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Short Communication

Revealing the Dengue Antiviral Activity of *Baper Tea* Polyherbal: *In Silico* and *In Vitro* Approaches for the NS2B/NS3 Protease Inhibitors

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ABSTRACT

Dengue virus serotype 4 (DENV-4) remains a significant global health burden, and no specific antiviral therapy has been approved to date. The exploration of plant-derived compounds as potential antiviral agents targeting the NS2B/NS3 protease complex represents a promising but largely underexplored therapeutic strategy. This study aimed to evaluate the antiviral activity of *Baper Tea* polyherbal against DENV-4 by targeting the NS2B/NS3 protease via *in silico* and *in vitro* approaches. The cytotoxicity and antiviral activity were evaluated via MTT and Viral ToxGlo assays in DENV-4-infected Vero cells to determine the CC_{50}, EC_{50}, and selectivity index (SI). Ten bioactive compounds from the *Baper Tea* polyherbal infusion were subjected to molecular docking against the NS2B/NS3 protease (PDB: 5YVU) via AutoDock Vina 1.2.0, and the binding interactions were analyzed via Discovery Studio. *Baper Tea* polyherbal infusion exhibited potent antiviral activity against DENV-4, with an EC_{50} of 77.57 µg/mL, a CC_{50} of 7,345 µg/mL, and a favorable selectivity index of 94.69. Molecular docking studies revealed tetraacetyl-D-xylonic acid as the most promising compound, demonstrating a binding affinity (ΔG = -6.75 kcal/mol; Ki 11.29 µM) approximately 457-fold stronger than that of the native ligand through six hydrogen bonds with catalytic residues (LYS B:201, ASN B:416, ALA B:197, GLY B:198, GLY B:196, and ARG B:463). The secondary candidates included dihydroxanthin (ΔG -6.38 kcal/mol; Ki 21.23 µM) and 2,7-diphenyl-1,6- (ΔG -6.31 kcal/mol; Ki 23.74 µM). This study provides preliminary computational and *in vitro* evidence of the potential of *Baper Tea* polyherbal infusion constituents to inhibit NS2B/NS3 protease.

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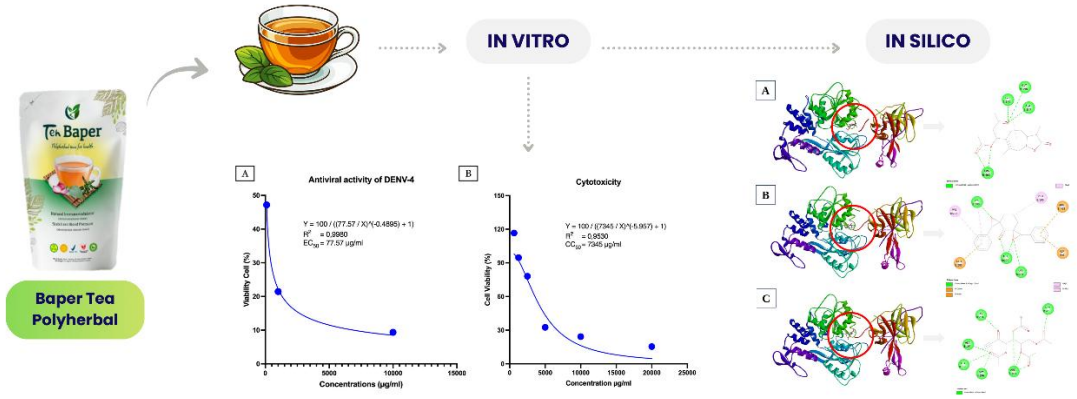
GRAPHICAL ABSTRACT



Introduction

Dengue virus (DVI) infection is an arboviral disease with an increasing global health burden [1–4]. In 2024, there were 14.1 million new cases and 9,508 deaths, which is a twofold increase compared to 2023 and a twelve-fold increase since 2014 [5]. Asia recorded 884,402 cases with 1,008 deaths (CFR 0.11%), the highest number of deaths worldwide [6]. Dengue virus (DENV), classified within the genus *Orthoflavivirus* of the family *Flaviviridae*, comprises four serotypes (DENV-1 to DENV-4) that can lead to severe or life-threatening disease [2,7,8]. Among these serotypes, DENV-4 has become the predominant serotype in recent Indonesian outbreaks but remains the least studied for antiviral development. Despite the availability of vaccines such as CYD-TDV (*Dengvaxia*®) and TAK-003 (*Qdenga*®), their efficacy against DENV-4 remains unclear, and in the absence of approved antiviral therapies, dengue treatment continues to depend on supportive therapy [9–12].

The nonstructural protein NS3 of dengue virus is a promising therapeutic target because of its role as a serine protease with the catalytic triad HIS51, ASP75, and SER135, which requires the cofactor NS2B for full activity in viral polyprotein processing, RNA genome replication, and viral particle maturation [13,14]. The NS2B/NS3 protease complex processes eight of the 13 cleavage sites of viral polyproteins, making it a prime target for the development of antiviral inhibitors against DENV and related flaviviruses [15,16]. An integrative in silico and in vitro approach has been widely used to identify candidate NS2B/NS3 protease inhibitors from natural sources with various phytoconstituents that show promising binding affinity through molecular docking in the range of -5.2-8.7 kcal/mol [17,18].

Herbal compounds such as extracts from *Curcuma longa* (IC_{50} 17.91 µg/mL; SI 4.8), *Taraxacum officinale* (IC_{50} 126.1 µg/mL; SI 6.01), and geraniin from *Nephelium lappaceum* (IC_{50} 1.75 µM) have demonstrated antiviral activity with acceptable safety profiles [19,20]. However, the majority of inhibitor candidates are still in the preclinical stage due to limited pharmacokinetic data, bioavailability, and translation to *in vivo* and clinical trials, which represent significant research gaps [21]. The previous research on formulations of *Baper Tea* polyherbal containing *Allium cepa* L., *Physalis angulata* leaves, and *Phyllanthus urinaria* identified forty bioactive compounds with antiviral potential through GC–MS and FT–IR characterization [22,23]. However, significant knowledge gaps persist concerning the

antiviral potential of Baper Tea polyherbal against DENV-4. To date, no experimental studies have validated the anti-DENV-4 activity of this formulation, nor have the molecular mechanisms of NS2B/NS3 protease inhibition been elucidated. Furthermore, the structure–activity relationships of lead compounds within this polyherbal mixture remain uncharacterized, hindering evidence-based therapeutic development. This study aimed to evaluate the antiviral activity of *Baper Tea* polyherbal against dengue virus serotype 4 (DENV-4) by targeting the NS2B/NS3 protease through integrated *in silico* and *in vitro* inhibition approaches. The antiviral activity of *Baper Tea* polyherbal was evaluated *in vitro* by determining the EC_{50}, CC_{50}, and selectivity index and identifying the binding affinity and molecular interactions of bioactive compounds against the DENV-4 NS2B/NS3 protein through an *in-silico* approach. The findings of this study are expected to provide scientific evidence of the potential of *Baper Tea* polyherbal as a DENV-4 antiviral candidate that can be developed into an alternative therapy or adjuvant while also providing mechanistic insights into compound–protein interactions that contribute to global efforts to develop natural-source dengue antivirals.

Experimental

Research design and ethics

Experimental research using an integrative *in vitro* and *in silico* approach was conducted at the Medical Biology Laboratory, Universitas Hindu Indonesia; the Dengue Laboratory of the Institute of Tropical Diseases at Universitas Airlangga; and the Laboratorium Satwa Sehat (May–December 2024). This research protocol was approved by the Health Research Ethics Committee of the Faculty of Medicine and Health Sciences, Warmadewa University (213.01.05.2024-37/Unwar/FKIK/EC-KEPK/V/2024).

Preparation of baper tea polyherbal infusion

The polyherbal formulation of the *Baper Tea* polyherbal used in this study is a standardized product registered with the Indonesian Food and Drug Administration (P-IRT 5105101010166-30) and manufactured by PT Mega Science Indonesia [22]. The formulation contained three botanical components, including the skin of *Allium cepa* L., the leaves of *Physalis angulata* L., and *Phyllanthus urinaria* at a 2:1:1 ratio, which underwent a standardization process involving collection, sorting, washing, and drying via an FDH-16 food dehydrator with 16 trays, grinding, packaging, and storage [23]. *Baper tea* polyherbal infusions were prepared by steeping 5 g of powder in 100 mL of sterile water at 90 °C for 10 min, filtering it through a 0.22 μm sterile membrane, lyophilizing it, and storing it at -20°C until use. The experimental procedure was carried out by collecting the lyophilized extract reconstituted in complete MEM to prepare a 10 mg/mL stock solution, which was then serially diluted to obtain the required concentrations [22,24].

Culture and viruses

Vero cells (ECACC 84113001; lot 19E011) were cultured in MEM supplemented with 10% heat-inactivated fetal bovine serum (FBS) and 1% penicillin–streptomycin at 37 °C in a 5% CO_2 incubator. Subcultures were performed at 80–100% confluence via the use of 0.25% trypsin-EDTA. Local Indonesian DENV-4 strains (from the collection of the Institute of Tropical Disease, Universitas Airlangga) were propagated in Vero cells, and titers were determined via a focus-forming assay (FFU).

MTT assay

Vero cells (5×10^4 cells/mL, 100 μL/well) were seeded into 96-well microplates and incubated for 24 h. After washing with PBS, the cells were exposed to serial concentrations of *Baper Tea*

polyherbal infusion (3.31–200 µg/mL) in triplicate for 24 h. Cell viability was measured via the MTT assay with the addition of 10 µL of MTT (5 mg/mL) and 100 µL of medium, incubation for 4 h at 37 °C, dissolution of formazan with 50 µL of DMSO, and absorbance reading at 595 nm. The percentage of viability was calculated as [(Abs treatment - Abs medium)/(Abs control cells - Abs medium)] × 100%. The cell viability at 50% CC_{50} was determined via linear regression.

DENV-4 antiviral activity

Vero cells (5×10^4 cells/mL, 100 µL/well) in a 96-well luminescent microplate were incubated for 24 h and then exposed to serial concentrations of *Baper Tea* polyherbal extract (1.56–100 µg/mL, 25 µL/well) in triplicate. A DENV-4 suspension (25 µL, 2×10^3 FFU/mL) was then added to each well. The controls included cells without the virus, virus without *Baper Tea* polyherbal infusion, and medium without cells. After incubation for 48–144 hours (37 °C, 5% CO_2), cell viability was quantified via the Viral ToxGlo Assay according to the manufacturer's protocol through luminescent readings (GloMax luminometer®). The percentage of CPE inhibition was calculated using the following equation: [(Lum treatment - Lum medium)/(Lum virus+cell control - Lum medium)] × 100%. EC_{50} values (concentration inhibiting 50% virus replication) and selectivity indices (SI = CC_{50}/EC_{50}) were determined [23]. In this study, Vero cells (African green monkey kidney cells) were employed for virus propagation and antiviral testing, as they are the standard model for dengue virus studies because of their high susceptibility to infection. However, these cells are not without their limitations, including the fact that Vero cells do not possess a functional type I interferon response due to a defect in the interferon signaling pathway. Consequently, the antiviral effects observed in this system may not fully represent the response in human cells with intact innate immunity.

Molecular docking

The structure of the bioactive compound of *Baper Tea* polyherbal, which was previously identified through GC–MS and FT-IR testing, was used in this study [23]; it was downloaded from PubChem (*sdf format) and optimized using the MMFF94 force field (Open Babel). The ligands were prepared in PDBQT format (AutoDockTools 1.2.0). The full-length NS3 DENV-4 protein structure (PDB ID: 5YVU) was prepared by removing water molecules and adding polar hydrogen. The crystal structure of the DENV-4 NS2B/NS3 protease was obtained from the RCSB Protein Data Bank. The selection of this structure was based on several considerations, including its high resolution, which provides accurate atomic coordinates for docking calculations, and the fact that it was crystallized with an inhibitor, thereby validating the binding site. All residues in the active site are complete with no missing atoms. This structure represents the DENV-4 serotype and has been widely used in previous virtual screening studies, allowing direct comparison of the docking results with previously published data. Molecular docking was performed using AutoDock Vina 1.2.0, with a grid box covering the active site of methyltransferase. The docking protocol was validated through the redocking of native ligands (RMSD criterion of 2.0 Å). The compounds with the highest binding affinities were prioritized for interaction analysis via Discovery Studio Visualizer 2021. Interaction characterization included hydrogen bonds (distance ≤3.5 Å, angle ≥120°), hydrophobic interactions, π–π stacking, and salt bridges. Drug-likeness and ADME profiles were predicted via SwissADME (in compliance with Lipinski's rule of five).

Analyzed data

Cell viability, virus inhibition, and binding affinity data were analyzed using nonlinear regression (GraphPad Prism 10.3.1 version

MacOS Tahoe 26.0.1). The EC_{50}, CC_{50}, and SI values are reported as the means with 95% confidence intervals. Comparisons of the binding affinities between the compounds and native ligands were performed descriptively. The molecular interaction profile data were visualized in 2D and 3D formats.

Results and Discussion

DENV-4 antiviral activity, cytotoxicity, and selectivity index

In vitro evaluation revealed that the *Baper Tea* polyherbal infusions had inhibitory effects on DENV-4, with an EC_{50} value of 77.57 µg/mL (95% CI: 3.785–211.3 µg/mL; R^2 = 0.9980), indicating strong inhibition. The dose–response curve revealed a concentration-dependent inhibition pattern, which was consistent with a progressive decline in virus viability from approximately 47% at the lowest concentration to 9% at 10,000 µg/mL (Figure 1A). A nonlinear regression model with a coefficient of determination (R^2 of 0.9980) indicated a model fit. Cytotoxicity testing via the MTT assay in Vero cells yielded a CC_{50} of 7,345 µg/mL. The cell viability curve showed a gradual decrease in viability with increasing concentrations, with cells maintaining >90% viability at concentrations up to 20,000 µg/mL (Figure 1B). The high CC_{50} value indicates that *Baper Tea* polyherbal has the potential to be used as an antiviral candidate against DENV-4. Furthermore, calculation of the selectivity index ($SI = CC_{50}/EC_{50}$) revealed a value of 94.69 for anti-DENV-4 activity. A high SI value indicates that the *Baper Tea* polyherbal is highly specific to the target cells and has minimal side effects on normal cells.

Analyzed data ADME profile and drug likeness

Ten compounds from the *Baper Tea* polyherbal infusion exhibit diverse pharmacokinetic characteristics.

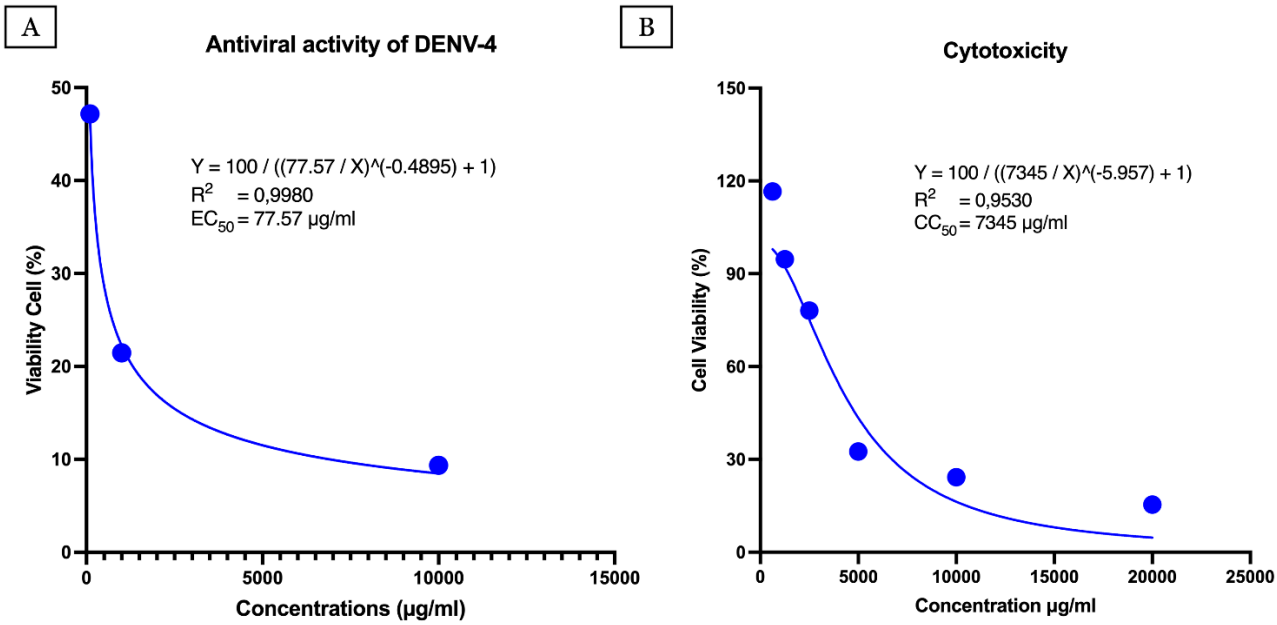


Figure 1. Antiviral activity and cytotoxicity profile of the *Baper Tea* polyherbal infusion. Remarks: (A) Concentration-dependent dose–response curve of anti-DENV-4 activity; (B) cytotoxicity profile in Vero cells. Data are presented from three independent experiments via a nonlinear regression model [inhibitor] vs. normalized response-variable slope

Tetraacetyl-D-xylonic acid has a molecular weight of 343.29 g/mol with a log P of -0.85, indicating that high hydrophilicity may affect membrane permeability. In contrast, N-propyl 9- showed excessive lipophilicity (log P 4.80), which violates Lipinski's rules and has the potential to reduce oral bioavailability.

The other nine compounds met the drug likeness criteria without violation, with a molecular weight range of 116.07–421.90 g/mol. Alpha-D-galactopyranose has the highest hydrophilicity (log P -2.75), with five hydrogen donors and six hydrogen acceptors.

Terpinen-4-ol presented the most balanced profile, with a log P of 2.30 and minimal structural complexity, indicating optimal absorption potential (Table 1).

Validation of the molecular docking methods

Validation of the method against the DENV-4 NS3 protein yielded an RMSD of 1.932 Å, which met the validity criteria (≤2 Å). The native ligand glycerol showed a binding affinity of -3.12 kcal/mol, with an inhibition constant of 5.16 mM. The interaction involves conventional hydrogen bonds with residues GLYB 198 and GLYB 196, as well as unfavorable donor–donor bonds with ASPB 6. This interaction pattern establishes a baseline for the evaluation of the test compounds (Table 2 and Figure 2).

Table 1. ADME profile and drug likeness

Compound	Molecular weight (g/mol)	Log P	Donor H	Akseptor H	Druglikeness
Standard	<500	≤ 5	<10	<5	≤1
Tetraacetyl-d-xylonic	343.29	-0.85	0	10	Yes; 0 violation
Terpinen-4-ol	154.25	2.30	1	1	Yes; 0 violation
Dihydroxanthin	308.37	2.00	5	5	Yes; 0 violation
1,4-dioxane-2,6-dione	116.07	-0.93	0	4	Yes; 0 violation
N-propyl 9-	296.49	4.80	0	2	Yes; 1 violation: MLOGP>4.15
2,7-Diphenyl-1,6-	306.36	2.51	0	3	Yes; 0 violation
Phenanthrene, 9-	288.38	2.65	3	3	Yes; 0 violation
Alpha-D-Galactopyranose (alpha-D-galactose)	180.16	-2.75	5	6	Yes; 0 violation
Prost-13-en-1-oic-acid	354.48	1.98	3	5	Yes; 0 violation
W-18 ((*E*)-4-Chloro-*N*-(1-(4-nitrophenethyl) piperidin-2-ylidene) benzenesulfonamide)	421.90	3.11	0	5	Yes; 0 violation

Table 2. Validation of the molecular docking method

Serotype	PDB	Types of protein	Native ligand	RMSD (Å)	ΔG (kcal/mol)	Ki
DENV-4	5YVU	Full Length NS3	GLYCEROL (GOL) $C_3H_8O_3$	1.932	-3.12	5.16 mM

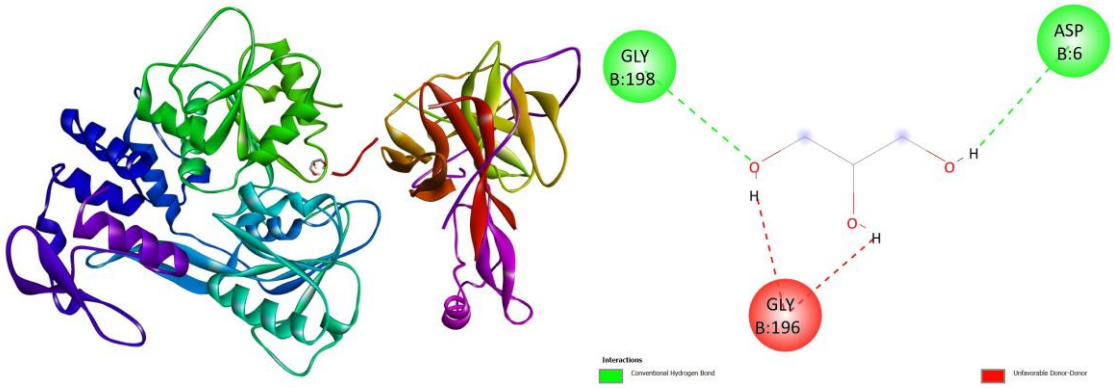


Figure 2. Interaction of the native DENV-4 ligand

Binding affinity of the Baper Tea polyherbal compound for DENV-4

The binding affinity of the *Baper Tea* polyherbal compound for DENV-4 varies (Table 3). Tetraacetyl-d-xylonic acid has the highest affinity (ΔG -6.75 kcal/mol; Ki 11.29 μM) with six hydrogen bonds at LYS B:201, ASN B:416, ALA B:197, GLY B:198, GLY B:196, and ARG B:463, which exceeded the native glycerol ligand by 457 times. Dihydroxanthin occupies the second position (ΔG -6.38 kcal/mol; Ki 21.23 μM) through four hydrogen bonds with GLY B:198, LYS B:199, ALA B:197, and ARG B:463, exhibiting a binding affinity that is 243 times greater. In contrast, 2,7-diphenyl-1,6- (ΔG -6.31 kcal/mol; Ki 23.74 μM) adopts a multimodal strategy comprising hydrogen bonds on LYS B:199, THR B:200, LYS B:201, π-cation interactions with ARG B:463, and π-anion interactions with GLU B:285 and ASP B:6. This results in a binding affinity that is 217 times stronger than that of the native ligand. The W-18((E)-4-chloro-N-(1-(4-nitrophenethyl)piperidin-2-ylidene) benzenesulfonamide compound has moderate affinity (ΔG -5.86 kcal/mol; Ki 50.64 μM) but forms an unfavorable salt bridge at LYS B:198, which is compensated for by interactions with GLU B:230, GLY B:194, THR B:200, and ASP B:6. The compound with the lowest affinity was prost-13-en-1-oic acid (ΔG -4.36 kcal/mol; Ki 632.94 μM). In terms of ligand efficiency, calculated as the ratio of affinity to molecular weight, dihydroxanthin compounds had the highest ligand efficiency (0.0207 kcal/mol/Da), followed by 2,7-diphenyl-1,6- (0.0206 kcal/mol/Da) and tetraacetyl-d-xylonic (0.0197 kcal/mol/Da) as candidates with the best structural effectiveness, where the inhibition constant in the micromolar range indicates the potential for pharmacologically relevant inhibition of NS3 protease activity in the DENV-4 replication cycle. The binding affinities of the three compounds are shown in Figure 3.

Molecular interactions of Baper Tea polyherbal compounds with DENV-4

Residue analysis revealed LYS B:199, GLY B:198, and ARG B:463 as interaction hotspots for the majority of the active compounds in the *Baper Tea* polyherbal compound against DENV-4 (Table 4). LYS B:199 interacted with five different compounds, indicating a key role in ligand recognition. Hydrophobic interactions (alkyl, π-alkyl) appear as secondary contributions to aromatic compounds such as 2,7-diphenyl-1,6- and phenanthrene-9-, complementing primary hydrogen bonds. Terpinen-4-ol showed a unique pattern with a dominance of alkyl interactions (LYS B:201, LYS B:199) over hydrogen bonds (ASP B:6), indicating that an alternative binding mechanism still produces adequate affinity.

Table 3. Bond affinities of *Baper Tea* polyherbal compounds against DENV-4

Compound	ΔG (kcal/mol)	Ki
Tetraacetyl-d-xylonic	-6.75	**11.29 uM**
Terpinen-4-ol	-5.70	65.98 uM
Dihydroxanthin	-6.38	**21.23 uM**
1,4-dioxane-2,6-dione	-5.35	119.72 uM
N-propyl 9-	-4.71	350.55 uM
2,7-Diphenyl-1,6-	-6.31	**23.74 uM**
Phenanthrene, 9-	-5.35	120.07 uM
Alpha-D-Galactopyranose (alpha-D-galactose)	-4.69	364.77 uM
Prost-13-en-1-oic-acid	-4.36	632.94 uM
W-18 ((*E*)-4-Chloro-*N*-(1-(4-nitrophenethyl) piperidin-2-ylidene) benzenesulfonamide)	-5.86	50.64 uM

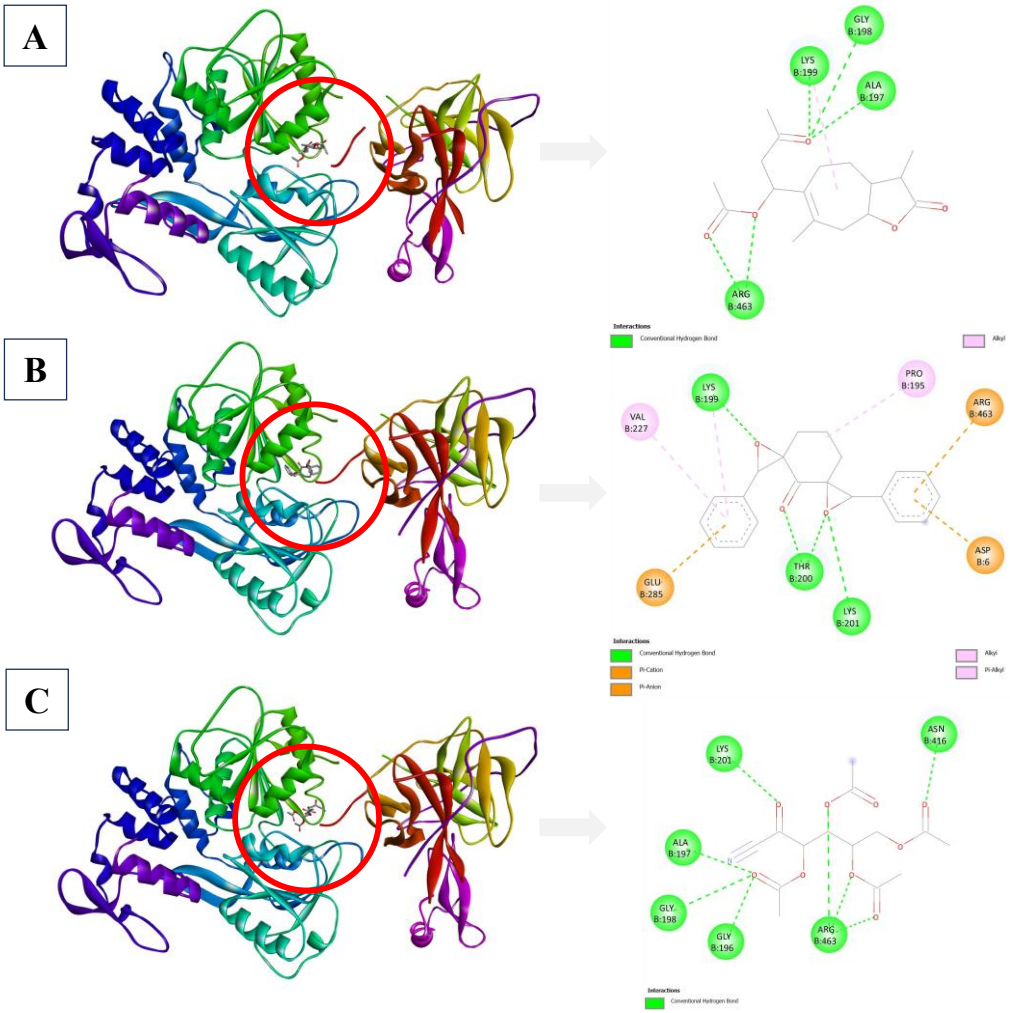


Figure 3. Interaction of polyherbal compounds in tea baper with DENV-4. Remarks: (A) Dihydroxanthin, (B) 2,7-diphenyl-1,6-, and (C) tetraacetyl-d-xylonic

Table 4. Binding area of the *baper tea* polyherbal compound to DENV-4

Compound	Binding site of compounds	
	Interaction	**Amino acid residues**
Tetraacetyl-d-xylonic	Conventional hydrogen bond	**LYS B:201**, **ASN B:416**, **ALA B:197**, **GLY B:198**, **GLY B:196**, **ARG B:463**
Terpinen-4-ol	Conventional hydrogen bond	**ASP B:6**
	alkyl	**LYS B:201**, **LYS B:199**
Dihydroxanthin	Conventional hydrogen bond	**GLY B:198**, **LYS B:199**, **ALA B:197**, **ARG B:463**
	alkyl	**LYS B:199**
1,4-dioxane-2,6-dione	Conventional hydrogen bond	**ARG B:463**, **ALA B:197**, **LYS B:199**, **GLY B:198**
	Carbon hydrogen bond	**ASP B:6**
N-propyl 9-	Conventional hydrogen bond	THR B:200, **LYS B:201**
	alkyl	**LYS B:199**, PRO B:195, VAL B:227
2,7-Diphenyl-1,6-	Conventional hydrogen bond	**LYS B:199**, THR B:200, **LYS B:201**
	π-Cation	**ARG B:463**
	π-Anion	GLU B:285, **ASP B:6**
	Alkyl	VAL B:227
	π-Alkyl	PRO B:195, **ARG B:463**, **ASP B:6**
Phenanthrene, 9-	Conventional hydrogen bond	LEU B:193
	Carbon hydrogen bond	**LYS B:199**
	π-Cation	**LYS B:199**
	Alkyl	**ALA B:197**
	π-Alkyl	PRO B:195, **ALA B:197**
Alpha-D-Galactopyranose (alpha-D-galactose)	Conventional hydrogen bond	**LYS B:199**, LEU B:193, **ASP B:6**
Prost-13-en-1-oic-acid	Conventional hydrogen bond	**LYS B:201**, **ASN B:416**, **ASP B:6**, **LYS B:199**
	Alkyl	**ALA B:197**, PRO B:195
	Salt bridge	**LYS B:198**
W-18 ((*E*)-4-Chloro-*N*-(1-(4-nitrophenethyl) piperidin-2-ylidene) benzenesulfonamide)	Conventional hydrogen bond	GLU B:230, GLY B:194, THR B:200, **ASP B:6**
	π-Donor hydrogen bond	**LYS B:198**
	Unfavorable donor-donor	**ASP B:6**
	π-Cation	**ARG B:463**
	Alkyl	PRO B:195, **LYS B:201**, **GLY B:196**, GLY B:190, **ALA B:197**

Structure-activity and antiviral potential

The structure–activity correlations revealed that compounds with multiple hydrogen acceptors (≥5) and complex structures presented the best binding affinity [25–27]. The tetraacetyl-d-xylonic compound with 10 hydrogen acceptors showed maximum binding capacity despite having a negative log P value. This finding indicates that hydrophilicity is not a limitation of the NS3 DENV-4 binding pocket. Conversely, simple planar aromatic structures such as phenanthrene-9 yield moderate affinity (ΔG -5.35 kcal/mol) with limited interactions, indicating the need for polar functionality for optimized binding. The ligand efficiency was calculated as the ratio of the affinity

to the molecular weight, which revealed that tetraacetyl-d-xylonic acid had the highest value (0.0197 kcal/mol/Da), followed by dihydroxanthin (0.0207 kcal/mol/Da). This parameter identifies candidates with optimal structural effectiveness. The inhibitory potential of tetraacetyl-d-xylonic and dihydroxanthin in the micromolar range indicated the presence of DENV-4 replication inhibition activity, suggesting that these compounds have potential as dengue antiviral therapy candidates.

Discussion

In this study, it was demonstrated that the *Baper Tea* polyherbal infusion has antiviral activity against DENV-4, with an EC_{50} value of 77.57 µg/mL, a CC_{50} value of 7,345 µg/mL, and a selectivity index of 94.69, indicating the ability to inhibit viral replication at concentrations that are still safe for host cells. Furthermore, the tetraacetyl-d-xylonic compound showed the highest affinity (ΔG -6.75 kcal/mol; Ki 11.29 µM) with six hydrogen bonds at LYS B:201, ASN B:416, ALA B:197, GLY B:198, GLY B:196, and ARG B:463, resulting in an affinity 457 times stronger than that of the native ligand, followed by dihydroxanthin in second place (ΔG -6.38 kcal/mol; Ki 21.23 µM) and 2,7-diphenyl-1,6- (ΔG -6.31 kcal/mol; Ki 23.74 µM). These three compounds had optimal ligand efficiency ratios and showed potential for inhibiting NS3 protease activity. These findings are consistent with the research of Altamish *et al*. [19], who reported that *Curcuma longa* has an IC_{50} of 17.91 µg/mL with an SI of 4.8, as do extracts of *Taraxacum officinale* and *Urtica dioica,* with IC50 values of 126.1 µg/mL (SI 6.01) and 165.7 µg/mL (SI 5.59) against DENV-2, respectively. Flores-Ocelotl *et al*. [20] confirmed that the polyherbal formulation has antiviral potential with an acceptable safety profile. The strong to very strong 50% effective concentration (EC_{50}) values indicate promising therapeutic potential, although the selectivity index still needs to be improved for safer clinical applications [28,29]. The mechanism of viral inhibition is thought to involve the inhibition of NS3 protease activity, a multifunctional enzyme that plays a role in viral polyprotein processing and viral RNA genome replication [24].

The molecular docking approach confirmed that bioactive compounds in the *Baper Tea* polyherbal could interact with the catalytic domain of NS3 DENV-4 through stable and specific bonds. In silico analysis revealed tetraacetyl-d-xylonic acid as the compound with the highest binding affinity for NS3 DENV-4, with a Gibbs free energy of -6.75 kcal/mol and an inhibition constant of 11.29 µM. This compound forms six hydrogen bonds with residues LYS B:201, ASN B:416, ALA B:197, GLY B:198, GLY B:196, and ARG B:463 at the active site of NS3 methyltransferase and produces a protein–ligand complex that is 457 times greater than the native glycerol ligand. The bond affinity of tetraacetyl-d-xylonic compounds (-6.75 kcal/mol) is greater than that of phytocompounds in the study by Purohit *et al*. [17], which reported binding energies ranging from -5.2 to -7.8 kcal/mol for the NS2B-NS3 protease of DENV, as well as that reported by Abdullah *et al*. [18], who identified compounds with optimal binding affinities in the range of -7.2 to -8.5 kcal/mol. Dihydroxanthin and 2,7-diphenyl-1,6- occupied the second and third positions, with affinities of -6.38 kcal/mol and -6.31 kcal/mol, respectively. These three compounds showed optimal ligand efficiency, with values above 0.019 kcal/mol/Da, indicating a favorable affinity–to–molecular weight ratio for drug development. The validation of the docking method with an RMSD of 1.932 Å met the standard validity criteria, reinforcing the reliability of the bond affinity predictions obtained. Molecular interactions revealed that LYS B:199, GLY B:198, and ARG B:463 are binding hotspots that participate in the stabilization of the majority of protein–ligand complexes. The identification of these key residues is consistent with the findings of Norshidah *et al*. [14],

emphasizing the role of HIS51, ASP75, and SER135 as the catalytic triad of the NS2B/NS3 protease, as well as the research by Goyal *et al*. [30], which indicates that interactions at the conserved residues of the NS3 protease are the main determinants of inhibitor activity in all DENV serotypes. The dominance of hydrogen bonds in compounds with multiple hydrogen acceptors, such as tetraacetyl-d-xylonic, indicates that hydrophilicity does not limit penetration into the DENV-4 NS3 binding pocket, contrary to the general drug design paradigm that emphasizes the lipophilicity–hydrophilicity balance [31]. Aromatic compounds such as 2,7-diphenyl-1,6- adopt a multimodal strategy with a combination of hydrogen bonds, π-cation interactions, and π-anion interactions that contribute to the stability of the complex. These findings are consistent with the research of Zhang *et al*. [32], who reported that NS3 protease inhibitors with complex structures and multiple binding modes exhibit superior antiviral activity against flaviviruses. This study provides further evidence to support the hypothesis that Indonesian medicinal plants possess therapeutic potential in the treatment of dengue virus infection. *Baper tea* polyherbal infusion, a traditional remedy for general health benefits, has been shown to exhibit multicompound activity against validated antiviral targets. This polypharmacological profile offers an advantage over single-compound approaches because it can reduce the likelihood of viral resistance developing [33]. The identified compounds, primarily flavonoids and phenolic acids, exhibit favorable ADME properties, suggesting that their oral bioavailability is consistent with the oral route of drug administration [34].

From a drug discovery perspective, these results confirm that the NS2B/NS3 protease is a viable target for the development of natural-based dengue antivirals. The binding mode revealed through molecular docking shows that these compounds occupy the substrate binding pocket and form interactions with catalytic residues. These interactions disrupt the proteolytic processing of dengue virus polyproteins, thereby significantly inhibiting viral replication. Moreover, the integration of computational screening with *in vitro* validation has been identified as a cost-effective strategy for identifying antiviral leads from complex plant matrices. This approach has the potential to be extended to other traditional medicines and other viral proteases with the aim of accelerating the discovery of new antivirals.

Limitations and recommendations

The present study is limited to in silico predictions and *in vitro* testing; consequently, its clinical application must take into account *in vivo* test results and further relevant testing. Second, the mechanistic evidence is indirect, particularly with respect to NS2B/NS3 protease inhibition; as a result, direct enzymatic assays and structural biology techniques, such as X-ray crystallography or cryo-electron microscopy, are needed to experimentally confirm the binding mode. Third, bioactive compounds were predicted on the basis of the previously reported phytochemical profile of *Baper Tea*; however, the isolation, purification, and structural characterization of individual compounds from the extract used have not been performed. Further research is needed to evaluate the pharmacokinetics, pharmacodynamics, and potential chronic toxicity in animal models before clinical trials in humans. The development of formulations with optimized bioavailability is necessary, given the hydrophilic nature of tetraacetyl-d-xylonic acid, which may limit oral absorption. Nevertheless, these findings open up opportunities for exploring lead compounds from natural sources as DENV-4 antiviral candidates that can be developed into alternative therapies or adjuvants for dengue infection.

Conclusion

In conclusion, this study demonstrated that *Baper Tea* polyherbal infusions have antiviral activity against DENV-4. They have a strong inhibitory effect on viral replication at concentrations that are safe for host cells. The molecular docking approach identified tetraacetyl-d-xylonic acid as the compound with the highest binding affinity to the DENV-4 NS2B/NS3 protease, forming six hydrogen bonds at key residues with protein–ligand complex stability 457 times stronger than that of the native glycerol ligand, followed by dihydroxanthin in second place and 2,7-diphenyl-1,6-. These three compounds had optimal ligand efficiency ratios and showed potential for inhibiting NS3 protease activity. These findings indicate the potential of *Baper Tea* polyherbal infusions as DENV-4 antiviral candidates that can be further developed for therapeutic applications through *in vivo* testing and pharmacokinetic optimization.

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Disclosure Statement

No potential conflict of interest was reported by the authors.

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References

[1] Fong, S.-L., Wong, K.-T., Tan, C.-T. Dengue virus infection and neurological manifestations: An update. *Brain*, **2024**, 147(3), 830-838.
[2] Adnyana, I.M.D.M., Utomo, B., Sulistiawati, S., Adiwinoto, R.P., Sudaryati, N.L.G. Climate matters: Integrating bioclimatology into dengue vector control within the one health framework. *Journal of Arthropod-Borne Diseases*, **2025**, 19(1), 93.
[3] Adnyana, I.M.D.M., Sudaryati, N.L.G., Eljatin, D.S., Adiwinoto, R.P., da Cruz, Z.V. One health approach worldwide and challenges in collaboration. *One Health Integration: Global Perspectives on Animal Health and Sustainable Agriculture*, **2025**, 33-62.
[4] Adnyana, I.M., Utomo, B., Eljatin, D.S., Sudaryati, N.L. One health approach and zoonotic diseases in indonesia: Urgency of implementation and challenges. *Narra J*, **2023**, 3(3), e257.
[5] Kementerian Kesehatan Republik Indonesia. Profil Kesehatan Indonesia 2024. Jakarta: 2024. Kementerian Kesehatan Republik Indonesia.
[6] Haider, N., Hasan, M.N., Onyango, J., Billah, M., Khan, S., Papakonstantinou, D., Paudyal, P., Asaduzzaman, M. Global dengue epidemic worsens with record 14 million cases and 9,000 deaths reported in 2024.

International Journal of Infectious Diseases, **2025**, 107940.
[7] WHO. Dengue and severe dengue. World Health Organization **2025**.
[8] Adnyana, I.M.D.M., Utomo, B. Dengue elimination challenges in bali: A one health perspective. *National Journal of Community Medicine*, **2023**, 14(08), 544-546.
[9] Hou, J., Ye, W., Chen, J. Current development and challenges of tetravalent live-attenuated dengue vaccines. *Frontiers in Immunology*, **2022**, 13, 840104.
[10] Thomas, S.J. Is new dengue vaccine efficacy data a relief or cause for concern? *NPJ Vaccines*, **2023**, 8(1), 55.
[11] Flacco, M.E., Bianconi, A., Cioni, G., Fiore, M., Calò, G.L., Imperiali, G., Orazi, V., Tiseo, M., Troia, A., Rosso, A. Immunogenicity, safety and efficacy of the dengue vaccine tak-003: A meta-analysis. *Vaccines*, **2024**, 12(7), 770.
[12] Tarasuk, M., Songprakhon, P., Muhamad, P., Panya, A., Sattayawat, P., Yenchitsomanus, P. Dual action effects of ethyl-p-methoxycinnamate against dengue virus infection and inflammation via nf-κb pathway suppression. *Scientific Reports*, **2024**, 14(1), 9322.
[13] Starvaggi, J., Previti, S., Zappalà, M., Ettari, R. The inhibition of NS2B/NS3 protease: A new therapeutic opportunity to treat dengue and zika virus infection. *International Journal of Molecular Sciences*, **2024**, 25(8), 4376.
[14] Norshidah, H., Leow, C.H., Ezleen, K.E., Wahab, H.A., Vignesh, R., Rasul, A., Lai, N.S. Assessing the potential of NS2B/NS3 protease inhibitors biomarker in curbing dengue virus infections: In silico vs. In vitro approach. *Frontiers in Cellular and Infection Microbiology*, **2023**, 13, 1061937.
[15] Lin, K.H., Ali, A., Rusere, L., Soumana, D.I., Yilmaz, N.K., Schiffer, C.A. Dengue virus NS2B/NS3 protease inhibitors exploiting the prime side. *Journal of Virology*, **2017**, 91, 17.
[16] Kronenberger, T., Sa Magalhaes Serafim, M., Kumar Tonduru, A., Goncalves Maltarollo, V., Poso, A. Ligand accessibility insights to the dengue virus NS3 - NS2B protease assessed by long-timescale molecular dynamics simulations. *ChemMedChem*, **2021**, 16(16), 2524-2534.
[17] Purohit, P., Sahoo, S., Panda, M., Sahoo, P.S., Meher, B.R. Targeting the denv NS2B-NS3 protease with active antiviral phytocompounds: Structure-based virtual screening, molecular docking and molecular dynamics simulation studies. *Journal of Molecular Modeling*, **2022**, 28(11), 365.
[18] Abdullah, Z.L., Chee, H.-Y., Yusof, R., Mohd Fauzi, F. Finding lead compounds for dengue antivirals from a collection of old drugs through in silico target prediction and subsequent in vitro validation. *ACS Omega*, **2023**, 8(36), 32483-32497.
[19] Altamish, M., Khan, M., Baig, M.S., Pathak, B., Rani, V., Akhtar, J., Khan, A.A., Ahmad, S., Krishnan, A. Therapeutic potential of medicinal plants against dengue infection: A mechanistic viewpoint. *ACS Omega*, **2022**, 7(28), 24048-24065.
[20] Flores-Ocelotl, M.R., Rosas-Murrieta, N.H., Moreno, D.A., Vallejo-Ruiz, V., Reyes-Leyva, J., Domínguez, F., Santos-López, G. Taraxacum officinale and urtica dioica extracts inhibit dengue virus serotype 2 replication in vitro. *BMC Complementary and Alternative Medicine*, **2018**, 18(1), 95.
[21] Saqallah, F.G., Abbas, M.A., Wahab, H.A. Recent advances in natural products as potential inhibitors of dengue virus with a special emphasis on NS2B/NS3 protease. *Phytochemistry*, **2022**, 202, 113362.
[22] Sudaryati, N.L.G., Adnyana, I., Wahyudi, I.W., Dewi, I.A.U., Agustini, D.G.D.A., Putra, I. Polyherbal teh baper untuk obat tradisional (bioaktivitas, potensi dan prospeknya). CV. Media Sains Indonesia, UNHI Press, **2024**.
[23] Sudaryati, N.L.G., Utomo, B., Adnyana, I.M.D.M., Fauziyah, S., Hari, T., Sucipto, P.L.C., Dewi, I.G.A.G.S., Kautsar, R., Gede, D., Agustini, D. Phytochemical profiling, dengue antiviral properties, and cytotoxicity of novel baper tea polyherbal infusion: Insights from in silico and in vitro studies. *Salud Cienc Tecnol*, **2025**, 6, 1791.
[24] Adnyana, I.M.D.M., Sudaryati, N.L.G. Phytochemical analysis of the antioxidant compounds of baper tea and its potential as an immunomodulatory agent and candidate for standardized herbal medicine. *Trends in Sciences*, **2023**, 20(1), 6391-6391.
[25] Mohanty, M., Mohanty, P.S. Molecular docking in organic, inorganic, and hybrid systems: A tutorial review. *Monatshefte für Chemie-Chemical Monthly*, **2023**, 154(7), 683-707.
[26] Ramli, S., Wu, Y.S., Batumalaie, K., Guad, R.M., Choy, K.W., Kumar, A., Gopinath, S.C., Rahman Sarker, M.M., Subramaniyan, V., Sekar, M. Phytochemicals of withania somnifera as a future promising drug against sars-cov-2: Pharmacological role, molecular mechanism, molecular docking evaluation, and efficient delivery. *Microorganisms*, **2023**, 11(4), 1000.
[27] Kumah, R.T., Omondi, R.O., Smith, G.S., Ojwach, S.O. Molecular structures, dft calculations, in silico molecular docking, predicted pharmacokinetics and biological activities of n-(quinolin-8-yl)-2-pyridine/pyrazine carboxamide compounds. *Journal of Molecular Structure*, **2025**, 1319, 139425.

[28] Sebaugh, J. Guidelines for accurate ec50/ic50 estimation. *Pharmaceutical statistics*, **2011**, 10(2), 128-134.
[29] Cahyaningrum, P.L., Sudaryati, N.L.G., Bogoriani, N.W., Asih, I.A.R.A., Adnyana, I.M.D.M., Jenar, P.D. Phytochemical profiling, antibacterial properties and toxicity of amla fruit tea (phyllanthus emblica l.): An in vitro and in silico study. *Journal of Multidisciplinary Applied Natural Science*, **2025**, 5(3), 799-822.
[30] Hanson, G., Adams, J., Kepgang, D.I., Zondagh, L.S., Tem Bueh, L., Asante, A., Shirolkar, S.A., Kisaakye, M., Bondarwad, H., Awe, O.I. Machine learning and molecular docking prediction of potential inhibitors against dengue virus. *Frontiers in Chemistry*, **2024**, 12, 1510029.
[31] Lipinski, C.A., Lombardo, F., Dominy, B.W., Feeney, P.J. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Advanced Drug Delivery Reviews*, **2012**, 64, 4-17.
[32] Zhang, W.-X., Zhao, T.-Y., Wang, C.-C., He, Y., Lu, H.-Z., Zhang, H.-T., Wang, L.-M., Zhang, M., Li, C.-X., Deng, S.-Q. Assessing the global dengue burden: Incidence, mortality, and disability trends over three decades. *PLoS Neglected Tropical Diseases*, **2025**, 19(3), e0012932.
[33] Zainul, R., Ansori, A.N.M., Murtadlo, A.A.A., Sucipto, T.H., Kharisma, V.D., Widyananda, M.H., Muchtaromah, B., Fadholly, A., Kusala, M.K.J., Jakhmola, V. The recent development of dengue vaccine: A review. *Journal of Medicinal and Pharmaceutical Chemistry Research*, **2024**, 6(4), 362-382.
[34] Hossain, M.A., Dewan, P., Kawsar, S.M.A., Dangwal, A., Kalra, K., Kalra, J.M., Ashok, P.K., Parashar, T., Jakhmola, V., Saha, S. Chemical descriptors, admet, molecular docking and molecular dynamics simulation of mannopyranoside derivatives against smallpox virus proteins. *Advanced Journal of Chemistry, Section A*, **2025**, 8, 1-16.

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