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ORIGINAL

Phytochemical profiling, dengue antiviral properties, and cytotoxicity of novel Baper tea polyherbal infusion: Insights from in silico and in vitro studies

Perfil fitoquímico, propiedades antivirales contra el dengue y citotoxicidad de una nueva infusión polibotánica de té Baper: conclusiones de estudios in silico e in vitro

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ABSTRACT

Introduction: dengue virus infection remains a significant global health challenge with limited therapeutic options, necessitating the development of natural antiviral agents.
Objective: this study aimed to evaluate the phytochemical composition, antiviral efficacy, and safety profile of the Baper Tea polyherbal infusion against DENV-3 using integrated computational and experimental methodologies.
Method: gas chromatography-mass spectrometry (GC-MS) was used to identify bioactive compounds, and Fourier transform infrared (FT-IR) spectroscopy was used to characterize functional groups. In vitro antiviral assays were used to determine the effective concentration (EC_{50}) and cytotoxic concentration (CC_{50}) values. Molecular docking simulations were used to evaluate the binding affinity between the identified compounds and DENV-3 NS5 protein, followed by drug-likeness assessment and toxicity prediction.
Results: GC-MS analysis revealed 40 bioactive compounds, predominantly tetraacetyl-d-xylonic nitriles (11,73 %). FT-IR spectroscopy confirmed the characteristic hydroxyl, aliphatic C-H, C=C, and C-O functional groups of flavonoids, terpenoids, and glycosidic structures. In vitro assays demonstrated potent anti-DENV-3 activity (EC_{50}=19,02 µg/mL) with minimal cytotoxicity (CC_{50}=4,897,6 µg/mL), yielding an exceptional selectivity index (SI) of 257,5. The ten selected compounds exhibited drug-like properties with favorable toxicity profiles and organ safety parameters. Molecular docking revealed that W-18 exhibited the strongest binding affinity (-9,93 kcal/mol, Ki=52,35 nM) for the DENV-3 NS5 protein, forming complex interaction networks through conventional hydrogen bonds, Pi-donor hydrogen bonds, and pi-sulfur interactions, followed by phenanthrene and dihydroxanthin.

Conclusions: Baper Tea polyherbal infusion exhibits significant antiviral potential against DENV-3 through synergistic interactions of bioactive compounds targeting the NS5 protein methyltransferase domain, potentially disrupting viral RNA capping and replication mechanisms. These findings highlight the potential of Baper tea as a candidate for developing novel anti-dengue therapeutic agents.

Keywords: Antiviral Agents; NS5 Protein; Baper Tea; Infectious Disease; Molecular Docking Simulation; Cytotoxicity.

RESUMEN

Introducción: la infección por el virus del dengue sigue siendo un importante problema de salud mundial con opciones terapéuticas limitadas, lo que hace necesario el desarrollo de agentes antivirales naturales.
Objetivo: el objetivo de este estudio fue evaluar la composición fitoquímica, la eficacia antiviral y el perfil de seguridad de la infusión polibotánica Baper Tea contra el DENV-3 utilizando metodologías computacionales y experimentales integradas.
Método: se utilizó cromatografía de gases-espectrometría de masas (GC-MS) para identificar los compuestos bioactivos y espectroscopia de infrarrojo por transformada de Fourier (FT-IR) para caracterizar los grupos funcionales. Se utilizaron ensayos antivirales in vitro para determinar los valores de concentración efectiva (EC_{50}) y concentración citotóxica (CC_{50}). Se utilizaron simulaciones de acoplamiento molecular para evaluar la afinidad de unión entre los compuestos identificados y la proteína NS5 del DENV-3, seguidas de una evaluación de la similitud con los fármacos y una predicción de la toxicidad.
Resultados: el análisis GC-MS reveló 40 compuestos bioactivos, predominantemente nitrilos tetraacetil-d-xilónicos (11,73 %). La espectroscopia FT-IR confirmó los grupos funcionales característicos hidroxilo, C-H alifático, C=C y C-O de flavonoides, terpenoides y estructuras glicosídicas. Los ensayos in vitro demostraron una potente actividad anti-DENV-3 (EC_{50} = 19,02 µg/mL) con una citotoxicidad mínima (CC_{50} = 4897,6 µg/mL), lo que dio lugar a un índice de selectividad (SI) excepcional de 257,5. Los diez compuestos seleccionados mostraron propiedades similares a las de los fármacos, con perfiles de toxicidad favorables y parámetros de seguridad para los órganos. El acoplamiento molecular reveló que el W-18 exhibía la mayor afinidad de unión (-9,93 kcal/mol, Ki = 52,35 nM) por la proteína NS5 del DENV-3, formando complejas redes de interacción a través de enlaces de hidrógeno convencionales, enlaces de hidrógeno pi-donor e interacciones pi-azufre, seguido por el fenantreno y la dihidroxantina.
Conclusiones: la infusión polibotánica de té Baper muestra un importante potencial antiviral contra el DENV-3 a través de interacciones sinérgicas de compuestos bioactivos que se dirigen al dominio metiltransferasa de la proteína NS5, lo que podría alterar los mecanismos de encapsulación y replicación del ARN viral. Estos hallazgos destacan el potencial del té Baper como candidato para el desarrollo de nuevos agentes terapéuticos contra el dengue.

Palabras clave: Agentes Antivirales; Proteína NS5; Té Baper; Enfermedades Infecciosas; Simulación de Acoplamiento Molecular; Citotoxicidad.

INTRODUCTION

Dengue virus infection (DVI) is a significant global health challenge with limited therapeutic options available. The widespread decline in immune system function leaves individuals vulnerable to pathogenic agents, including viruses, bacteria, and fungi.[1] This vulnerability has contributed to the resurgence of infectious diseases such as dengue virus infection, chikungunya, and Zika in Indonesia, which may escalate into epidemics or pandemics.[2,3] Concurrently, increasing reports of antibiotic resistance and adverse effects of long-term medication use present additional challenges.[4,5] These circumstances highlight the need to develop natural plant-based medicines, particularly for immune enhancement and antiviral applications against dengue infection. This phenomenon is attributed to the heightened awareness of pharmacological safety and drug accessibility among the population.[6,7]

Owing to their antiviral, antioxidant, anti-inflammatory, and antimicrobial properties, plants have served as effective sources of traditional medicines that strengthen the immune system and address various diseases, including dengue.[8,9,10] Several native Indonesian plants, including A. cepa L., P. angulata L., and P. urinaria, have shown immunomodulatory and anti-DENV properties. A. cepa L. contains quercetin compounds, allicin and derivatives with multitarget antiviral effects on the dengue virus, blocking virus binding and suppressing NF-B activation, heat shock proteins, and viral protease inhibitors.[11,12,13,14,15] P. angulata leaves contain physalucoside, withanolides, physalin, epigallocatechin gallate, and other compounds with antimicrobial, antioxidant, and

dengue antiviral activities through mechanisms involving NF-κB activation, downregulation of Mcl-1 expression, inhibition of viral replication cycle stages, and anti-adsorption to DENV.[16,17,18,19] P. urinaria leaves contain nirtetralin, niranthin, hinokinin, phyllanthosterol, astragalin, ethyl gallate, and (-)-epicatechin, which have antioxidant, hepatoprotective, anti-inflammatory, antiviral and immunomodulatory activities. Their antiviral mechanisms affect early viral infection phases, inhibit viral entry and replication, induce B and T lymphocyte proliferation, and increase phagocytic activity, TNF-α release, and macrophage nitric oxide (NO) release.[20,21]

Previous studies have demonstrated that bioactive compounds from these plants can serve as potential antiviral agents.[22] However, research has primarily focused on crude extract identification, compound characterization, reviews, and single preclinical applications without the development of ready-to-use products. Lebdah et al.[13] demonstrated that oral administration of A. cepa essential oil in combination with a Newcastle disease virus (NDV) vaccine significantly attenuated or eliminated lethal clinical manifestations, pathological lesions, and viral shedding. Nur et al.[23] reported that A. cepa extract exhibited inhibitory activity against DENV-2 in vero cells indicating promising antiviral potential. Furthermore, withanolides derived from P. angulata L. have been documented to suppress HIV transcriptional activity,[18,24,25,26] while P. urinaria demonstrated broad-spectrum antiviral efficacy through inhibition of viral replication and genetic material synthesis across in vitro, in vivo, and in silico experimental paradigms.[27,28,29]

Our previous research revealed the antioxidant activity, phytoconstituents, and potential immunomodulatory properties of a formulation combining A. cepa L., P. angulata and P. urinaria leaves packaged as "Baper Tea polyherbal". The results revealed that this polyherbal tea contains various phytoconstituents, including flavonoids, tannins, polyphenols, and other compounds with strong antioxidant activity (IC50 of 24,27 ppm), a water content of 4,43 %, and α-glucosidase inhibition of 12,18 ppm.[20] Building on these preliminary findings, the present study addresses the critical need for standardized, evidence-based natural therapeutic interventions against dengue virus infections. Therefore, this study aimed to comprehensively evaluate the phytochemical profile, antiviral mechanisms, and therapeutic potential of Baper Tea polyherbal infusion against DENV-3 using systematic computational modeling and experimental validation approaches, specifically targeting the NS5 protein as the primary therapeutic target.

DENV-3 selection for this study was particularly relevant, as this serotype has been associated with severe clinical manifestations and higher hospitalization rates than other serotypes in Indonesia, accounting for ~40 70 % of dependent areas over the past five decades and proving challenging to manage and eradicate.[30,31,32,33,34] The structural distinctiveness of the DENV-3 NS5 protein, characterized by unique binding pocket configurations and allosteric sites distinct from those of other serotypes, provides exceptional opportunities for developing plant-derived, serotype-specific therapeutic interventions with enhanced selectivity and reduced off-target effects. This comprehensive investigation provides essential preclinical evidence supporting the rational development of standardized, nature-derived therapeutic formulations with validated antiviral mechanisms, addressing critical gaps in dengue management strategies for tropical disease-control programs.

METHOD

Type of study

This study employed an experimental research design with a completely randomized design to evaluate the anti-dengue properties of Baper Tea polyherbal infusion through integrated phytochemical profiling, computational analysis, and biological testing.[35] The investigation was structured as a three-phase experimental approach: phase one involved comprehensive phytochemical screening of bioactive compounds; phase two encompassed in silico molecular docking analysis against dengue virus serotype 3 (DENV-3) targets; and phase three comprised in vitro antiviral efficacy and cytotoxicity assessment.

Universe and sample

The study universe comprised polyherbal formulations with documented anti-dengue potential in the literature. The sample consisted of a standardized Baper Tea polyherbal infusion registered with the Indonesian Food and Drug Administration (BPOM number P-IRT 5105101010166-30) and produced by PT Mega Science Indonesia. The polyherbal formulation contains three botanical components: A. cepa L., P. angulata, and P. urinaria leaves in a 2:1:1 ratio, processed through standardized procedures, including collection, sorting, washing, drying using a 16 tray FDH-16 food dehydrator, grinding, packaging, and storage.[22] For cellular studies, Vero cells (African green monkey kidney epithelial cells; ECACC accession number 84113001; lot number 19E011) were used as the biological model system.

Variables

The primary independent variable was the Baper Tea polyherbal infusion concentration, ranging from 1,56 to 200 µg/mL, depending on the specific assay requirements. The dependent variables included phytochemical compound identification and quantification via gas chromatography-mass spectrometry, functional group

characterization through Fourier transform infrared spectroscopy, cell viability percentage, cytotoxic concentration causing 50 % cell death (CC_{50}), effective concentration inhibiting 50 % viral cytopathic effects (EC_{50}), selectivity index (SI), and molecular binding affinity scores from docking simulations. The controlled variables included incubation conditions (37°C, 5 % CO_2, specified time intervals), cell density (5×10^4 cells/mL), viral load (2×10^3 FFU/mL), and standardized laboratory protocols.

Data collection and processing

The study was conducted between May and August 2024 at the Medical Biology Laboratory, Universitas Hindu Indonesia, and the Dengue Laboratory, Institute of Tropical Disease, Universitas Airlangga, Indonesia.

Phytochemical profiling with gas chromatography–mass spectrophotometry

Comprehensive phytochemical profiling was conducted using gas chromatography-mass spectrometry (GC-MS) to identify and quantify the bioactive compounds. Sample preparation involved hot water extraction of 5 g of the polyherbal mixture at 90°C for 10 min using 100 mL of deionized water, followed by filtration through a 0,45 µm membrane, rotary evaporation concentration, and reconstitution in 1 mL of HPLC-grade methanol. Chromatographic separation was performed using a Perkin Elmer Clarus 500 system with a Clarus SQ 8S mass spectrometer and Elite-5 ms capillary column (30 m × 0,25 mm I.D. × 0,25 µm film thickness), helium carrier gas at 1,0 mL/min, and temperature programming from 110°C to 280°C. Mass spectral identification was performed using the NIST 2017 and Wiley Registry 11th edition libraries with a similarity index >85 % acceptance criteria. Semiquantitative analysis was expressed as the percentage contribution of each compound to the total ion chromatogram area (TICA).

FT-IR spectroscopic analysis

Fourier transform infrared (FT-IR) spectroscopy was used to characterize the functional groups in the Baper Tea polyherbal infusion using a Bruker Tensor II spectrometer with OPUS 8,0 software. The analysis used attenuated total reflectance (ATR) sampling with a universal accessory for direct liquid-sample measurements. A droplet of the freshly prepared infusion was placed on the surface of an ATR crystal. The spectra were recorded in the mid-infrared region (5000-400 cm^{-1}) with a 45 s scan time. Three replicate measurements were performed to ensure the reproducibility of the results. The spectra were processed using baseline correction, smoothing algorithms, and normalization to eliminate interference and enhance the signal quality. Peak identification and assignment were performed by comparing the spectral patterns with reference libraries and published data on herbal constituents. Characteristic absorption bands for functional groups, such as hydroxyl (-OH), carbonyl (C=O), aromatic ring, and glycosidic linkages, were identified and correlated with known bioactive compounds in the herbs of the Baper Tea polyherbal formulation.

Cell maintenance and propagation protocol

Vero cells (African green monkey kidney epithelial cells, ECACC accession number 84113001, lot number 19E011) were cultivated in minimum essential medium (MEM) enriched with 10 % heat-inactivated fetal bovine serum (FBS), and 1 % penicillin–streptomycin solution. The cells were maintained under standard physiological conditions in a humidified incubator at 37°C with a 5 % CO_2 atmosphere. Upon reaching 80–100 % confluence, which typically occurred every 3–4 days, the cells were subjected to subculturing procedures. The established monolayer was washed twice with phosphate-buffered saline (PBS; pH 7,4) to remove residual medium components. The cells were subsequently exposed to 0,25 % trypsin-EDTA solution and incubated for 5 min at 37°C to facilitate cell detachment from the culture surface. Following cell dissociation, the enzymatic reaction was terminated by the addition of complete MEM. The resulting cell suspension was centrifuged at 3500 rpm for 5 min, and the supernatant was removed. The resulting cell pellet was resuspended in fresh complete medium for subsequent experiments.[36]

Preparation of Baper tea polyherbal infusion

Five grams of Baper Tea polyherbal was infused in 100 mL of sterile distilled water at 90°C for 10 min. The infusion was filtered through a 0,22 µm sterile membrane filter, lyophilized, and the resulting powder was stored at -20°C until further use. For the experimental procedures, the lyophilized extract was reconstituted in complete MEM to prepare a stock solution (10 mg/mL), which was serially diluted to obtain the required concentration.

Cytotoxicity assay in Vero cells

The cytotoxic potential of Baper Tea polyherbal infusion was assessed via a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) colorimetric assay. Vero cells in the exponential growth phase were harvested, and their viability was confirmed using trypan blue exclusion. The cell suspension was standardized

to 5×10^4 cells/mL in complete minimum essential medium (MEM). A total of 100 µL of this suspension was dispensed into each well of a 96-well microplate, followed by incubation for 24 h at 37°C in 5 % CO_2 to facilitate cellular attachment. After incubation, the culture supernatant was aspirated, and the cell layer was washed thrice with phosphate-buffered saline (PBS).

The baper tea infusion was serially diluted in MEM to generate concentrations ranging from 3,31 to 200 µg/mL. Each concentration (100 µL) was added to the wells in triplicates. The controls included wells with complete medium alone or without cells. The treated cultures were then incubated for 24 h under standard conditions. After treatment, the medium containing the samples was removed, and the samples were washed with phosphate-buffered saline (PBS). Viable cell metabolic activity was determined by adding 10 µL of MTT solution (5 mg/mL in PBS) and 100 µL of complete medium to each well and incubating for 4 h at 37°C. Metabolically active cells reduce tetrazolium to form insoluble formazan crystals. After aspiration and washing, 50 µL of dimethyl sulfoxide (DMSO) was added to solubilize the formazan crystals. The microplates were agitated for 10 min, and the absorbance was measured at 595 nm using a spectrophotometer. The percentage of viable cells was calculated relative to that of the untreated control.

% Cell viability = (Absorbance of treated cells - Absorbance of medium control)/(Absorbance of cell control - Absorbance of medium control) × 100 % (1)

The cytotoxic concentration that reduced cell viability by 50 % (CC_{50}) was determined by plotting the percentage of cell viability against the sample concentration and applying a linear regression analysis. The formula y = Bx + A (where y represents % cell viability and x represents concentration) was used to calculate the CC_{50} value by substituting 50 % for y and solving for x.

Anti-dengue virus serotype 3 (DENV-3) activity assay

The antiviral activity of the Baper Tea polyherbal extract against DENV-3 was evaluated using the Viral ToxGlo Assay, which measures cell viability based on ATP quantification. Vero cells were cultivated in 96-well flat-bottom luminescence microplates at 5×10^4 cells/mL, with 100 µL of cell suspension per well. The plates were incubated for 24 h at 37°C with 5 % CO_2 to establish cellular adherence. The culture medium was aspirated, and the cells were exposed to the polyherbal extract at concentrations ranging from 1,56 to 100 µg/mL, at 25 µL per well in triplicate. The controls received 50 µL of complete medium (cell control), DENV-3 suspension (virus control), or a medium without cells (medium control). Following extract administration, 25 µL of DENV-3 suspension (2×10^3 FFU/mL) was added to the experimental wells, except for the cell and culture medium controls. The plates were incubated for 48–144 h at 37°C with 5 % CO_2. After incubation, 100 µL of Viral ToxGlo Assay reagent was added to each well, according to the manufacturer's protocol, to quantify cell viability. The plates were shaken for 10 min at room temperature and incubated for 10 min at 37°C, and luminescence was measured using a GloMax luminometer in relative light units (RLUs). The percentage of cytopathic effect (CPE) inhibition was calculated using the following formula:

% CPE Inhibition= (Luminescence of treatment - Luminescence of medium control)/(Luminescence of (virus control + cell control) - Luminescence of medium control) × 100 % (2)

The effective concentration required to inhibit 50 % of the virus-induced CPE (EC_{50}) was determined by plotting the percentage of CPE inhibition against the sample concentration and applying a linear regression analysis. The selectivity index (SI), which indicates the relative safety of the extract as an antiviral agent, was calculated as the ratio of CC_{50} to EC_{50}.

Molecular docking analysis, validation protocol, and complex visualization

A comprehensive in silico analysis was conducted to evaluate the potential inhibitory activity of the bioactive compounds in the Baper Tea polyherbal infusion against DENV-3. The study employed a workflow that included ligand and protein preparation, molecular docking simulation, analysis of binding interactions, toxicity and allergenicity prediction, and validation of docking results using multiple scoring functions and redocking procedures. The chemical structures of the major bioactive compounds in Baper tea polyherbal were obtained from PubChem in *sdf format based on previous GC–MS phytochemical profiling. Three-dimensional structures were optimized using the MMFF94 force field in Open Babel, with Gasteiger–Hückel partial charges assigned and rotatable bonds defined. Ligands were prepared in the PDBQT format using AutoDockTools 1.2.0 with the appropriate torsional degrees of freedom. The DENV-3 target protein structures were retrieved from the RCSB PDB using the PDB ID 5JJS.

A computational toxicity assessment was conducted using ProTox-II to generate toxicity profiles (hepatotoxicity, carcinogenicity, immunotoxicity, mutagenicity, and cytotoxicity). Drug-likeness and ADME

properties were predicted using SwissADME. The physicochemical parameters assessed included molecular weight, TPSA, number of hydrogen bond donors and acceptors, and cLogP. Drug likeness was evaluated based on compliance with Lipinski's rule of five. Molecular docking was performed using AutoDock Vina 1.2.0 and visualized using Discovery Studio Visualizer 2021. The 2D structures of the test compounds were downloaded from PubChem in the *pdb format. 3D and conformational optimizations were performed using ChemDraw. The original ligand was separated from the protein complex structure and reassembled during the method validation. Validation was performed by redocking the cocrystallized ligands (RMSD < 2,0 Å was considered acceptable), and high-ranking compounds were prioritized for further analysis.[37]

Binding interactions were characterized based on hydrogen bonds, hydrophobic interactions, π–π stacking, cation–π interactions, and salt-bridge interactions. The distance threshold for the hydrogen bonds was 3,5 Å, with a minimum bond angle of 120°. Two-dimensional interaction diagrams illustrating the specific amino acid residues involved in ligand binding. A comparison with the native ligand-binding patterns was performed to assess the similarity of the interaction profiles between the Baper Tea compounds and the known inhibitors. Compounds with interaction patterns similar to those of established antiviral agents are promising candidates for further investigation.

Statistical and data analysis

The data of this study in the form of phytochemical profiling via GC–MS and FT–IR were analyzed qualitatively, whereas the data of dengue antiviral activity (EC50) and cytotoxicity (CC50) in vitro were analyzed via nonlinear regression analysis (curve fit) with the model [Inhibitor] vs. normalized response–variable slope using GraphPad Prism software, LLC. Premium version 10.3.1 on a MacBook. In silico dengue antiviral activity data were analyzed through physicochemical analysis and drug-likeness assessment, ADME profiling, prediction of toxicity levels and organ toxicity of the compounds, and types of interactions between ligands and target proteins using molecular docking.

Ethical Standards

This study was approved by the Health Research Ethics Commission of the Faculty of Medicine and Health Sciences, Universitas Warmadewa (protocol number 213.01.05.2024–37/Unwar/ FKIK/EC-KEPK/V/2024).

RESULTS

Gas chromatography-mass spectrometry

Table 1. Bioactive compounds found in Baper tea polyherbal infusion

Bioactive compounds	RT (Min)	MF	MW (g/mol)	% Area	FG
Tetraacetyl-d-xylonic nitrile	2,013	$C_{14}H_{17}NO_9$	343,29 g/mol	11,726	Nitriles, ester, and ketone
Dihydroxanthin	2,524	$C_{17}H_{24}O_5$	308,4 g/mol	3,007	Dehydrofuran, heptacycle, ester, ketone, and unsaturated C-H
1,4-Dioxane-2,6-dione	2,739	$C_4H_4O_4$	116,07 g/mol	2,168	Dioxane, and Ketone
Terpinen-4-ol	7,756	$C_{10}H_{18}O$	154,25 g/mol	1,16	Hydroxy, Aliphatic C-H, and Unsaturated C-H
(1S,3R,6R)-(-)-4-Carene	2,949	$C_{10}H_{16}$	136,23 g/mol	1,09	Aliphatic C-H, Nitriles, and Unsaturated C-H
Tetraacetyl-d-xylonic nitrile	3,189	$C_{14}H_{17}NO_9$	343,29 g/mol	0,832	Ester and Ketone
n-Propyl 9-tetradecenoate	3,039	$C_{17}H_{32}O_2$	268,4 g/mol	0,762	Aliphatic C-H, Ester, and Unsaturated C-H
O-Trimethylsilyl-N-Methyl nicotinimidate	3,309	$C_{10}H_{16}N_2OSi$	180,16 g/mol	0,421	Pyridine, and Trimethylsilyl Imine
1-(2-Acetoxyethyl)-3,6-diazahomoadamantan-9-one oxime	3,234	$C_{13}H_{21}N_3O_3$	267,32 g/mol	0,287	Hydroxy, and Ester
(8xi,12xi)-9-Methoxyimino-11,15-bis (trimethylsilyloxy) prost-13-en-1-oic acid trimethylsilyl ester	39,038	$C_{30}H_{61}NO_5Si_3$	421,90 g/mol	0,254	Trimethylsilyl, Aliphatic C-H, Ester, and Methyloxime Unsaturated C-H

GC–MS characterization of the Baper Tea polyherbal infusion revealed 40 primary compounds (table 1), with 10 major components identified at various retention times (RTs), peak areas, and area percentages. The

retention times ranged from 2,013 to 39,038 min. Tetraacetyl-d-xylonic nitriles were the most abundant, with an area percentage of 11,726 %. Other compounds presented lower area percentages, ranging from 3,007 % (dihydroxanthin) to 0,254 % ((8xi,12xi)-9-methoxyimino-11,15-bis(trimethylsilyloxy) prost-13-en-1-oic acid trimethylsilyl ester). Chemical structure analysis identified aliphatic, cyclic, aromatic, and terpene compounds containing nitrile, ester, ketone, alcohol, double bonds, cyclic ether, silyl, and oxime functional groups.

Fourier transform infrared (FT-IR) spectroscopic analysis

Fourier transform infrared spectroscopy (FT-IR) analysis identified the presence of hydroxyl groups (3279,20 cm^{-1}), aliphatic C–H stretching (2917,72 and 2849,73 cm^{-1}), C=C stretching (1633,80 cm^{-1}), and C-O stretching (1012,05 cm^{-1}). Additional peaks were observed at 516,18 cm^{-1} and 414,07 cm^{-1}. The functional group analysis of Baper tea polyherbal infusions via FT-IR is presented in figure 1.

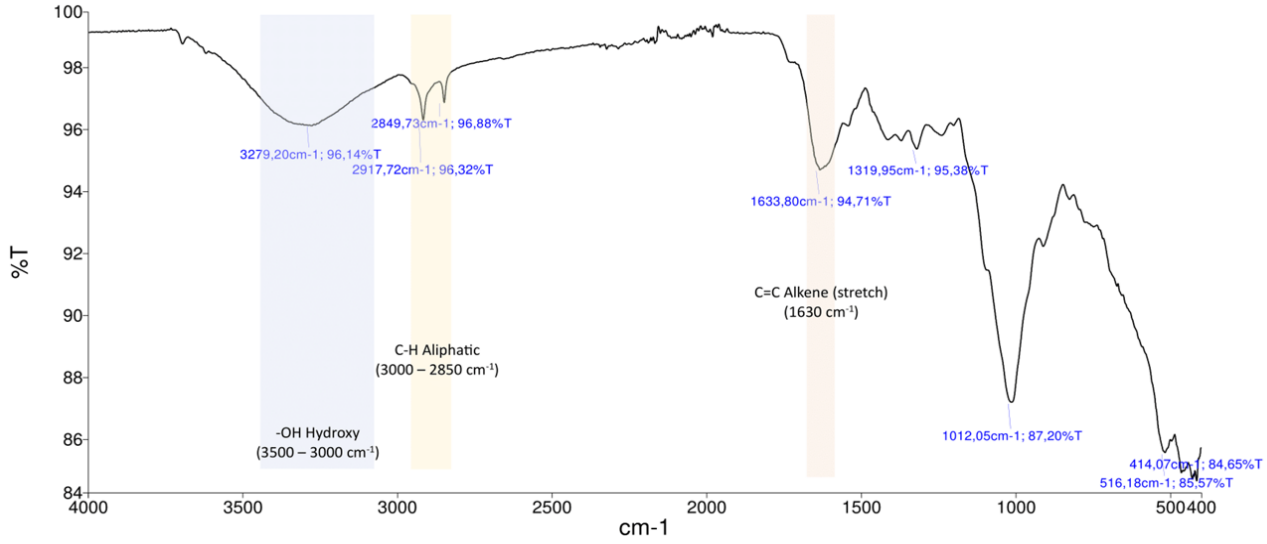

Figure 1. Functional group analysis of Baper tea polyherbal infusions using FT-IR

Anti-DENV-3 activity and Cytotoxicity assay

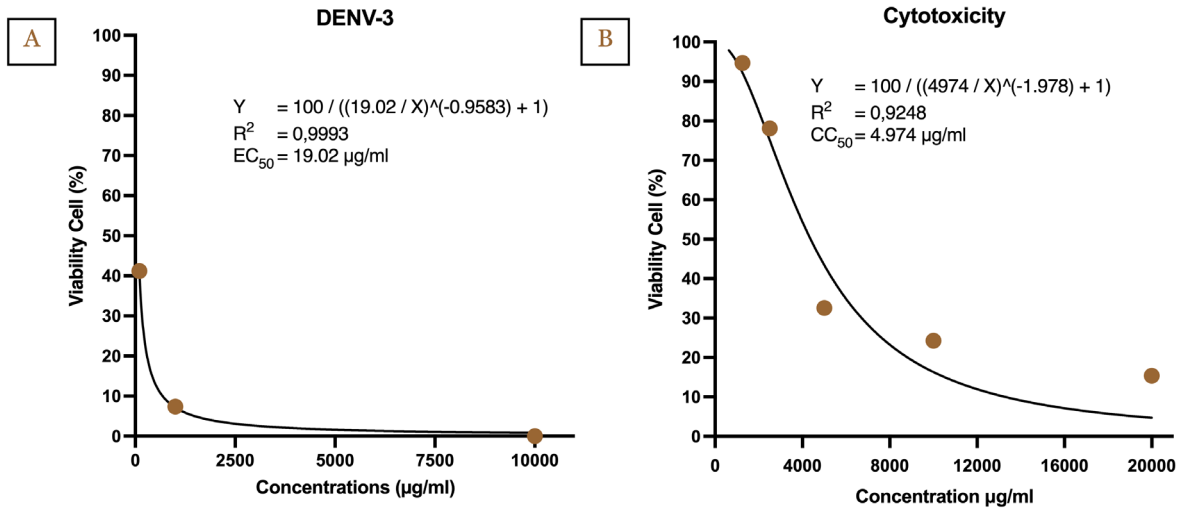

Figure 2. Dose-response relationship of Baper Tea polyherbal infusion against DENV-3. The EC_{50} value was determined via nonlinear regression analysis (curve fit) with the [inhibitor] vs. normalized response-variable slope model (A), dose-response curve for assessing the cytotoxicity of Baper Tea polyherbal infusion in Vero cells. The sigmoidal curve illustrates the relationship between the concentration of Baper Tea polyherbal infusion (µg/ml) and cell viability (%) (B)

In vitro efficacy assessment of the Baper Tea polyherbal infusion revealed inhibitory activity against DENV-3 at concentrations of 100, 1000, and 10 000 µg/ml. DENV-3 exhibited an EC50 of 19,02 µg/ml with a dose-

response curve fit of R^2 = 0,9993 (99,93 %). The inhibition pattern demonstrated a dose-dependent trend, where viral viability decreased from approximately 40 % at lower concentrations to nearly 0 % at 10 000 µg/ml (figure 2A). Cytotoxicity testing in Vero cells yielded a CC_{50} value of 4897,6 µg/ml. The Pearson correlation coefficient (r) of -0,8210 demonstrated a strong negative correlation between the concentration and cell viability. The R^2 value of 0,9248 indicated that 92,48 % of the variation in cell viability was explained by concentration changes (figure 2B).

Molecular docking analysis

Physicochemical analysis and drug-likeness assessment

A comprehensive analysis of the physicochemical properties of the compounds isolated from the Baper Tea polyherbal infusion revealed complete compliance with Lipinski's rule for all five parameters (table 2). All ten selected compounds demonstrated favorable drug-like properties. This unanimous adherence to Lipinski's parameters provides strong evidence for their potential pharmaceutical applications. All examined compounds, including structurally diverse constituents, presented favorable parameters for molecular weight (<500 g/mol), consensus Log P (≤5), H-bond donors (<10), and H-bond acceptors (<5). α-D-galactopyranose, despite having six H-bond acceptors (slightly exceeding the standard threshold of <5), still maintains its classification as drug-like with zero violations.

Table 2. Physicochemical analysis and drug-likeness assessment

Compound	Molecular weight (D) (g/mol)	Consensus Log P	H-bond donor	H-bond Acceptor	Violation of Lipinski's rule
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; 0 violation
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

Predicted toxicity levels of the compounds from Baper tea polyherbal infusion

Table 3. Predicted toxicity levels of the compounds from Baper tea polyherbal infusion

Compound	Predicted LD_{50} (mg/kg)	Predicted toxicity class	Average similarity	Prediction accuracy
Tetraacetyl-d-xylonic	7000 mg/kg	Six (nontoxic)	69,85	68,07
Terpinen-4-ol	1016 mg/kg	Four (harmful)	100	100
Dihydroxanthin	1410 mg/kg	Four (harmful)	71,2	69,26
1,4-dioxane-2,6-dione	2000 mg/kg	Four (harmful)	68,41	68,07
N-propyl 9-	339 mg/kg	Four (harmful)	97,73	72,9
2,7-Diphenyl-1,6-	2300 mg/kg	Five (Possibly Hazardous)	66,93	68,07
Phenanthrene, 9-	2000 mg/kg	Four (harmful)	100	100
Alpha-D-Galactopyranose (alpha-D-galactose)	23 000 mg/kg	Six (nontoxic)	54,27	67,38
Prost-13-en-1-oic-acid	186 mg/kg	Three (toxic swallowed)	100	100
W-18((E)-4-Chloro-N-(1-(4-nitrophenethyl) piperidin-2-ylidene)benzenesulfonamide)	300 mg/kg	Three (toxic swallowed)	50,57	67,38

The prediction of toxicity levels (table 3) revealed that the toxicity classes of the compounds in the Baper Tea polyherbal infusion ranged from toxic (class 3) to non-toxic (toxicity class 6). Specifically, there are two compounds belonging to the nontoxic class, namely, tetraacetyl-xylonic and-D-galactopyranose. Moreover, five compounds were categorized as harmful (toxicity class 4), including terpinen-4-ol, dihydroxanthin, 1,4-dioxane-2,6-dione, N-propyl 9-, and phenanthrene 9. One compound was potentially hazardous (toxicity class 5), namely, 2,7-diphenyl-1,6. Additionally, two compounds exhibited significant toxicity (class 3), classified as "toxic when swallowed": Prost-13-en-1-oic acid and W-18((E)-4-Chloro-N-(1-(4-nitrophenethyl) piperidin-2-ylidene) benzenesulfonamide).

Toxicological evaluation of the compounds in the Baper Tea polyherbal infusion revealed diverse safety profiles for the different compounds. This assessment documented toxicity classifications from non-toxic to toxic, highlighting the complex pharmacological properties of these compounds. α-D-galactopyranose exhibited a favorable safety profile, with an LD_{50} of 23 000 mg/kg, indicating minimal toxicity at high doses. However, this requires cautious interpretation because of its low prediction similarity index (54,27 %) compared to that of the other constituents. In contrast, compounds such as N-propyl 9- derivatives and W-18 analogs were classified as harmful or toxic. The reliability of these predictions is supported by high prediction accuracy values (67,38–100 %) and similarity indices of 100 % for several compounds, including terpinen-4-ol and phenanthrene 9-.

Toxic doses and toxicity classes were defined according to the Globally Harmonized System of Classification of Labeling of Chemicals (GHS).

Predicted organ toxicity of the compounds from Baper tea polyherbal infusion

The results of organ toxicity prediction (table 4) revealed that the compounds in the Baper Tea polyherbal infusion exhibited varying degrees of specific organ toxicity potential. The assessment revealed that among the 10 compounds analyzed, several demonstrated activity in specific areas of toxicity. With respect to hepatotoxicity, only tetra-O-acetyl-d-xylonic acid showed potential liver-damaging properties. Regarding carcinogenicity, no compounds were predicted to exhibit significant cancer-inducing potential, with all values marked as inactive. However, immunotoxicity appears to be a concern for multiple compounds, including tetraacetyl-d-xylonic, dihydroxanthin, phenanthrene 9-, and Prost-13-en-1-oic acids, all of which demonstrate predicted immunological toxicity. In terms of mutagenicity, W-18 exhibited borderline activity, indicating potential DNA-damaging properties, whereas all other compounds were classified as inactive.

Table 4. Physicochemical analysis and drug-likeness assessment

Compounds	Organ Toxicity				
	Hepato-toxicity	Carcino-genicity	Immuno-toxicity	Muta-genicity	Cyto-toxicity
Tetraacetyl-d-xylonic	0,84**	0,53*	0,99**	0,67*	0,76*
Terpinen-4-ol	0,8*	0,72*	0,99*	0,83*	0,88*
Dihydroxanthin	0,77*	0,51*	0,56**	0,88*	0,8*
1,4-dioxane-2,6-dione	0,85*	0,52*	0,99*	0,72*	0,79*
N-propyl 9-	0,76*	0,53**	0,96*	0,98*	0,76*
2,7-Diphenyl-1,6-	0,69*	0,53**	0,99*	0,56*	0,63*
Phenanthrene, 9-	0,79*	0,67*	0,97**	0,93*	0,87*
Alpha-D-Galactopyranose (alpha-D-galactose)	0,98*	0,82*	0,99*	0,81*	0,87*
Prost-13-en-1-oic-acid	0,93*	0,76*	0,51**	0,97*	0,63*
W-18((E)-4-Chloro-N-(1-(4-nitrophenethyl)piperidin-2-ylidene)benzenesulfonamide)	0,61 *	0,51*	0,97*	0,50**	0,71*

Molecular docking compounds targeted by DENV-3

The validation method used in the molecular docking analysis is presented in table 5, while the Native Ligand-NS5 Protein (S-adenosyl-L-homocysteine (SAH) $C_{14}H_{20}N_6O_5S$) DENV-3 interactions in both 2-D and 3-D forms are visualized in figure 3. The validation process confirmed the reliability of the docking protocol, with an RMSD value of 0,936 Å for DENV-3, indicating excellent re-docking accuracy (RMSD < 2 Å). The native ligand exhibited a binding affinity of -6,84 kcal/mol, with an inhibition constant (Ki) of 9,73 μM against the NS5 protein of DENV-3. The 2D interaction analysis revealed five interactions between the native ligand and the NS5 protein of DENV-3. These interactions included two salt bridges and pi-cation interactions (TRP87 and ASP146), five conventional hydrogen bond interactions (SER56, GLY85, GLY86, THR104, and ASP131), two unfavorable donor–donor interactions, and two unfavorable acceptor–acceptor interactions (CYS82 and VAL132).

Tabel 5. Method validation in docking molecular methods			
Serotype	**RMSD (Å)**	**Binding Energy (ΔG) (kcal/mol)**	**Inhibition Constant (Ki)**
DENV-3	0,936	-6,84	9,73 uM

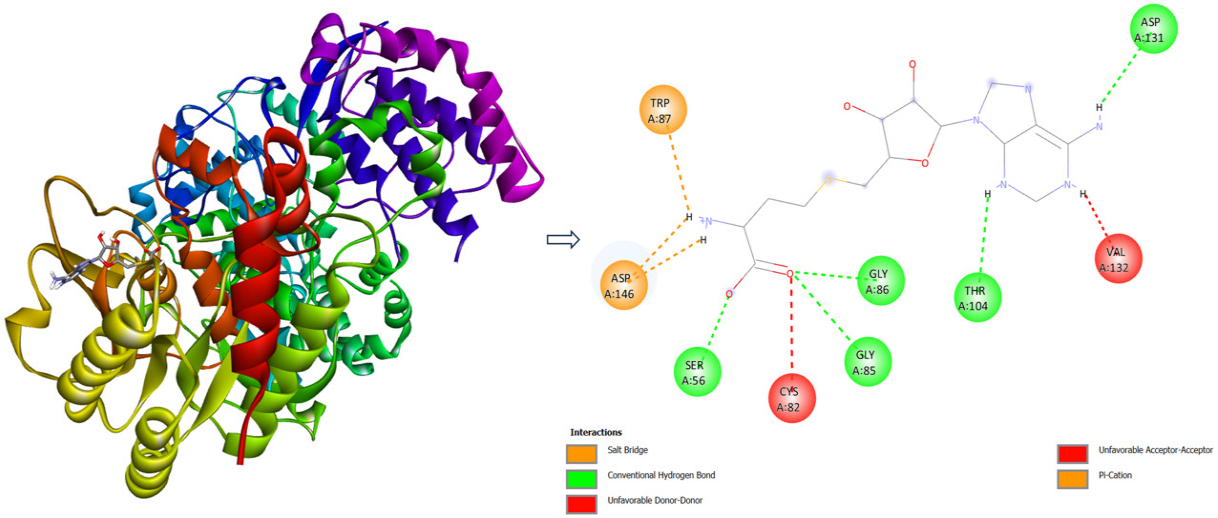

Figure 3. Visualization of the native ligand-NS5 protein interaction of DENV-3

Molecular docking analysis of the compounds in the Baper Tea polyherbal infusion revealed varying degrees of binding affinity for DENV-3 (table 6). Among all the tested compounds, W-18 demonstrated the strongest binding affinity, with a ΔG value of -9,93 kcal/mol and an inhibition constant (Ki) of 52,35 nM, indicating its potential as a potent inhibitor. Three compounds exhibited comparable moderate binding strengths: phenanthrene (-7,41 kcal/mol, Ki = 3,70 μM), dihydroxanthin (-7,40 kcal/mol, Ki = 3,74 μM), and 2,7-diphenyl-1,6- (-7,40 kcal/mol, Ki = 3,75 μM). Prost-13-en-1-oic acid also showed promising interactions, with a binding energy of -7,17 kcal/mol (Ki = 5,54 μM). The remaining compounds displayed moderate to weak binding affinities: N-propyl 9- (-5,81 kcal/mol, Ki = 54,70 μM), terpinen-4-ol (-5,63 kcal/mol, Ki = 75,23 μM), and tetraacetyl-d-xylonic (-6,25 kcal/mol, Ki = 26,16 μM), and considerably weaker interactions for 1,4-dioxane-2,6-dione (-4,66 kcal/mol, Ki = 383,28 μM) and alpha-D-galactopyranose (-4,46 kcal/mol, Ki = 540,69 μM). These results suggest that W-18 may serve as a lead compound for DENV-3 inhibitor development, with a binding affinity approximately 14-fold stronger than that of the next most potent compound and significantly stronger than that of the native ligand interaction. Molecular visualization revealed specific interaction patterns between the compounds and the NS5 protein binding pocket, highlighting their exceptional potential as antiviral candidates against the dengue virus.

Table 6. Summary of the molecular docking results		
Compound	**Binding Energy (ΔG) (kcal/mol)**	**Inhibition Constant (Ki)**
Tetraacetyl-d-xylonic	-6,25	26,16 μM
Terpinen-4-ol	-5,63	75,23 μM
Dihydroxanthin	-7,40	3,74 μM
1,4-dioxane-2,6-dione	-4,66	383,28 μM
N-propyl 9-	-5,81	54,70 μM
2,7-Diphenyl-1,6-	-7,40	3,75 μM
Phenanthrene, 9-	-7,41	3,70 μM
Alpha-D-Galactopyranose (alpha-D-galactose)	-4,46	540,69 μM
Prost-13-en-1-oic-acid	-7,17	5,54 μM
W-18 ((E)-4-Chloro-N-(1-(4-nitrophenethyl) piperidin-2-ylidene)benzenesulfonamide)	-9,93	52,35 nM

Binding sites of Baper tea polyherbal infusion compounds against DENV-3

According to the binding site analysis (table 7), the compounds in the Baper Tea polyherbal infusion demonstrated diverse interaction patterns with the DENV-3 protein. Figure 4 shows the three compounds that best inhibited development and bound to DENV-3 protein. W-18, which exhibited the strongest binding affinity, formed the most comprehensive interaction network comprising conventional hydrogen bonds with ASP79, CYS82, THR104, HIS110, and GLU111 and carbon–hydrogen bonding with GLY83. Notably, W-18 was the only compound that engaged in π-donor hydrogen bonding with LYS105 and π-sulfur interactions with HIS110 while also forming a π-π T-shaped interaction with TRP87, multiple alkyl/π-alkyl interactions with ILE147, VAL132, and PHE133, and π-sigma bonding with ILE147.

Table 7. The binding sites of compounds from Baper tea polyherbal infusion

Compound	Binding site of compounds	
	Interaction	Amino acid residues
Tetraacetyl-d-xylonic	Conventional hydrogen Bond	VAL132, GLY148, ARG163, LYS105, GLY106
Terpinen-4-ol	Conventional hydrogen Bond	TRP87, GLY86, CYS82
	Pi-Alkyl	TRP87
	Pi-Sigma	TRP87
Dihydroxanthin	Conventional hydrogen Bond	TRP87, GLY86, ARG84, GLY148
1,4-dioxane-2,6-dione	Conventional hydrogen Bond	SER56, TRP87, GLY85, ARG84
	Carbon Hydrogen Bond	GLY58
N-propyl 9-	Conventional hydrogen Bond	GLY86, SER56,
	Alkyl	ILE147, VAL132, LYS105
	Pi-Alkyl	TRP87
	Pi-Sigma	TRP87
2,7-Diphenyl-1,6-	Conventional hydrogen Bond	SER56, ARG84, GLY85
	Carbon Hydrogen Bond	GLY83
	Alkyl	ILE147
	Pi-Alkyl	TRP87
Phenanthrene, 9-	Conventional hydrogen Bond	GLU149, THR104, ARG163
	Carbon Hydrogen Bond	HIS110
	Pi-Pi T Shaped	ILE147
	Alkyl	ILE147, PHE133, LYS105
	Pi-Alkyl	ILE147
Alpha-D-Galactopyranose (alpha-D-galactose)	Conventional hydrogen Bond	ASP146, GLY81, ARG84, CYS82, TRP87
	Carbon Hydrogen Bond	CYS82
	Unfavorable Donor-Donor	GLY85
Prost-13-en-1-oic-acid	Conventional hydrogen Bond	LYS105, THR104, ARG84, GLY86, GLY85, SER56
	Alkyl	VAL132, ILE147
W-18 ((E)-4-Chloro- N-(1-(4-nitrophenethyl) piperidin-2-ylidene) benzenesulfonamide)	Conventional hydrogen Bond	ASP79, CYS82 THR104, HIS110, GLU111
	Carbon Hydrogen Bond	GLY83
	Pi-Donor Hydrogen Bond	LYS105
	Pi-Sulfur	HIS110
	Pi-Pi T Shaped	TRP87
	Alkyl	ILE147, VAL132, PHE133,
	Pi-Alkyl	ILE147

Phenanthrene 9 also displayed a multifaceted interaction pattern, forming conventional hydrogen bonds with GLU149, THR104, and ARG163, carbon hydrogen bonding with HIS110, π–π interactions with ILE147, alkyl interactions with ILE147, PHE133, and LYS105, and π–alkyl interactions with ILE147. Other compounds with moderate binding affinities exhibited fewer types of interactions. Dihydroxanthin interacted primarily through conventional hydrogen bonds with TRP87, GLY86, ARG84, and GLY148, whereas 2,7-diphenyl-1,6- formed conventional hydrogen bonds with SER56, ARG84, and GLY85, complemented by carbon hydrogen bonding with GLY83 and alkyl/Pi-alkyl interactions. Interestingly, α-D-galactopyranose was the only compound that exhibited unfavorable donor–donor interactions with GLY85, which may explain its weak binding affinity. Tetraacetyl-xylonic acid engaged solely through conventional hydrogen bonds with multiple residues (VAL132, GLY148, ARG163, LYS105, and GLY106), limiting its binding.

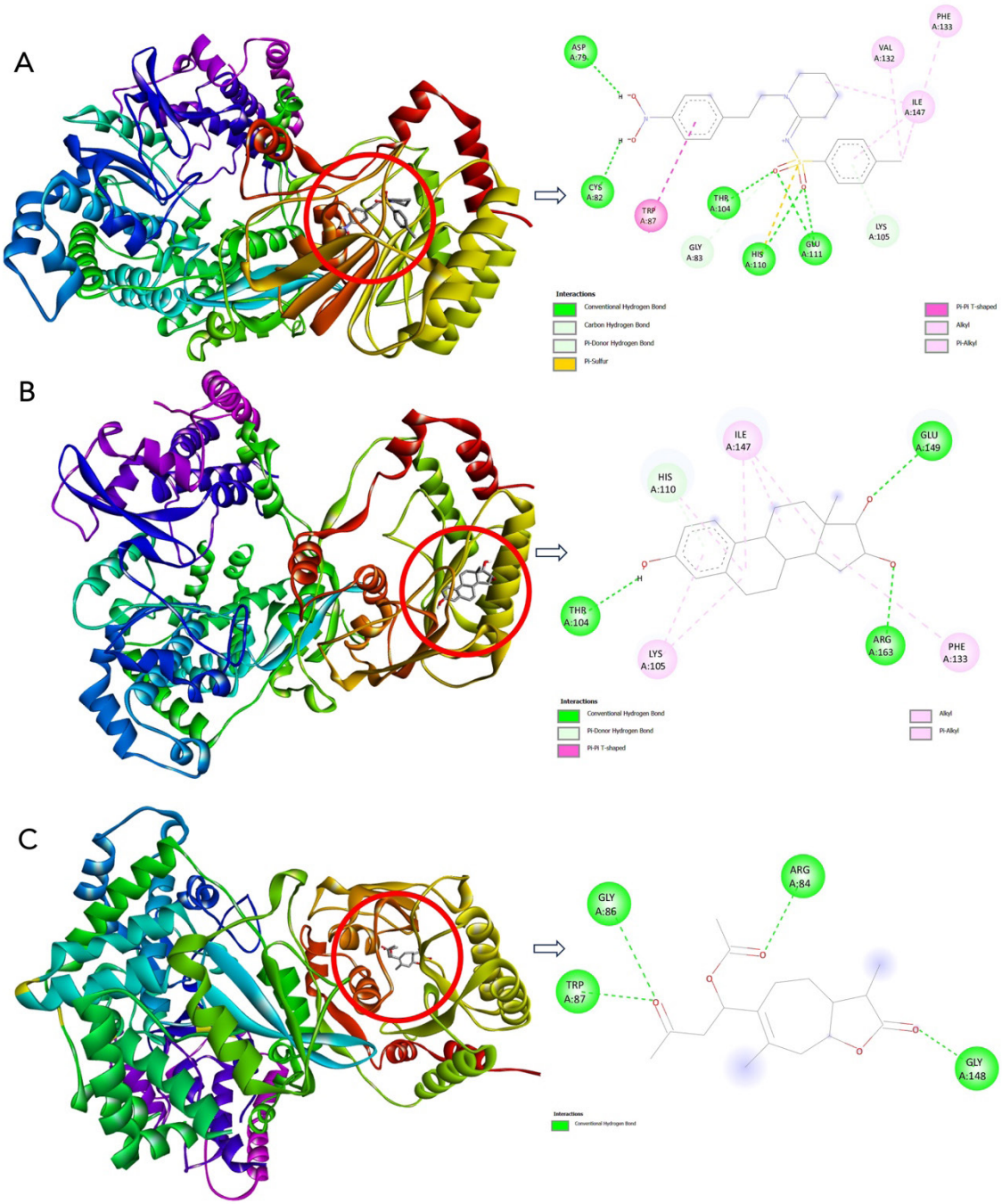

Figure 4. 3D (left) and 2D (right) visualization and binding site (red circle) of the Baper Tea Polyherbal infusion compounds with DENV-3 NS5 protein. (A) W-18 ((E)-4-chloro-N-(1-(4-nitrophenethyl) piperidin-2-ylidene) benzenesulfonamide), (B) phenanthrene, 9-, (C) dihydroxanthin

DISCUSSION

A comprehensive analysis of Baper Tea polyherbal infusion via GC–MS and FT–IR spectroscopy revealed a complex mixture of bioactive compounds with diverse chemical structures and functional groups. GC–MS identified 40 primary compounds, with tetraacetyl-d-xylonic nitriles being the most abundant (11,726 % area). The retention times ranged from 2,013–39,038 min, indicating variations in volatility and polarity, with lower RT compounds indicating more volatile and less polar compounds, whereas higher RT compounds are less volatile and more polar.[38] The diversity of structures, including aliphatic, cyclic, aromatic, terpene, and complex compounds featuring functional groups such as nitriles, esters, ketones, hydroxyl groups, and unsaturated C–H bonds, provides information on the complexity of the therapeutic effects resulting from Baper Tea polyherbal infusion.[39,40] The presence of nitrile groups indicates antiviral properties via interference with viral protein function or host–virus interactions.[41,42]

FT-IR analysis confirmed the presence of hydroxyl groups (broad peaks at 3500–3000 cm^{-1}), aliphatic C–H stretching (2917,72 cm^{-1} and 2849,73 cm^{-1}), C=C stretching (1633,80 cm^{-1}), and C–O stretching (1012,05 cm^{-1}), indicating the presence of bioactive compounds such as flavonoids, polyphenols, saponins, terpenoids, and glycosidic structures that are known to have therapeutic effects, such as antioxidant, antiviral, antibacterial, anticancer, and other effects.[8,13,43] The presence of hydroxyl groups indicates the ability of a compound to form hydrogen bonds, which can increase aqueous solubility and interactions with biological targets. The hydroxyl-rich composition suggests the potential for hydrogen bonding with viral proteins.[44]

The presence of strong C-O groups indicates the presence of glycosidic or ester structures that affect the pharmacokinetics and pharmacodynamics of the compound, including its absorption and distribution in the body. The identified aliphatic hydrocarbon chains may contribute to the lipophilic nature of the compound, which is important for cell membrane penetration and interaction with lipophilic targets.[45] The presence of unsaturated bonds may facilitate π-stacking interactions with aromatic amino acid residues, a mechanism commonly associated with antiviral compounds. These findings are consistent with those of previous studies, highlighting the therapeutic potential of polyherbal formulations. Anwar et al.[46] identified similar bioactive compounds in herbal infusions with antiviral properties.

This study confirmed the antiviral effect of Baper Tea polyherbal infusion in vitro against dengue virus serotype 3 (DENV-3). The findings revealed very strong antiviral activity (EC50= 19,02 µg/mL) after inoculation of Baper Tea polyherbal infusion on DENV-3. This finding provides evidence that this polyherbal formulation provides good antiviral insights for its development as an antiviral candidate. This result is comparable to the findings of Alagarasu et al.[36], who reported an EC50 of 18,5 µg/mL for a polyherbal extract against DENV-3, as did Halim et al.[47], Low et al.[48], and Rosmalena et al.[49], who reported that several herbal compounds have strong antiviral activity against DENV-3. These findings suggest that the Baper Tea polyherbal infusion has the same potential as an antidengue agent. The constituents of Baper Tea polyherbal in the form of A. cepa L., P. angulata, and P. urinaria leaves are believed to be antivirals. Lim et al.[8] revealed that these three plants had moderate to very strong antiviral effects against different DENV serotypes.

The inhibition pattern was dose dependent, indicating that the antiviral effect of Baper Tea polyherbal infusion increased with increasing concentration, which was supported by a very strong model fit (R^2 = 0,9993). This serotype-specific activity could be attributed to the unique interaction between DENV-3 viral proteins and the bioactive compounds identified in the Baper Tea polyherbal infusion. Similar serotype-specific efficacy was observed by Rosmalena et al.[49] and Rehman et al.[50], who noted that herbal extracts often exhibit varying potencies against DENV serotypes due to structural differences in viral proteins. The cytotoxicity assay in Vero cells revealed a high CC50 value (4897,6 µg/mL), indicating low toxicity and a favorable therapeutic index. A high selectivity index (SI) (>10), calculated as the CC50/EC50 ratio, was used to evaluate the antiviral activity selectivity. The high SI value of the Baper Tea polyherbal infusion (SI = 257,5) indicates good selectivity for further antiviral drug development.[51,52]

The limited availability of preclinical animal models in biopharmaceutical research on dengue virus infection has made it difficult to develop DENV antiviral drugs. This has led to the need for in vitro permeability measurements coupled with in silico modeling and prediction as a practical approach in molecular property determination and biopharmaca prediction for the determination of the ability to develop new DENV antiviral drug candidates.[53,54,55,56] In this study, the ability of Baper Tea polyherbal infusion compounds to inhibit the NS5 DENV-3 protein was evaluated in silico. Physicochemical and drug similarity assessments revealed that all 10 major compounds conformed to Lipinski's rule of five, indicating good absorption, distribution, metabolism, and excretion (ADME) properties. However, all the test compounds were predicted to have drug-like properties and could be actively administered via the oral route.[39,57,58]

The results of toxicity level prediction testing revealed that compounds W-18 and Prost-13-en-1-oic acid were toxic (class 3), whereas tetraacetyl-d-xylonic acid and alpha-D-galactopyranose were nontoxic (class 6). This variability in toxicity profiles is similar to previous findings, which reported a spectrum of toxicity classes among bioactive compounds in herbal extracts while remaining within good safety limits and having medicinal

effects.[59,60] Organ toxicity predictions highlighted immunotoxicity as a concern for some compounds, especially tetraacetyl-d-xylonic and dihydroxanthin, but no significant carcinogenicity or cytotoxicity was observed. Despite the possibility of immunotoxicity, the index value was <1, which indicates harmless amounts.[46] These findings underscore the need for further investigation into the safety profile of this compound, as emphasized by several previous studies evaluating herbal drugs/products.[61,62]

Molecular docking analysis revealed that W-18 had the strongest binding affinity (ΔG = -9,93 kcal/mol, Ki = 52,35 nM) for the DENV-3 NS5 protein. This binding affinity surpasses that of the native ligand S-adenosyl-L-homocysteine (SAH), which has a ΔG of -6,84 kcal/mol, indicating the potential of compound W-18 as a stronger inhibitor. These findings indicate that W-18 has better inhibitory potential than the native ligand. The binding effectiveness was also assessed via the inhibition constant (Ki). The Ki value represents the effectiveness of a compound and the strength of the test compound in inhibiting the activity of the target receptor. The lower the Ki value is, the greater the inhibitory power. In this study, the best Ki value was derived from compound W-18. This compound formed the most comprehensive interaction network with the NS5 protein, comprising conventional hydrogen bonds with ASP79, CYS82, THR104, HIS110, and GLU111 and carbon–hydrogen bonding with GLY83.

This extensive hydrogen-bonding network likely contributes significantly to the high binding affinity of the compound by providing multiple points of attachment to the binding pocket. W-18 was the only compound that engaged in π-donor hydrogen bonding with LYS105 and π-sulfur interactions with HIS110 while also forming a π–π T-shaped interaction with TRP87 and multiple alkyl/π–alkyl interactions with ILE147, VAL132, and PHE133, along with π–sigma bonding with ILE147. These diverse noncovalent interactions collectively stabilize the ligand–protein complex and may explain the exceptional binding affinity observed. Binding site analysis revealed that W-18 interacts with key residues within the N-terminal domain of the NS5 protein, which contains the methyltransferase enzyme crucial for viral RNA cap methylation. By binding to this domain, W-18 may interfere with methyltransferase function, preventing proper capping of viral RNA and subsequently inhibiting viral replication.[63,64]

The number of hydrogen bonds in a compound is directly proportional to the energy required during the absorption process. The greater the number of hydrogen bonds is, the greater the energy required for absorption. Conversely, the lower the number of hydrogen bonds is, the lower the energy required in the absorption process.[65] These polar groups can decrease the affinity for hydrophobic membrane regions and increase water solubility when the drug penetrates the lipid bilayer membrane. Other compounds, such as phenanthrene, 9-, and dihydroxanthin, exhibit moderate binding affinities, contributing to overall antiviral activity.[66] This multicompound synergy is in line with the findings of Aladejana[45] and Casanova et al.[67], which suggests that polyherbal formulations often show increased efficacy because of the combined action of multiple bioactive compounds.

The antiviral mechanism of Baper Tea involves the synergistic effect of its bioactive compounds, such as flavonoids, alkaloids, and terpenoids, which have been previously reported to inhibit viral attachment and replication and modulate the host immune response.[55,68,69] The presence of quercetin and kaempferol in A. cepa L., withanolides in P. angulata leaves, and lignans in P. urinaria leaves likely contributes to their antiviral activity.[58,68,70,71] The interaction of W-18 with the DENV-3 NS5 protein receptor complex creates various types of bonds, including hydrogen, electrostatic, and hydrophobic bonds. Hydrogen bonds are electrostatic bonds formed by the interaction of hydrogen atoms with atoms that have high electronegativity values, such as oxygen (O), fluorine (F), and nitrogen (N). The nitrogen bonds formed during molecular tethering actively play a role in supporting protein stability.[72]

The W-18 forms hydrogen bonds with amino acid residues. These residues are in the N-terminal domain, where there is a methyltransferase protein that plays a role in methylating the viral RNA genome cap. The bond formed due to the interaction of compound W-18 with the NS5 protein complex in this domain interferes with the work process of the methyltransferase enzyme because it cannot bind to its substrate on the active side.[73] Most compounds from Baper Tea polyherbal infusion target the N-terminal domain of the NS5 protein, where the methyltransferase enzyme is located. Methyltransferases play a critical role in the viral lifecycle by catalyzing the methylation of the viral RNA cap structure, a process essential for RNA stability, efficient translation, and evasion of host immune responses.[64] Inhibition of this enzyme prevents proper viral RNA capping, leading to reduced viral protein synthesis and attenuated viral replication.

The presence of electron-withdrawing groups (chlorine and nitro groups) likely enhances the electrostatic interactions of the compound with polar amino acid residues, whereas its extended conjugated system facilitates π-stacking interactions with aromatic residues in the binding pocket.[74] Additionally, the piperidine ring provides conformational flexibility, allowing the molecule to adopt an optimal binding orientation within the NS5 protein cavity. Electrostatic bonds play an important role in protein–ligand interactions. The formation of electrostatic bonds in protein–ligand interactions can improve ligand efficiency. Compound W-18 interacts through electrostatic bonds with the residue HIS110. Histidine is one of the most versatile amino acid residues

that affects protein structure and function. The functional importance of this amino acid is evident in many proteins.[75] The interaction of W-18 with this electrostatic bond interferes with the action of proteins in the N-terminal complex. The interaction of W-18 also results in the formation of hydrophobic bonds that minimize interactions with water through the positioning of nonpolar compounds. This bond plays an important role in protein stability.[56,76]

The compounds that exhibited moderate binding affinities, such as phenanthrene, 9- and dihydroxanthin, also interacted with key residues in the NS5 protein but formed fewer types of interactions than W-18 did. Despite their individually lower binding affinities, these compounds may contribute to the overall antiviral effect of Baper Tea through different mechanisms or by targeting different stages of the viral life cycle, resulting in synergistic inhibition of DENV-3. This multitarget approach is a common advantage of polyherbal formulations over single-compound drugs. On the basis of these results, the compounds present in the samples in this study collaboratively and comprehensively inhibited DENV-3 replication; therefore, they have the potential to be developed as new anti-DENV drug candidates.

CONCLUSIONS

Based on the comprehensive research conducted, it can be concluded that the polyherbal infusion of Baper Tea shows significant potential as a natural antiviral agent against dengue virus serotype 3 (DENV-3). Baper tea polyherbal contains 40 bioactive compounds and functional groups characteristic of flavonoids, terpenoids, and glycosidic structures. Evaluation of antiviral activity in vitro obtained EC_{50} of 19,02 µg/mL, CC_{50} of 4897,6 µg/mL, and selectivity index (SI) of 257,5. Additionally, the ten selected compounds met the drug-likeness criteria, with acceptable toxicity profiles and adequate organ safety parameters. Molecular docking analysis revealed that compound W-18 exhibited the strongest binding affinity towards the NS5 DENV-3 protein, with a binding energy value of -9,93 kcal/mol and an inhibition constant of 52,35 nM. It formed a complex interaction network through conventional hydrogen bonding, π-donor hydrogen bonding, and π-sulfur interactions with the methyltransferase domain of the NS5 protein. These findings provide a strong scientific foundation for the development of nature-based standardized formulations as anti-dengue therapeutic candidates, with validated mechanisms of action and favorable safety profiles. Further research is needed for preclinical and clinical evaluation to optimize the therapeutic potential of Teh Baper polyherbal infusion in the management of dengue virus infection.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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