic acid, have been originate to bind to the S-Adenosylmethionine (SAM)-binding pocket of the enzyme, rival with the natural substrate for binding. This competitive inhibition principals to reduced MTase activity, which can meaningfully impair the virus's ability to produce infectious particles. The potential of these compounds as

dual inhibitors, affecting both the viral enzyme and host cellular pathways, has been emphasised in numerous studies, suggesting a multifaceted mechanism of action.[29]

Nevertheless, current studies have revealed that confident phytocompounds can inhibit NS5 MTase athwart several serotypes, signifying their broad-spectrum antiviral potential. For example, flavonoids like rutin and catechin have been shown to effectively inhibit DENV-2 & DENV-4 NS5 MTase in investigational assays, through some compounds demonstrating cross-serotype efficacy. This broad-spectrum activity is particularly valuable, as it could deliver a treatment option for infections caused by different DENV serotypes (Serotype 1, 2 and 3).[30-32] Despite the fact the inhibitory effects of phytocompounds on NS5 MTase are promising, several challenges remain in translating these findings into clinical applications.[33]

LIMITATIONS & FUTURE DIRECTIONS

Despite the promising insights provided by this study, several limitations must be acknowledged.

First, the exclusion of DENV4 and DENV5 from the analysis was due to the lack of high-resolution crystallographic or cryo-EM structures of their NS5-MTase proteins. Since molecular docking and Molecular Dynamics (MD) simulations rely on accurate structural data, including unreliable or low-resolution models could compromise the validity of the predictions. Future studies should consider employing homology modeling to predict the three-dimensional conformations of DENV4 and DENV5 NS5-MTase, enabling their inclusion in computational and experimental screening efforts.

Second, this study is based entirely on computational methods, including molecular docking, QSAR modeling, and MD simulations, without experimental validation. While computational approaches provide valuable insights into molecular interactions, *in vitro* enzyme inhibition assays and cell-based experiments remain necessary to confirm the predicted inhibitory effects of Baicalin and other phytocompounds. Future work should incorporate experimental techniques to validate the binding affinities, structural interactions, and pharmacokinetic properties of the identified inhibitors.

Finally, while the QSAR model used in this study has been externally validated and tested against independent datasets, further improvements could be made by expanding the training dataset and integrating more diverse chemical scaffolds. Additionally, pharmacokinetics and toxicity assessments should be conducted to ensure the clinical viability of Baicalin as a potential antiviral agent against multiple DENV serotypes.

By addressing these limitations, future research can further enhance the reliability and applicability of computationally identified inhibitors, advancing the development of broad-spectrum anti-dengue therapeutics.

Importance of the Research and Current Trends

Significance of Dengue Virus NS5 Methyltransferase (MTase) as a Therapeutic Target

The study of NS5 Methyltransferase (MTase) in Dengue Virus (DENV) has become increasingly crucial due to the rising global incidence of mosquito-borne diseases and the current lack of effective antiviral treatments. Each year, millions of individuals contract dengue fever, necessitating the exploration of novel therapeutic strategies. The highly conserved nature of the NS5 protein, which is essential for viral replication and immune evasion, makes it a compelling target for broad-spectrum antiviral drug development.[33]

Computational Drug Discovery and the Potential of Isoquercitrin

This research underscores the potential of Isoquercitrin, a naturally occurring flavonoid, as a potent inhibitor of NS5 MTase. The molecular properties and pharmacokinetic profile of this compound have been analyzed using advanced computational techniques, including molecular docking and Density Functional Theory (DFT).[34] Previous docking studies, such as those identifying inhibitors for Japanese Encephalitis Virus proteins, further validate the reliability of these computational approaches in antiviral drug discovery.[35]

Insights from gene expression profiling and functional annotation of dengue virus proteins have significantly contributed to understanding the molecular mechanisms governing viral pathogenicity. These studies serve as a robust framework for designing targeted antiviral interventions[36]. Moreover, research investigating methyltransferase activity in DENV RNA viruses has reinforced the critical role of this enzyme in viral replication, solidifying its therapeutic potential as a drug target.[37]

Structural and Biomechanical Perspectives in Antiviral Research

Current Trends in Antiviral Drug Discovery: Emerging Trends in Dengue Virus (DENV) Drug Development> Integration of Computational and Experimental Techniques.

The synergistic integration of computational and experimental approaches has revolutionized modern drug discovery. Advanced computational methodologies, including Density Functional Theory (DFT), molecular docking, and Molecular Dynamics (MD) simulations, enable rapid identification and optimization of potential drug candidates. These techniques have been successfully applied in studies targeting DENV NS5 MTase as well as other viral proteases, such as SARS-CoV-2 main protease, demonstrating their broad applicability in antiviral research.[36]

Natural Products as Promising Antiviral Agents

Natural compounds, particularly flavonoids such as Isoquercitrin, have attracted considerable interest due to their antiviral,

anti-inflammatory, and antioxidant properties. Their ability to selectively bind conserved viral proteins makes them promising candidates for the development of broad-spectrum antiviral therapies. Investigations into these bioactive molecules have reinforced their potential role in targeting essential viral enzymes and minimizing viral resistance.[37]

Advancements in Methyltransferase Research and Molecular Docking

Recent studies exploring the methyltransferase activity of DENV RNA viruses using purified proteins have underscored the pivotal role of this enzyme in viral replication and pathogenesis. The findings from these studies provide a foundation for the development of highly selective inhibitors, offering a new therapeutic avenue for dengue virus treatment.[38,39]

AI-Driven Drug Discovery and Computational Optimization

The integration of Artificial Intelligence (AI) and Machine Learning (ML) in drug discovery has significantly enhanced docking algorithms, protein-ligand interaction predictions, and structure-based drug design. These computational tools not only accelerate the screening process for potential drug candidates but also optimize their pharmacokinetic and pharmacodynamic properties, improving the efficiency of drug repurposing and lead compound selection.[40]

Microtubule Biomechanics and Drug Design

The mechanical properties of protein microtubules have emerged as a novel consideration in antiviral drug design. Research employing nonlocal elasticity theory has provided critical insights into how antiviral agents interact with cytoskeletal components to modulate viral replication. Furthermore, investigations into thermal fluctuations, vibrational properties, and microtubule instability have revealed new opportunities for designing drugs that target microtubule-associated viral processes without disrupting normal cellular functions.[41]

Structural analyses of protein microtubules at the nanoscale level provide valuable insights into cellular mechanics and drug interactions. The investigation of microtubule buckling and vibrational properties aids in designing drugs that selectively inhibit viral replication while preserving cellular integrity.[42] Additionally, research on thermal stress effects on microtubules has enhanced our understanding of how cellular mechanics are influenced under disease conditions, potentially informing future antiviral therapeutic strategies.[43]

FUTURE IMPLICATIONS

Future Directions and Implications in Antiviral Drug Development

The findings of this study establish a strong scientific foundation for future preclinical and clinical investigations targeting DENV NS5 Methyltransferase (MTase). The favorable binding affinity and pharmacokinetic properties of Isoquercitrin position it as a promising candidate for further exploration. Moreover, the integration of computational approaches with experimental validation enhances the efficiency and cost-effectiveness of drug discovery pipelines, ensuring a streamlined transition from *in silico* predictions to *in vitro* and *in vivo* evaluations.

The continued exploration of microtubule interactions, thermal stress effects, and the role of natural compounds in antiviral therapies offers new opportunities for developing broad-spectrum antiviral strategies. These areas of research may lead to novel drug formulations that effectively inhibit viral replication while minimizing cytotoxic effects, particularly for mosquito-borne flaviviruses such as DENV, Zika, and Chikungunya.

This study underscores the therapeutic significance of targeting NS5 methyltransferase as a strategy for combating dengue virus infections. By leveraging bioactive natural products like Isoquercitrin and employing state-of-the-art computational methodologies, researchers are advancing the field of antiviral drug discovery. The integration of molecular docking, Density Functional Theory (DFT), and experimental validation provides a comprehensive and multidisciplinary approach to tackling global health threats posed by dengue and other flaviviruses. These advancements not only reinforce current antiviral research efforts but also lay the groundwork for future breakthroughs in drug development.

CONCLUSION

In this study, phytocompounds were systematically evaluated for their potential interaction with NS5 Methyltransferase (MTase) across multiple Dengue Virus (DENV) serotypes. The approach incorporated molecular docking, Molecular Dynamics (MD) simulations, and Quantitative Structure-Activity Relationship (QSAR) modeling to assess binding affinity and predicted inhibitory activity. Among the screened compounds, Baicalin emerged as the most promising candidate, exhibiting a highly stable binding affinity across DENV1, DENV2, and DENV3 and an IC_{50} value of 76.0 nM against MTase. These findings support the potential of Baicalin as a broad-spectrum antiviral agent targeting DENV NS5-MTase, warranting further preclinical validation and pharmacokinetic assessments.

ACKNOWLEDGEMENT

This project was funded by the Deanship of Scientific Research (DSR) at King Abdulaziz University, Jeddah, under grant No. (G-511-662- 1443). The authors, therefore, acknowledge with thanks DSR for technical and financial support.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest

ABBREVIATIONS

DENV: Dengue virus; **MTase:** Methyltransferase; **QSAR:** Quantitative Structure Activity Relationship; **CID:** Compound; **SAM:** S-adenosyl-L-methionine; **MD:** Molecular Dynamics; **Rg:** Radius of gyration; **SASA:** Solvent Accessible Surface Area; **RMSD:** Root Mean Square Deviation; **RMSF:** Root Mean Square Fluctuations.

SUMMARY

This study presents a comprehensive computational analysis of phytocompounds targeting DENV NS5-MTase, integrating molecular docking, MD simulations, and QSAR techniques. Baicalin, a flavonoid compound, exhibited high binding affinity and strong molecular interactions across major DENV serotypes, reinforcing its therapeutic potential. With an IC_{50} value of 76.0 nM, Baicalin demonstrates promising inhibitory activity against NS5-MTase, a critical enzyme in DENV replication. These results lay the groundwork for future experimental validation and potential drug development efforts.

REFERENCES

1. Verma P, Baskey U, Choudhury KR, Dutta S, Bakshi S, Das R, *et al.* Changing pattern of circulating dengue serotypes in the endemic region: an alarming risk to the healthcare system during the pandemic. J Infect Public Health. 2023; 16(12): 2046-57. doi: 10.1016/j.jiph.2023.10.014, PMID 37944366.
2. Sah R, Siddiq A, Padhi BK, Mohanty A, Rabaan AA, Chandran D, *et al.* Dengue virus and its recent outbreaks: current scenario and counteracting strategies. Int J Surg. 2023; 109(9): 2841-45. doi: 10.1097/JS9.0000000000000045, PMID 36906765.
3. Rocklöv J, Tozan Y. Climate change and the rising infectiousness of dengue. Emerg Top Life Sci. 2019; 3(2): 133-42. doi: 10.1042/ETLS20180123, PMID 33523146.
4. Ten Bosch QA, Clapham HE, Lambrechts L, Duong V, Buchy P, Althouse BM, *et al.* Contributions from the silent majority dominate dengue virus transmission. PLOS Pathog. 2018; 14(5): e1006965. doi: 10.1371/journal.ppat.1006965, PMID 29723307.
5. Stanley SM, Khera HK, Chandrasingh S, George CE, Mishra RK. A comprehensive review of dengue with a focus on emerging solutions for precision and timely detection. Int J Biol Macromol. 2024; 254(1): 127613. doi: 10.1016/j.ijbiomac.2023.127613, PMID 37875186.
6. Burley SK, Bhikadiya C, Bi C, Bittrich S, Chao H, Chen L, *et al.* RCSB Protein Data bank: tools for visualizing and understanding biological macromolecules in 3D. Protein Sci Publ Protein Soc. 2020; 31(12): e4482. doi: 10.1002/pro.4482, PMID 36281733.
7. Dwivedi VD, Tripathi IP, Tripathi RC, Bharadwaj S, Mishra SK. Genomics, proteomics and evolution of dengue virus. Brief Funct Genomics. 2017; 16(4): 217-27. doi: 10.1093/bfgp/elw040, PMID 28073742.
8. Bitencourt-Ferreira G, de Azevedo Jr. WF. Molecular docking simulations with ArgusLab. Methods Mol Biol. 2019; 2053: 203-20. doi: 10.1007/978-1-4939-9752-7_13, PMID 31452107.
9. Benmansour F, Trist I, Coutard B, Decroly E, Querat G, Brancale A, *et al.* Discovery of novel dengue virus NS5 methyltransferase non-nucleoside inhibitors by fragment-based drug design. Eur J Med Chem. 2017; 125: 865-80. doi: 10.1016/j.ejmech.2016.10.007, PMID 27750202.
10. Dwivedi VD, Arya A, Yadav P, Kumar R, Kumar V, Raghava GP. DenvInD: dengue virus inhibitors database for clinical and molecular research. Brief Bioinform. 2021; 22(3): bbaa098. doi: 10.1093/bib/bbaa098, PMID 32510549.
11. Lim SP, Noble CG, Shi PY. The dengue virus NS5 protein as a target for drug discovery. Antiviral Res. 2015; 119: 57-67. doi: 10.1016/j.antiviral.2015.04.010, PMID 25912817.
12. Lim SP, Sonntag LS, Noble C, Nilar SH, Ng RH, Zou G, *et al.* Small molecule inhibitors that selectively block dengue virus methyltransferase. J Biol Chem. 2011; 286(8): 6233-40. doi: 10.1074/jbc.M110.179184, PMID 21147775.
13. Podvinec M, Lim SP, Schmidt T, Scarsi M, Wen D, Sonntag LS, *et al.* Novel inhibitors of dengue virus methyltransferase: discovery by *in vitro*-driven virtual screening on a desktop computer grid. J Med Chem. 2010; 53(4): 1483-95. doi: 10.1021/jm900776m, PMID 20108931.
14. Ullah A, Atia-tul-Wahab, Gong P, Mateen Khan A, and Iqbal Choudhary M. Identification of new inhibitors of NS5 from dengue virus using saturation transfer difference (STD-NMR) and molecular docking studies. RSC Adv. 2023; 13(1): 355-69. doi: 10.1039/D2RA04836A, PMID 36605638.
15. Chavda VP, Patel AB, Vihol D, Vaghasiya DD, Ahmed KM, Trivedi KU, *et al.* Herbal remedies, nutraceuticals, and dietary supplements for COVID-19 management: an update. Clin Complement Med Pharmacol. 2022; 2(1): 100021. doi: 10.1016/j.ccmp.2022.100021, PMID 36620357.
16. Loe MW, Hao E, Chen M, Li C, Lee RC, Zhu IX, *et al.* Betulinic acid exhibits antiviral effects against dengue virus infection. Antiviral Res. 2020; 184: 104954. doi: 10.1016/j.antiviral.2020.104954, PMID 33080251.
17. Moghaddam E, Teoh BT, Sam SS, Lani R, Hassandarvish P, Chik Z, *et al.* Baicalin, a metabolite of baicalein with antiviral activity against dengue virus. Sci Rep. 2014; 4: 5452. doi: 10.1038/srep05452, PMID 24965553.
18. Peng M, Watanabe S, Chan KW, He Q, Zhao Y, Zhang Z, *et al.* Luteolin restricts dengue virus replication through inhibition of the proprotein convertase furin. Antiviral Res. 2017; 143: 176-85. doi: 10.1016/j.antiviral.2017.03.026, PMID 28389141.
19. Powers CN, Setzer WN. An *In-Silico* Investigation of Phytochemicals as Antiviral Agents Against Dengue Fever. Comb Chem High Throughput Screen. 2016; 19(7): 516-36. doi: 10.2174/1386207319666160506123715, PMID 27151482.
20. Sánchez I, Gómez-Garibay F, Taboada J, Ruiz BH. Antiviral effect of flavonoids on the dengue virus. Phytother Res PTR. 2000; 14(2): 89-92. doi: 10.1002/(sici)1099-1573(200003)14: 2<89: aid-ptr569>. PMID 10685103.
21. Takhampunya R, Ubol S, Houng HS, Cameron CE, Padmanabhan R. Inhibition of dengue virus replication by mycophenolic acid and ribavirin. J Gen Virol. 2006; 87(7): 1947-52. doi: 10.1099/vir.0.81655-0, PMID 16760396.
22. Kim S, Thiessen PA, Bolton EE, Chen J, Fu G, Gindulyte A, *et al.* PubChem Substance and Compound databases. Nucleic Acids Res. 2016; 44((Database issue)):D1202-13. doi: 10.1093/nar/gkv951, PMID 26400175.
23. Eberhardt J, Santos-Martins D, Tillack AF, Forli S, AutoDock V. AutoDock Vina 1.2.0: New docking methods, expanded force field, and python bindings. J Chem Inf Model. 2021; 61(8): 3891-8. doi: 10.1021/acs.jcim.1c00203, PMID 34278794.
24. Salentin S, Schreiber S, Haupt VJ, Adasme MF, Schroeder M. PLIP: fully automated protein–ligand interaction profiler. Nucleic Acids Res. 2015; 43(W1) Web Server Issue:W443-7. doi: 10.1093/nar/gkv315, PMID 25873628.
25. Abraham MJ, Murtola T, Schulz R, Páll S, Smith JC, Hess B, *et al.* GROMACS: high performance molecular simulations through multi-level parallelism from laptops to supercomputers. SoftwareX. 2015; 1-2: 19-25. doi: 10.1016/j.softx.2015.06.001.
26. Yap CW. PaDEL-descriptor: an open-source software to calculate molecular descriptors and fingerprints. J Comp Chem. 2011; 32(7): 1466-74. doi: 10.1002/jcc.21707, PMID 21425294.
27. Witten IH, Frank E, Hall MA, Pal CJ. Data mining: practical machine learning tools and techniques. 4th ed; ISBN: 9780128042915. eBook ISBN: 9780128043578; 2016.
28. Podvinec M, Lim SP, Schmidt T, Scarsi M, Wen D, Sonntag LS, *et al.* Novel inhibitors of dengue virus methyltransferase: discovery by *in vitro*-driven virtual screening on a desktop computer grid. J Med Chem. 2010; 53(4): 1483-95. doi: 10.1021/jm900776m, PMID 20108931.
29. Yang W, Chen X, Li Y, Guo S, Wang Z, Yu X. Advances in pharmacological activities of terpenoids. Natural Product Communications. 2020; 15(3). doi: 10.1177/1934578X20903555.
30. Geraghty RJ, Aliota MT, Bonnac LF. Broad-spectrum antiviral strategies and nucleoside analogues. Viruses. 2021; 13(4): 667. doi: 10.3390/v13040667, PMID 33924302.
31. Setchell KD, Brown NM, Desai P, Zimmer-Nechemias L, Wolfe BE, Brashear WT, *et al.* Bioavailability of pure isoflavones in healthy humans and analysis of commercial soy isoflavone supplements. J Nutr. 2001; 131(4) Suppl:1362S-75S. doi: 10.1093/jn/131.4.1362S, PMID 11285356.
32. Tarique M, Ahmad S, Malik A, Ahmad I, Saeed M, Almatroudi A, *et al.* Correction to: Novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV2) and other coronaviruses: A genome-wide comparative annotation and analysis. Mol Cell Biochem. 2022; 477(2): 645. doi: 10.1007/s11010-021-04317-9, PMID 34882300.
33. Alwabli AS. Comparative evaluation of dengue virus (DENV) serotypes infections in humans (Homo sapiens). Int J Pharm Investigation. 2022; 12(4). doi: 10.4103/jpi.jpi_12_22. Doi:483-8. doi: 10.5530/ijpi.2022.4.83.
34. Alwabli AS. Lead identification against 3C-like protease of SARS-CoV-2 via target-based virtual screening and molecular dynamics simulation. J Young Pharm. 2022; 14(2): 179-86. doi: 10.5530/jyp.2022.14.34.

35. Alwabli AS, Qadri I. Identification of possible inhibitor molecule against NS5 MTase and RdRp protein of dengue virus in Saudi Arabia. Indian J Pharm Educ Res. 2021; 55(4): 1028-36. doi: 10.5530/ijper.55.4.203.
36. Alwabli AS, Alattas SG, Mohammed N, Alhebshi A, Alkenani N, Al-ghmady K, *et al.* Methylytransferase Activity of Dengue RNA virus by Using Purified Protein. Int J Drug Dev Res. 2019; 11(2). doi: 10.36648/0975-9344.11.2.131.
37. Alwabli AS, Alattas SG, Alhebshi AM, Zabermawi NM, Alkenani N, Ghmady KA, *et al.* Molecular docking analysis of netropsin and novobiocin with the viral protein targets HABD, MTD and RCD. Bioinformation. 2019; 15(4): 233-9. doi: 10.6026/97320630015233, PMID 31285639.
38. Malik A, Afaq S, Alwabli AS, Al-Ghmady K. Networking of predicted post-translational modification (PTM) sites in human EGFR. Bioinformation. 2019; 15(7): 448-56. doi: 10.6026/97320630015448, PMID 31485130.
39. Alwabli AS. Deciphering the molecular landscape of dengue infection: insights from gene expression profiling and Protein Interactions. Microbe. 2024; 5: 100195. doi: 10.1016/j.microb.2024.100195.
40. Alwabli AS. Fluorescence emission and molecular docking studies identified novobiocin as a potent inhibitor of the Japanese encephalitis virus (JEV) envelope protein. Int J Pharm Investigation. 2023; 13(2). doi: 10.4103/jpi.jpi_13_23. Doi:321-8. doi: 10.5530/ijpi.13.2.042.
41. Alhebshi AM, Metwally AM, Al-Basyouni KS, Mahmoud SR, Al-Solami HM, Alwabli AS. Biomechanical behavior of protein microtubules in living cells using the nonlocal beam theory. Phys Mesomech. 2022; 25(2): 181-6. doi: 10.1134/S1029959922020096.
42. Taj M, Khadimallah MA, Hussain M, Fareed K, Safeer M, Khedher KM, *et al.* Thermal stress effects on microtubules based on orthotropic model: vibrational analysis. Adv Concr Constr. 2021; 11(3): 255-60. doi: 10.12989/acc.2021.11.3.255.
43. Alwabli AS, Kaci A, Bellifa H, Bousahla AA, Tounsi A, Alzahrani DA, *et al.* The nanoscale buckling properties of isolated protein microtubules based on modified strain gradient theory and a new single variable trigonometric beam theory. Adv Nano Res. 2021; 10(1): 15-24. doi: 10.12989/anr.2021.10.1.015.

Cite this article: Alwabli AS. Exploring Phytocompounds' Interaction with Multi-Serotype Dengue Virus NS5-MTase: Insights into Binding Affinity and Activity. Indian J of Pharmaceutical Education and Research. 2025;59(3s):s930-s944.