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Uncovering the potential of Niraga Tea Polyherbal™ as an antioxidant and antiviral agent against dengue: Insights from in vitro and in silico approaches

Revelando o potencial do Niraga Tea Polyherbal™como agente antioxidante e antiviral contra a dengue: conclusões a partir de abordagens in vitro e in sílico

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ABSTRACT

Dengue is a vector-borne disease with a high health burden in tropical regions, yet no specific antiviral therapy is currently available. This study aimed to investigate the antioxidant activity, cytotoxicity, and antiviral activity against dengue virus serotype 2 (DENV-2) of the Niraga Tea Polyherbal™ formulation and performed phytochemical profiling and molecular docking. GC–MS and FT–IR analyses were used to analyze the profiles and structures of bioactive compounds; antioxidant activity was evaluated using the DPPH, ABTS, and FRAP methods; dengue antiviral activity was evaluated using the Viral ToxGlo assay; and cytotoxicity was evaluated using Vero cells in an MTT assay. Molecular docking evaluations of the compounds targeting the DENV-2 NS5 protein in silico. GC–MS characterization revealed 100 compounds, predominantly methyl oleate (69.60%) and ethyl pentadecanoate (20.84%), along with quercetin (0.46%) and epigallocatechin gallate (0.19%). FT-IR analysis confirmed that phenolic hydroxyl groups were the dominant functional groups. DPPH, ABTS, and FRAP assays revealed IC_{50} values of 20.79, 21.34, and 18.80 µg/mL, respectively. MTT assays of Vero cells yielded a CC_{50} value of 1207 µg/mL. Niraga Tea Polyherbal™ significantly inhibited the cytopathic effects (F= 42.50; p=0.005), with an EC_{50} of 10.32 µg/mL and a selectivity index (SI) of 116.96 against DENV-2. Molecular binding studies of methyl oleate and

ethyl pentadecanoate to DENV-2 NS5 revealed weak binding affinities (ΔG -3.95 to -4.31 kcal/mol), which were weaker than those observed with more potent in vitro compounds. In conclusion, Niraga Tea Polyherbal™ has the potential to be developed as a candidate phytopharmaceutical for dengue antiviral therapy with a high safety profile against host cells.

Keywords: Niraga Tea Polyherbal™; dengue virus; antioxidants; cytotoxicity; molecular docking.

RESUMO

A dengue é uma doença transmitida por vetores que representa um grande ônus para a saúde em regiões tropicais; contudo, não existe, atualmente, uma terapia antiviral específica disponível. Este estudo teve como objetivo investigar a atividade antioxidante, a citotoxicidade e a atividade antiviral contra o sorotipo 2 do vírus da dengue (DENV-2) da formulação Niraga Tea Polyherbal™, além de realizar a caracterização fitoquímica e o docking molecular. Foram utilizadas análises por GC-MS e FT-IR para examinar os perfis e as estruturas de compostos bioativos; a atividade antioxidante foi avaliada por meio dos métodos DPPH, ABTS e FRAP; a atividade antiviral contra a dengue foi avaliada pelo ensaio Viral ToxGlo; e a citotoxicidade foi avaliada em células Vero por meio do ensaio MTT. Realizou-se, *in silico*, uma avaliação de docking molecular dos compostos tendo como alvo a proteína NS5 do DENV-2. A caracterização por GC-MS revelou a presença de 100 compostos, predominantemente oleato de metila (69,60%) e pentadecanoato de etila (20,84%), além de quercetina (0,46%) e galato de epigalocatequina (0,19%). A análise por FT-IR confirmou que os grupos hidroxila fenólicos eram os grupos funcionais predominantes. Os ensaios DPPH, ABTS e FRAP apresentaram valores de IC50 de 20,79, 21,34 e 18,80 µg/mL, respectivamente. O ensaio MTT em células Vero resultou em um valor de CC50 de 1207 µg/mL. O Niraga Tea Polyherbal™ inibiu significativamente os efeitos citopáticos (F = 42,50; p = 0,005), com uma CE50 de 10,32 µg/mL e um índice de seletividade (IS) de 116,96 contra o DENV-2. Estudos de ligação molecular do oleato de metila e do pentadecanoato de etila à proteína NS5 do DENV-2 revelaram afinidades de ligação fracas (ΔG de -3,95 a -4,31 kcal/mol), inferiores às observadas com compostos que apresentam maior potência *in vitro*. Em conclusão, o Niraga Tea PolyherbalTM tem potencial para ser desenvolvido como um candidato a fitofármaco para a terapia antiviral contra a dengue, apresentando um perfil de alta segurança em relação às células hospedeiras.

Palavras-chave: Niraga Tea Polyherbal™; vírus da dengue; antioxidantes; citotoxicidade; molecular docking.

1. INTRODUCTION

Dengue virus infection (DVI) remains a significant public health burden in tropical and subtropical regions. In Indonesia, 257,271 cases were recorded, with 1,461 deaths in 2024, more than double the number from the previous year (1,2), and a similar pattern was observed in Jambi Province, with an incidence rate of 62.32 per 100,000 residents in the same year (3). These morbidity figures are far above the national target of <10 per 100,000 population. Management of dengue virus disease has thus far been supportive, with no specific antiviral drugs that directly target the dengue virus (DENV) (4,5); however, the use of the Dengvaxia® vaccine, which is still in development, is limited because of the risk of severe disease in seronegative individuals (6), and other vaccine and monoclonal antibody strategies still face clinical translation challenges because of the concurrent circulation of four DENV serotypes and the risk of antibody-dependent enhancement (ADE) (7,8). Therefore, innovations and solutions are needed to control DVIs by developing herbal formulations with antioxidant and antiviral activity that are safe, affordable, and well tested.

To date, the development of natural-product-based drugs has generally targeted pathogenic viruses. The nonstructural proteins NS5 and NS3 of DENV have become widely studied molecular targets because they play crucial roles in viral replication and lack homologs in host cells, making their inhibition potentially selective against the virus (9). Combined in vitro and in silico approaches, including cytotoxicity assays, cell viability-based antiviral assays, and molecular binding to target proteins, have become the standard framework for the initial screening of natural-product-based antiviral candidates (10). Several previous studies have reported the antioxidant and antiviral potential of individual components of Niraga Tea Polyherbal™. Meniran leaves (*Phyllanthus niruri* L.) contain flavonoids, lignans, and tannins that have been confirmed to inhibit DENV replication in vitro (11). Corn silk (*Zea mays* L.) is rich in flavone C-glycosides with strong antioxidant activity (12,13), red onion peel (*Allium cepa* L.) contains quercetin and kaempferol, which exhibit antiviral potential through molecular binding(14), and *Stevia rebaudiana* Bertoni leaves have been shown through meta-analysis to alleviate oxidative stress (15). On the other hand, studies on polyherbal formulations generally indicate that combinations of plant extracts have the potential to produce stronger therapeutic effects with lower toxicity than single components (16); however, this evidence primarily stems from antimicrobial and anti-inflammatory indications rather than antiviral activity against dengue.

The identified research gap is the lack of studies that integrate these four ingredients, namely, meniran, corn silk, red onion peel, and stevia, into a single tea formulation to simultaneously test their antioxidant activity, cytotoxicity, and dengue antiviral activity, accompanied by mechanistic confirmation through molecular binding. This study aims to explore the phytochemical profile, antioxidant activity, cytotoxicity, antiviral activity against DENV-2, and molecular interactions of bioactive compounds in Niraga Tea Polyherbal™ with the DENV-2 NS5 protein through an integrated in vitro and in silico approach. These findings are expected to provide preliminary preclinical evidence supporting the development of Niraga Tea Polyherbal™ as a candidate for an Indonesian-originated phytopharmaceutical, as well as scientific evidence for the use of local herbal plant waste in the control of DVI.

2. MATERIALS AND METHODS

2.1 IDENTIFICATION AND ETHICAL APPROVAL

The plants used in this study including meniran, corn silk, red onion peel, and stevia, were identified at the Batu Herbal Materia Medica Laboratory under specimen number 000.9.3/2279/102.20/2026 dated June 15, 2026. This study received ethical approval from the Health Research Ethics Committee of the Faculty of Medicine and Health Sciences, Universitas Jambi, No. 130/DST/UN21.8.9/PT.01.04/2026, dated May 4, 2026.

2.2 MATERIALS AND FORMULATION

Niraga Tea Polyherbal™ is formulated from four raw materials at a 2:2:1:1 ratio: meniran leaves (*Phyllanthus niruri* L.) (500 mg), corn silk (*Zea mays* L.) (500 mg), red onion peel (*Allium cepa* L.) (250 mg), and *Stevia rebaudiana* Bertoni leaves (250 mg per tea bag). All the raw materials were sourced from the city of Jambi, Indonesia, to ensure product consistency; they were subsequently washed with running water, sorted by hand, and then dried using a food dehydrator (model FDH-16, 16-tray) at 50°C until the moisture content was <10% (17). The dried material was coarsely ground, homogenized, and packaged in nonwoven tea bags weighing 2 g per bag; it was stored at room temperature (25±2°C) with a moisture content of <10%. The infusion and stock solution samples were prepared by aseptically extracting 5 g of Niraga Tea Polyherbal™ in 100 mL of sterile distilled water (90°C, 10 min) under aseptic conditions, sequentially filtering through 0.45 µm and 0.22 µm porous membranes, and then lyophilizing them (−40°C, 0.1 mbar) to obtain a dry powder that was stored at −20°C. The powder was dissolved in sterile complete minimum essential medium (MEM) to a stock concentration of 1,000 µg/mL and serially diluted into eight concentration series (1.56; 3.13; 6.25; 12.5; 25; 50; 100; and 200 µg/mL) for all in vitro assays.

2.3 CHARACTERIZATION OF COMPOUND PROFILES AND STRUCTURES

The profiles of the bioactive compounds were analyzed using a Perkin Elmer Clarus 500 gas chromatography–mass spectrometry (GC–MS) system with a Clarus SQ 8S detector and an Elite-5

ms capillary column (30 m × 0.25 mm × 0.25 µm) (18). A total of 5 g of tea sample was extracted in 100 mL of deionized water (90°C, 10 min), filtered through a 0.45 µm porous membrane, and concentrated using a rotary evaporator, after which the residue was dissolved in 1 mL of HPLC-grade methanol. Analysis was performed using helium as the carrier gas (1.0 mL/min), a column temperature of 110–280°C, an injection volume of 1 µL, a split ratio of 10:1, and a mass-to-charge (m/z) detection range of 40–550 Da. Compound identification was based on mass spectrum matches against the NIST 2017 library and the 11th edition of the Wiley Registry, expressed as a percentage of the total ion chromatogram area (TICA). Functional group characterization was performed using Fourier transform infrared spectroscopy (FT-IR Bruker Tensor II, Attenuated Total Reflectance/ATR accessory, OPUS 8.0 software) in the wavenumber range of 4000–400 cm^{-1}, with a resolution of 4 cm^{-1}, and 32 accumulated scans were conducted in triplicate. Spectrum processing included baseline correction, smoothing, and normalization, with functional group identification based on characteristic absorption bands referenced against a database and the literature (14).

2.4 ANTIOXIDANT ACTIVITY ASSAY

Antioxidant activity was evaluated using three methods. In the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay, 40 µL of the sample was mixed with 40 µL of a 0.1 mM methanolic DPPH solution in a 96-well microplate and incubated for 30 min (25°C, in the dark), after which the absorbance was measured at 517 nm. In the ABTS (2,2'-azino-bis(-3-ethylbenzothiazoline-6-sulfonic acid)) assay, the $ABTS^{\bullet+}$ cation radical was prepared from a mixture of 7 mM ABTS and 140 mM potassium persulfate (incubated for 16 h in the dark at room temperature) and diluted to an absorbance of 0.70 ± 0.02 at 734 nm; 10 µL of the extract was mixed with 290 µL of the $ABTS^{\bullet+}$ solution and incubated for 6 min in the dark, after which the absorbance was measured at 734 nm. In the ferric reducing antioxidant power (FERG) assay, the reagent was prepared from 300 mM sodium acetate, 10 mM TPTZ, and 20 mM $FeCl_3$ (10:1:1, v/v/v) and was warmed to 37°C for 10 min; 20 µL of the extract was added to 280 µL of the reagent and incubated for 10 min (37°C), after which the absorbance was measured at 593 nm (19,20). The IC_{50} value was determined via variable-slope nonlinear regression (four parameters) using GraphPad Prism.

2.5 CYTOTOXICITY ASSAY

Vero cells (ECACC 84113001) were cultured in MEM supplemented with 10% fetal bovine serum (FBS, heat-inactivated at 56°C for 30 min) and 1% penicillin–streptomycin, incubated at 37°C with 5% CO_2, and subcultured when confluence reached 80–100% by washing with PBS (pH 7.4), detachment with 0.25% trypsin-EDTA (5 min at 37°C), centrifugation at 3,500 rpm for 5 min, and resuspension of the pellet in complete MEM. Cell viability was verified using the trypan blue exclusion method (target value ≥95%) prior to use. The cytotoxicity of Niraga Tea Polyherbal™ was evaluated by seeding Vero cells in a 96-well microplate (100 µL/well) and incubating them for 24 h (37°C, 5% CO_2); then, the medium was replaced with samples at eight concentrations (1.56–200 µg/mL) in triplicate, along with cell and medium controls, followed by a further 24-h incubation. Cell viability was measured using the MTT assay (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; 10 µL, 5 mg/mL; 4 h); formazan crystals were dissolved in 50 µL of DMSO, and the absorbance was read at 595 nm (14). The CC_{50} value was determined using variable-slope nonlinear regression (four parameters).

2.6 ANTIVIRAL ACTIVITY ASSAY AGAINST DENV-2

Antiviral activity was evaluated by seeding Vero cells (100 µL/well) into a 96-well luminescent microplate and incubating them for 24 h (37°C, 5% CO_2), after which they were treated with 25 µL of Niraga Tea Polyherbal™ at eight concentrations (1.56–200 µg/mL, in triplicate) and 25 µL of DENV-2 suspension (stock 2×10^3 FFU/mL), along with a cell control, a virus control, a medium control, and ribavirin as a positive control. The samples were incubated for 48–144 h. Cell viability was measured using Viral ToxGlo reagent (stirred for 10 min, incubated for 10 min at 37°C) and read on a GloMax luminometer (RLU). The EC_{50} value was determined via variable-slope nonlinear regression, and the selectivity index (SI) was calculated as the ratio of the CC_{50} to the EC_{50}, with a cutoff of the SI ≥ 3 (14).

2.7 MOLECULAR DOCKING

Ligand screening was performed on the dominant compounds identified by GC–MS on the basis of Lipinski's rule of five. The dominant compounds were optimized using the MMFF94 force field in

Open Babel and prepared in PDBQT format using AutoDockTools 1.2.0. The DENV-2 NS5 target protein (PDB ID: 5ZQK, resolution 2.3 Å) was downloaded from the RCSB Protein Data Bank and prepared by removing the native ligand and water molecules, adding polar hydrogens, and assigning Gasteiger charges, with a 20×20×20 Å grid box (spacing 0.375 Å) at the active site. Docking was performed using AutoDock Vina 1.2.0. Method validation was conducted by redocking the native ligand (S-adenosylmethionine) with an acceptance threshold of an RMSD ≤2.0 Å. Toxicity predictions and pharmacokinetic profiles were evaluated using ProTox-II and SwissADME (14,21). Visualization of ligand–protein interactions was performed using Discovery Studio Visualizer 2021. All the results are presented in terms of binding affinities and binding sites.

2.8 STATISTICAL ANALYSIS

The data are expressed as the mean ± standard deviation (SD). Data normality and homogeneity were tested using the Shapiro–Wilk test and Levene's test. Comparisons of antioxidant activity between methods were analyzed using one-way ANOVA. Differences in cell viability among dose groups in the cytotoxicity and antiviral assays were analyzed using a mixed-effects model (Geisser–Greenhouse correction), followed by Dunnett's multiple comparison (DMC) post hoc test, with a significance level of $p<0.01$ (99%). The results were considered valid if the coefficient of determination (R^2) was ≥0.95 for all regression curves, if the RMSD was ≤2.0 Å for docking validation, and if the coefficient of variation (CV) for replication was <15%.

3. RESULTS

3.1 GC–MS-BASED BIOACTIVE COMPOUND PROFILE

GC–MS analysis of Niraga Tea Polyherbal™ revealed 100 bioactive compounds whose total normalized area was 100% (Figure 1 and **Table 1**). The fatty acid group (methyl and ethyl esters of palmitic, oleic, elaidic, and isostearic acids) dominated the chromatogram profile, with the highest areas for methyl oleate (69.60%; RT 2.529 min) and ethyl pentadecanoate (20.84%; RT 2.447 min). Together, these compounds accounted for 90.44% of the total area of the ten dominant compounds. Flavonoids were detected as 3,3',4',5,7-pentahydroxy flavone (quercetin; 0.46%; RT 4.233 min) and epigallocatechin-3-gallate (0.19%; RT 24.582 min).

Figure 1. Total ion chromatogram (TIC) from GC–MS analysis of Niraga Tea Polyherbal™. Remarks: Retention times from 0 to 35 min resulted in the detection of 100 peaks. Three dominant peaks were visible at 2–4 min (peaks 1–3), with peak 3 having the highest intensity in the chromatogram.

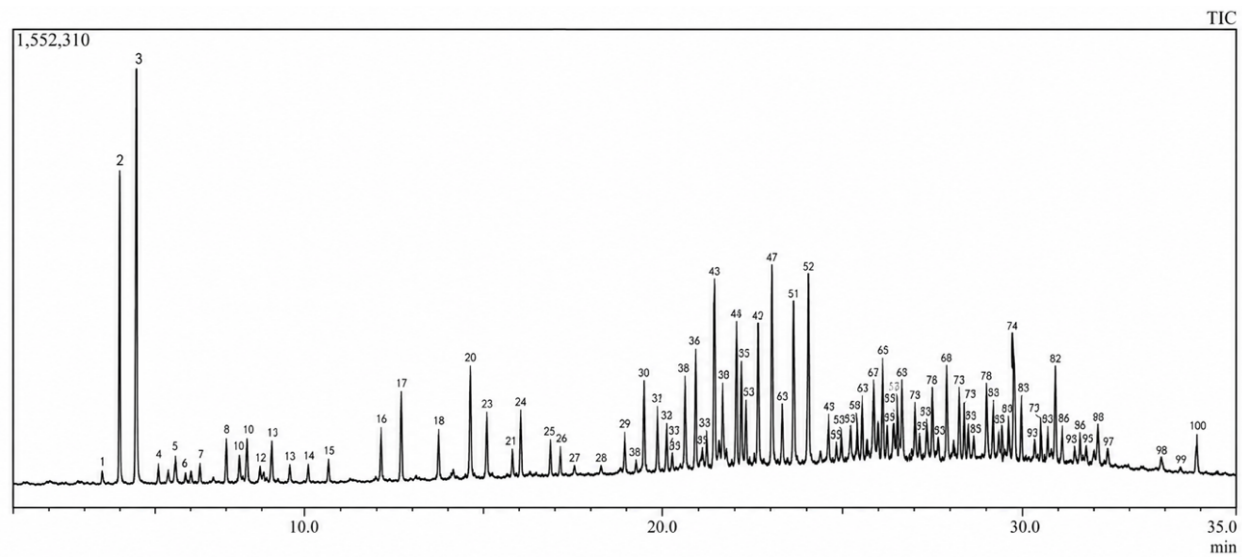


Table 1. Ten dominant compounds in Niraga Tea Polyherbal™ identified by GC–MS

Compound	Class	RT (min)	Area (%)	SI	Formula	CAS	MW (g/mol)
Methyl oleate	Unsaturated fatty acid	2.529	69.598	93	$C_{19}H_{36}O_2$	112-62-9	296.5
Ethyl pentadecanoate	Fatty acid ester	2.447	20.844	96	$C_{17}H_{34}O_2$	112-39-0	270.5
3,3',4',5,7-Pentahydroxy flavone (quercetin)	Flavonoid	4,233	0.461	88	$C_{15}H_{10}O_7$	117-39-5	302.23
Pentadecanoic acid, ethyl ester	Fatty acid ester	4.949	0.215	98	$C_{17}H_{34}O_2$	41114-00-5	270.5
Octadecanoic acid	Saturated fatty acid	24.342	0.192	96	$C_{18}H_{36}O_2$	628-97-7	284.5
Epigallocatechin-3-gallate	Flavonoid	24.582	0.186	95	$C_{22}H_{18}O_{11}$	989-51-5	458.4
Isopropyl palmitate	Epoxy ether	3.253	0.132	88	$C_{19}H_{38}O_2$	142-91-6	298.5
Di-(9-octadecenoyl)-glycerol	Diasylglycerol	17.468	0.131	90	$C_{39}H_{72}O_5$	2081110-78-1	621.0
Methyl isostearate	Branched-chain fatty acid	3.848	0.161	94	$C_{19}H_{38}O_2$	112-61-8	298.5
Methyl elaidate	Unsaturated fatty acid	2.369	0.041	90	$C_{19}H_{36}O_2$	1937-62-8	296.5

Remarks: RT, retention time; SI, similarity index (mass spectrum match score against the NIST/Wiley7 library, scale 0–100); MW, molecular weight.

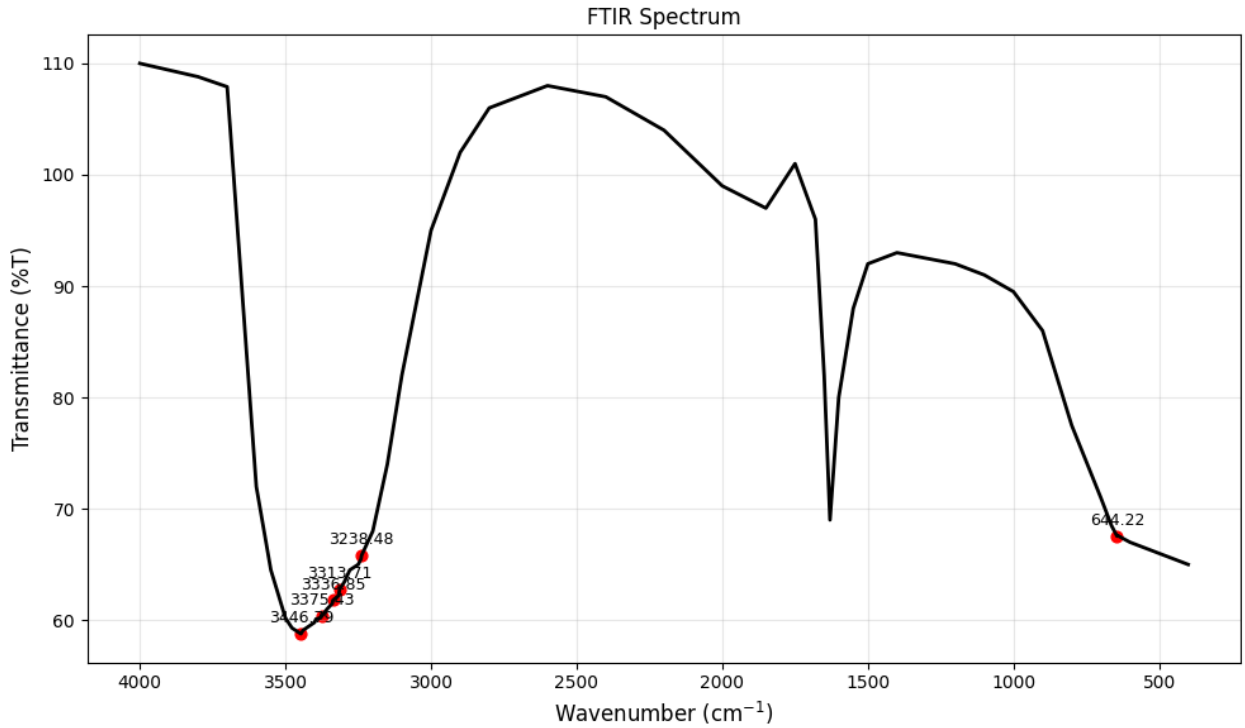
3.2 STRUCTURAL CHARACTERIZATION OF BIOACTIVE COMPOUNDS BY FT-IR

The FT-IR spectrum shows six main absorption peaks (**Figure 1** and **Table 2**) with dominant bands in the 3200–3500 cm^{-1} range (P1–P5), indicating that the vibrations of the hydroxyl group (O–H) from phenolic compounds and intramolecular hydrogen bonds are characteristic of fatty acids, catechins, flavonoids, and polyphenols. The peak with the highest intensity and area is located at 3446.79 cm^{-1} (58.77% T; area 26.054), confirming that the hydroxyl group is the most dominant functional group in the sample. The peak in the *fingerprint* region at 644.22 cm^{-1} indicates C–H bond deformation outside the aromatic ring plane, which is characteristic of the core structure of phenolic compounds.

Table 2. FT-IR absorption peaks of Niraga Tea Polyherbal™

Peak	Wavelength (cm^{-1})	%T	Area	Functional Group
P1	3446.79	58.77	26.054	O–H stretching (phenol/polyphenol)
P2	3,375.43	60.35	9,134	Hydrogen-bonded O–H (flavonoids)
P3	3,336.85	61.79	5,422	Phenolic O–H/Weak N–H
P4	3,313.71	62.79	9,393	Strongly bound phenolic O–H
P5	3,238.48	65.82	9,753	Strongly bound O–H (polyphenols)
P6	644.22	67.54	2,300	Aromatic C–H/ring deformation

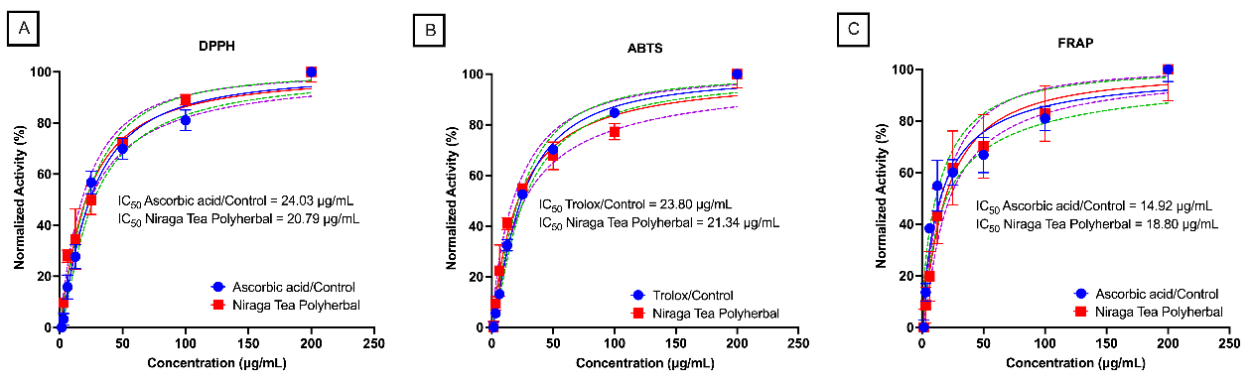
Figure 2. FT-IR Spectrum of Niraga Tea Polyherbal™



3.3 ANTIOXIDANT ACTIVITY OF NIRAGA TEA POLYHERBAL™

In the DPPH assay, Niraga Tea Polyherbal™ produced an IC_{50} of 20.79 µg/mL (99% CI: 17.71–24.37), which is lower than that of ascorbic acid (24.03 µg/mL; 99% CI: 21.20–27.25) (**Figure 3A**). In the ABTS assay, an IC_{50} of 21.34 µg/mL (99% CI: 17.21–26.51) was obtained, which was lower than that of Trolox at 23.80 µg/mL (99% CI: 21.72–26.08) (**Figure 3B**), whereas in the FRAP assay, an IC_{50} of 18.80 µg/mL (99% CI: 15.64–22.65) was obtained, which was higher than that of ascorbic acid at 14.92 µg/mL (99% CI: 11.26–19.85) (**Figure 3C**). All the parameters confirmed very strong free radical scavenging activity (IC_{50} < 50 µg/mL) indicating that Niraga Tea Polyherbal™ has antioxidant effects.

Figure 3. Dose–response curves for the antioxidant activity of Niraga Tea Polyherbal™ compared with that of the standard control. (A) DPPH, (B) ABTS, and (C) FRAP.



3.4 CYTOTOXICITY OF NIRAGA TEA POLYHERBALTM AGAINST VERO CELLS

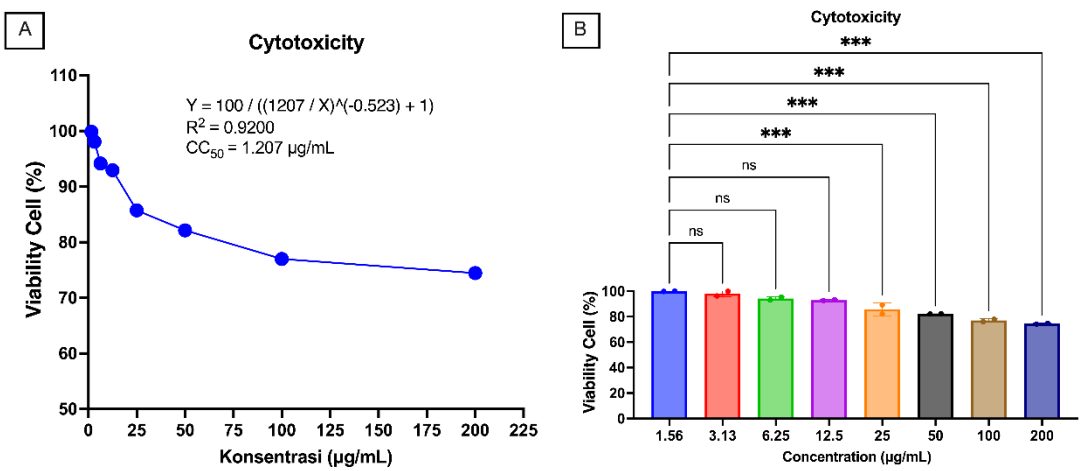
An MTT assay on Vero cells at eight concentrations (1.56–200 µg/mL) yielded a CC_{50} of 1207 µg/mL (99% CI: 602.1–3824; Hill slope = −0.523; R^2 = 0.9200) (**Figure 4**). Analysis using a mixed-effects model revealed a significant decrease in cell viability as the concentration increased (F = 40.84; R^2 = 0.9728; p<0.001); however, cell viability remained above 74% across the entire dose range (**Table 3**). These results indicate that Niraga Tea Polyherbal™ did not cause 50% cell death in Vero cells even at the highest dose tested and is considered safe as a candidate antiviral agent.

Table 3. Cytotoxicity of Niraga Tea Polyherbal™

Dose (µg/mL)	Mean ± SD	R^2	F	P value	*Dunnett's multiple comparisons test* (all doses vs. lowest dose)		
					Mean Diff.	q	Adjusted P value
1.56	99.86 ± 0.1561	0.9728	40.84	<0.001***	-	-	-
3.13	98.07 ± 2.548				-1.789	0.8400	0.92 ns
6.25	94.17 ± 1.560				-5.687	2.670	0.12 ns
12.5	92.94 ± 0.4943				-6.915	3,247	0.05 ns
25	85.73 ± 5.024				-14.13	6.635	<0.001***
50	82.15 ± 0.086				-17.71	8.316	<0.001***
100	76.99 ± 1.298				-22.87	10.74	<0.001***
200	74.46 ± 0.408				-25.40	11.92	<0.001***

Remarks: All groups met the assumption of normality (normally distributed, p>0.05), as determined by the Shapiro–Wilk test. The mean Diff., q, and Adj P value are the results of the Dunnett post hoc test for each group compared to the lowest dose group. The probability level was set at 99% (P < 0.01). (ns) 0.12; (*) 0.033; (**) 0.002; (***) <0.001.

Figure 4. Dose–response curve of the cytotoxicity of Niraga Tea Polyherbal™ in Vero cells. Legend: (**A**) CC_{50} values were obtained via nonlinear regression with a variable slope on cell viability data (% CPE), representing the mean ± SD of three replicates; and (**B**) results of *Dunnett's multiple comparisons test* (all doses vs. the lowest dose). All groups met the assumption of normality (normally distributed, P > 0.05). The significance level used was 99% (P < 0.01). (ns) 0.12; (*) 0.033; (**) 0.002; (***) < 0.001.



3.5 ANTIVIRAL ACTIVITY AGAINST DENV-2 AND SELECTIVITY INDEX

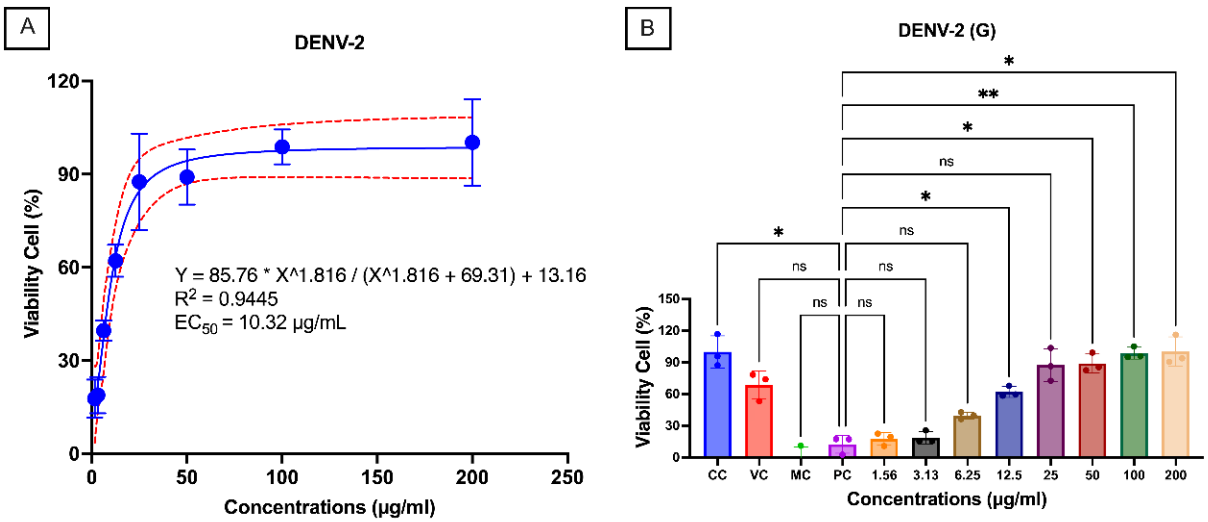
Nonlinear regression analysis yielded $_{an}EC_{50}$ of 10.32 µg/mL (99% CI: 4.757–15.53; R^2= 0.9445) (**Figure 5A**). Testing the antiviral activity of Niraga Tea Polyherbal™ against DENV-2 in Vero cells using the Viral ToxGlo assay revealed a significant dose-dependent effect on the inhibition of cytopathic effects (F = 42.50; p = 0.005) (**Figure 5B**). Based on a CC_{50} of 1,207 µg/mL, the selectivity index (SI) obtained was 116.96, well above the SI threshold of ≥3, indicating that the formulation is suitable as an antiviral candidate and has potential for development as a natural-product-based phytopharmaceutical. A summary of the mean difference, q statistic, and adjusted p value for each dose group of each serotype is presented in **Table 4**.

Table 4. Differences in cell viability (% CPE) after treatment with Niraga Tea Polyherbal™

Dose	Mean ± SD	GGE	F	P value	*Dunnett's multiple comparisons test* (all doses vs. KP)		
					Mean Diff.	q	Adjusted P value
CC	100.0 ± 15.26	0.1333	42.50	0.005**	9.696	87.52	0.03*
VC	68.64 ± 13.36				5.339	56.15	0.11 ns
MC	0.000 ± 10.10				1.861	-12.48	0.55 ns
PC	12.48 ± 8.455				-	-	-
1.56	17.68 ± 6.127				0.6693	5.197	0.98 ns
3.13	18.86 ± 5.901				0.7689	6.373	0.96 ns
6.25	39.61 ± 3.250				4.130	27.13	0.17 ns
12.5	62.10 ± 5.143				12.68	49.61	0.02*
25	87.54 ± 15.54				7.562	75.06	0.06 ns
50	89.11 ± 8.934				13.50	76.63	0.02*
100	98.80 ± 5.585				18.86	86.32	0.009**
200	100.2 ± 13.93				13.72	87.76	0.02*

Notes: CC, cell control; VC, virus control; MC, medium control; PC, positive control (ribavirin). The mean ± SD are expressed in relative light units (RLU). GGE, Geisser–Greenhouse epsilon, mean difference, q, and adjusted P value are the results of Dunnett's post hoc test for each group compared to the positive control. All groups met the assumption of normality (normally distributed, p>0.05) as determined by the Shapiro–Wilk test. The probability level used was 99% (P < 0.01). (ns) 0.12; (*) 0.033; (**) 0.002; (***) < 0.001.

Figure 5. Comparison of cell viability among the Niraga Tea Polyherbal™ dose groups and the positive control (ribavirin) for DENV-2. (A) EC_{50} values were obtained via nonlinear regression (agonist vs. response–variable slope (four-parameter)) on cell viability data (% CPE), representing the mean ± SD of three replicates; and (B) comparison using Dunnett's multiple comparisons test (all doses vs. ribavirin/positive control). Significance is indicated as (ns) 0.12; (*) 0.033; (**) 0.002; (***) <0.0001.



3.6 MOLECULAR BINDING OF THE TARGET COMPOUND TO THE DENV-2 NS5 PROTEIN

Based on molecular docking analysis, the compounds methyl oleate and ethyl pentadecanoate were docked to the DENV-2 NS5 protein (methyltransferase/RNA-dependent RNA polymerase) (PDB ID: 5ZQK; resolution 2.3 Å). Validation of the method by redocking the native lig e (S-adenosylmethionine) yielded an RMSD of 1.920 Å (meeting the acceptance threshold of <2 Å; ΔG -4.34 kcal/mol; Ki 658.32 µM) (**Table 5** and **Figure 6**). The binding affinities of both compounds for DENV-2 NS5 were weak, with ethyl pentadecanoate (ΔG −3.95 kcal/mol; Ki 1.28 mM) and methyl oleate (ΔG −4.31 kcal/mol; Ki 693.74 µM) (**Table 6 and Figure 7**). ADME and toxicity evaluations revealed that both compounds are nontoxic (toxicity class V; $_{LD_{50}}$ 3,000–5,000 mg/kg) and inactive across all organ toxicity models and clinical toxicity endpoints (**Table 7**). The interactions formed by the ethyl pentadecanoate compound include van der Waals (LYS A:105), conventional hydrogen (LYS A:105), and alkyl (ILE A:107) interactions, whereas those formed by the methyl oleate compound include van der Waals (LYS A:105), conventional hydrogen (LYS A:105), carbon–hydrogen (GLY A:81), alkyl (ILE A:107), and π-alkyl (VAL A:132 and TRP A:87) interactions, which play a role in inhibiting dengue virus replication.

Table 5. Target receptors and validation of the docking method for DENV-2 proteins

Serotype	PDB ID	Protein	Resolution	Native Ligand	RMSD (Å)	Validation ΔG (kcal/mol)	Validation Ki
DENV-2	5ZQK	NS5 Full-Length	2.3 Å	S-Adenosyl-methionine	1.920	-4.34	658.32 µM

Figure 6. Visualization of the target receptor and validation of the docking method for the DENV-2 protein

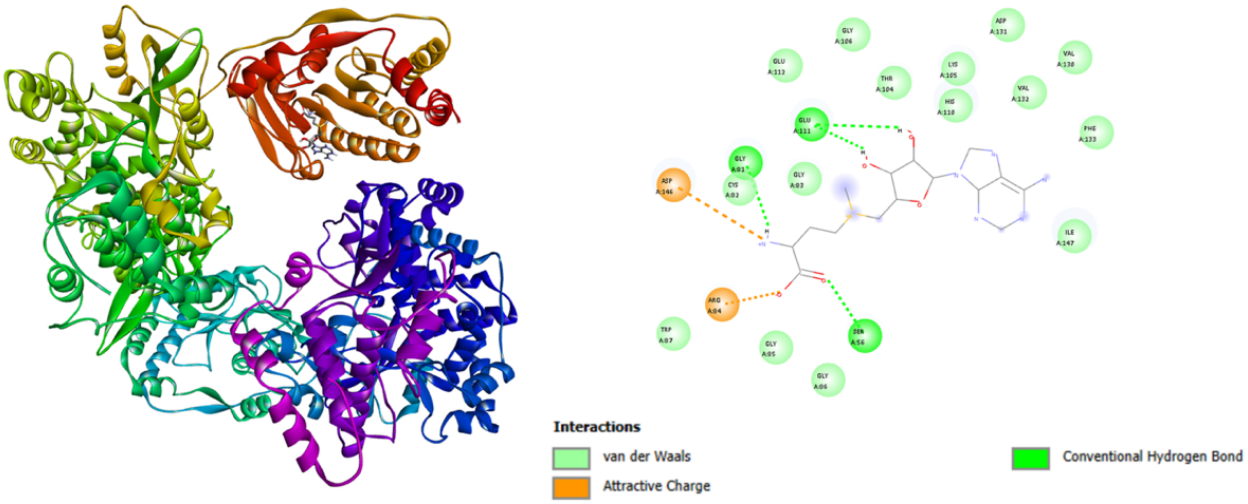


Table 6. Molecular binding of the Niraga Tea Polyherbal™ compound to DENV-2 NS5

Compound	ΔG (kcal/mol)	Ki
Ethyl Pentadecanoate	-3.95	1.28 mM
Methyl Oleate	-4.31	693.74 µM

Figure 7. Visualization of 2D and 3D binding poses of methyl oleate and ethyl pentadecanoate on NS5 DENV-2. (A) Ethyl pentadecanoate and (B) methyl oleate.

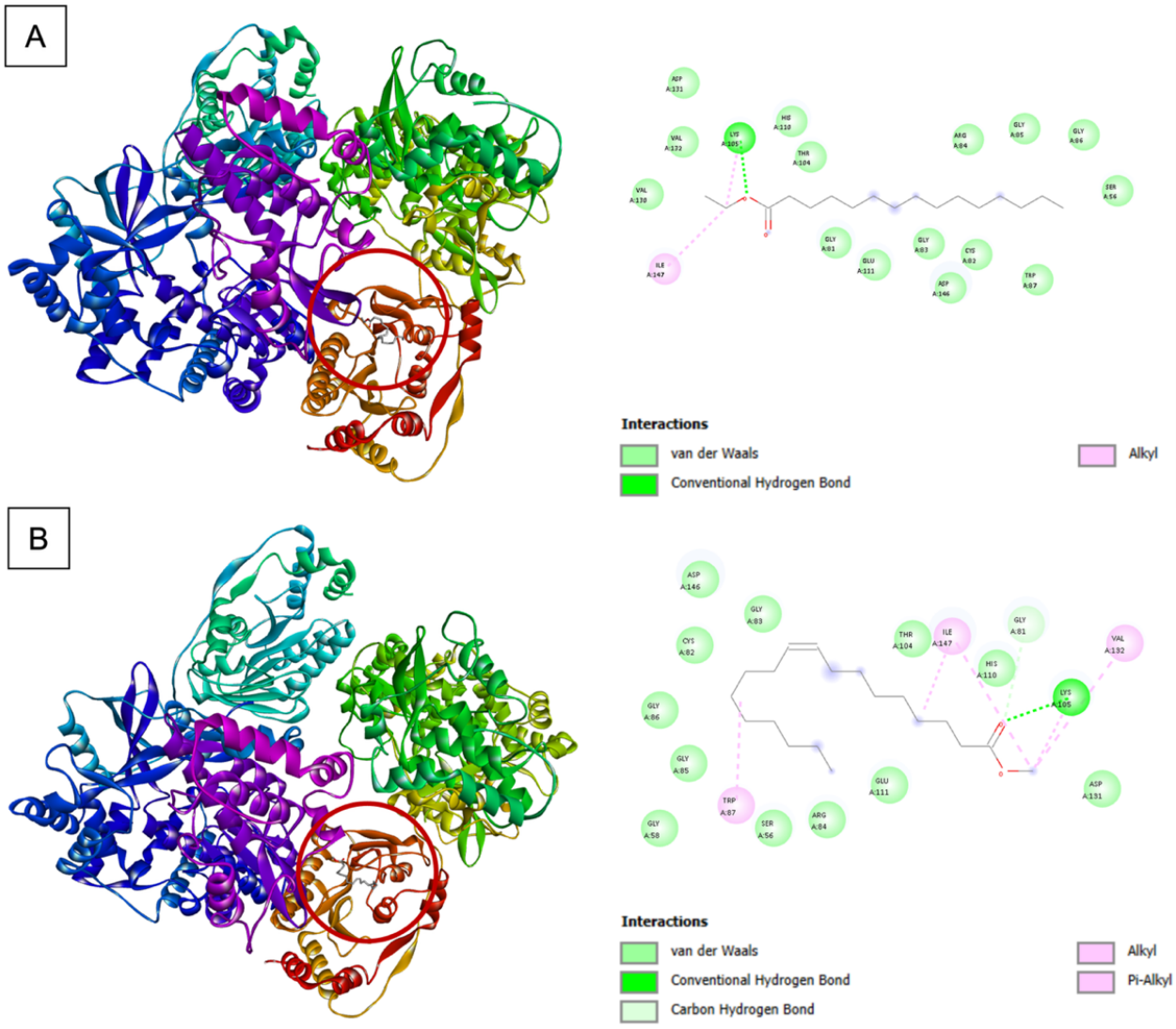


Table 7. ADME profiles and toxicity of dominant compounds

Parameter	Methyl Oleate	Ethyl Pentadecanoate
MW (g/mol)	270.45	296.49
Log P	4.44	4.80
Donor H	0	0
H Acceptor	2	2
Druglikeness	Yes; 1 violation (MLOGP > 4.15)	Yes; 1 violation (MLOGP > 4.15)
Predicted LD_{50} (mg/kg)	3000	5,000
Toxicity class	V	V
Hepatotoxicity/ Nephrotoxicity/ Cardiotoxicity/ Neurotoxicity	Inactivated	Inactivated
Carcinogenicity/ Mutagenicity/ Cytotoxicity	Inactivated	Inactivated
Blood–Brain Barrier	Active	Active

4. DISCUSSIONS

This study aims to confirm the antioxidant activity and antiviral activity against dengue serotype 2 (DENV-2) of Niraga Tea Polyherbal™, formulated from meniran leaves, corn silk, red onion peel, and stevia, and to elucidate the molecular basis of the interaction of its dominant compounds with the DENV-2 NS5 protein. In summary, these findings indicate that this formulation possesses antioxidant capacity, as confirmed through three testing methods, a selectivity index supporting its suitability as a candidate for dengue treatment, and a molecular interaction profile that is partially consistent with the observed in vitro activity.

With respect to the phytochemical profile, GC–MS and FT–IR characterization of this formulation confirmed the presence of compound classes that are structurally consistent with the secondary metabolites previously reported in each individual ingredient, without the emergence of any unexpected new compound classes resulting from the mixing or drying process. This consistency indicates that the formulation and drying processes using a food dehydrator did not cause thermal degradation that altered the major phytochemical groups, thus aligning with the principles of herbal tea preparation that preserve the structural integrity of active compounds during low-temperature drying (12). In terms of cytotoxicity, the CC_{50} values obtained for Vero cells fall within the range commonly reported for water-based polyherbal preparations (22–25). The aqueous extraction process used in this study yielded a cellular safety profile comparable to that of other characterized traditional herbal preparations (16) and serves as a prerequisite before antiviral selectivity values can be meaningfully interpreted (26).

The antioxidant findings in this study are consistent with patterns previously reported for the individual components of the Niraga Tea Polyherbal™ formulation. The very strong antioxidant activity of the Niraga Tea Polyherbal™ formulation indicates that it can effectively scavenge free radicals and is comparable to the positive controls used, namely, Trolox and ascorbic acid. The free radical scavenging activity of this formulation is conceptually supported by the presence of flavonoids and phenolic compounds, which have been confirmed in meniran (11,27), corn silk (12,13), red onion peel (28), and stevia (11,15). However, this study is the first to test the combination of these four ingredients in a single formulation; therefore, a direct comparison of the IC_{50} values with those in previous literature that exclusively used single-component formulations cannot be made. A similar pattern was observed in studies of other polyherbal formulations, which

revealed that multicomponent combinations can produce antioxidant effects equivalent to or better than those of single components because of additive or synergistic interactions among phytochemical groups (16); thus, the multicomponent formulation in this study is considered viable and shows promising results for development as a phytopharmaceutical and for further research.

The selectivity of the Niraga Tea Polyherbal™ formulation against DENV-2, as reflected by a selectivity index exceeding the threshold of ≥3, indicates that the Niraga Tea Polyherbal™ formulation is comparable to previously reported single-ingredient antidengue phytopharmaceutical candidates, including nano *P. niruri* (11) and the Baper tea polyherbal infusion, which target DENV nonstructural proteins through similar in silico and in vitro approaches (14,29). The methodological similarities across these studies, namely, the combination of luminescence-based cell viability assays and molecular binding assays against NS5/NS3, reinforce the external validity of the findings of this study as part of the trend toward integrated, evidence-based research on antidengue phytopharmaceuticals in Indonesia. A fundamental difference in this study lies in the formulation strategy, wherein the Niraga Tea Polyherbal™ preparation utilizes waste from four different botanical sources, including two commonly discarded materials (red onion peel and corn silk), thereby conceptually offering added value from the perspective of the circular economy and the utilization of agroindustrial waste, which has not been extensively explored in the literature on dengue antivirals.

Molecular interactions of the dominant compounds identified through phytochemical profiling with the DENV-2 NS5 protein showed weak to moderate binding affinities. This likely explains the inhibitory effects observed in in vitro studies, which result from the combination of phytochemical compounds in the Niraga Tea Polyherbal™ that act synergistically to produce very strong inhibition. These findings are consistent with those of Ghazali et al. (30), who reported a similar pattern for apigenin glycosides against SARS-CoV-2, where strong docking affinity does not always correlate directly with in vitro potency; they explained that these limitations may stem from the complexity of the intracellular biological environment, the limited membrane permeability of compounds with sugar groups, and the predictive nature of docking, which only estimates thermodynamic interactions without considering cellular pharmacokinetics (16). These methodological limitations are relevant to our study because molecular docking was performed on single compounds, whereas in vitro assays used whole extracts containing a mixture of multiple compounds with potential intercomponent interactions that are not represented in single-compound docking simulations.

4.1 CLINICAL AND PUBLIC HEALTH IMPLICATIONS

The results of this study provide preliminary preclinical evidence that Niraga Tea Polyherbal™ has the potential to be developed as a phytopharmaceutical candidate to complement supportive DVI management in endemic regions, particularly as an affordable and easily accessible preventive product based on local agroindustrial waste. The selectivity toward Vero cells, which supports the safety profile of Niraga Tea PolyherbalTM, provides an initial basis for the feasibility of conducting further testing.\

4.2 LIMITATIONS

This study was limited to in vitro models and in silico simulations without molecular dynamics validation or in vivo testing; therefore, the stability of ligand–protein interactions and systemic pharmacokinetics have not yet been confirmed. Molecular docking was performed on individual compounds, whereas antiviral testing used a whole multicomponent infusion; thus, the synergistic contributions of the individual components cannot yet be separated.

5. CONCLUSION

Niraga Tea Polyherbal™ demonstrates antioxidant activity and good selectivity against DENV-2, supporting its potential as a candidate for an antidengue phytopharmaceutical with dominant compound-NS5 molecular interactions. Niraga Tea Polyherbal™ has the potential to be used as a dengue antiviral agent on the basis of indigenous Indonesian natural ingredients and as an adjunct to supportive IVD therapy in the future. Further research is recommended to confirm the scientific evidence, including compound fractionation, molecular

dynamics, and in vivo studies, to strengthen the translational evidence toward a standardized phytopharmaceutical.

6. DECLARATIONS

6.1 FUNDING STATEMENT

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6.2 CONFLICTS OF INTEREST

The authors declare that they have no competing financial or nonfinancial interests related to the work described in this manuscript. No author has received payment, honoraria, or any form of compensation that could constitute a conflict of interest with respect to this study.

6.3 AUTHOR CONTRIBUTIONS (CREDIT TAXONOMY)

Conceptualization: M.F.R. and I.M.D.M.A. Methodology: M.F.R., N.F.A., K.N., A.K.R., and A.B.A. Software: R.K. Formal analysis: M.F.R. and R.K. Investigation: M.F.R., N.F.A., K.N., A.K.R., and A.B.A. Resources: I.M.D.M.A. and A.K.R. Data Curation: M.F.R. and R.K. Writing – Original Draft Preparation: M.F.R., N.F.A., and K.N. Writing – Review and Editing: M.F.R., I.M.D.M.A., R.K., A.K.R., and A.B.A. Visualization: M.F.R. and R.K. Supervision: I.M.D.M.A. Project Administration: M.F.R. and A.B.A. Funding Acquisition: M.F.R. and I.M.D.M.A. All the authors read and approved the final manuscript.

6.4 ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The experimental procedures, comprising Vero cell culture, cytotoxicity and antiviral assays, and molecular docking simulations, were conducted in accordance with standard laboratory biosafety practices. Ethical clearance for the overall research protocol, including the use of plant-derived materials and cell-based experimental methods, was obtained from the Health Research Ethics Committee of the Faculty of Medicine and Health Sciences, Universitas Jambi, under number 130/DST/UN21.8.9/PT.01.04/2026, dated May 4, 2026.

6.5 CONSENT TO PUBLISH

Not applicable.

6.6 DATA AVAILABILITY STATEMENT

The data generated and analyzed during this study are available from the corresponding author (I.M.D.M.A.) upon reasonable request.

6.7 ACKNOWLEDGMENTS

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6.8 LIST OF ABBREVIATIONS

ABTS, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid); ADE, antibody-dependent enhancement; ADME, absorption, distribution, metabolism, and excretion; ANOVA, analysis of variance; ATR, attenuated total reflectance; CC_{50}, half-maximal cytotoxic concentration; CI, confidence interval; CV, coefficient of variation; DENV, dengue virus; DENV-2, dengue virus serotype 2; DMSO, dimethyl sulfoxide; DPPH, 2,2-diphenyl-1-picrylhydrazyl; DVI, dengue virus infection; EC_{50}, half-maximal effective concentration; FBS, fetal bovine serum; FRAP, ferric reducing antioxidant power; FT-IR, Fourier transform infrared spectroscopy; GC–MS, gas chromatography–mass spectrometry; GGE, Geisser–Greenhouse epsilon; IC_{50}, half-maximal inhibitory concentration; LD_{50}, median lethal

dose; MEM, minimum essential medium; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; MW, molecular weight; NS3, nonstructural protein 3; NS5, nonstructural protein 5; PBS, phosphate-buffered saline; PDB, Protein Data Bank; PDBQT, Protein Data Bank, Partial Charge, and Atom Type file format; RdRp, RNA-dependent RNA polymerase; RLU, relative light unit; RMSD, root-mean-square deviation; RT, retention time; SD, standard deviation; SI, selectivity index (Tables 4–6) or similarity index (Table 1); TIC, total ion chromatogram; TICA, total ion chromatogram area; TPTZ, 2,4,6-tris(2-pyridyl)-s-triazine.

REFERENCES

1. Adnyana IMDM, Nastiti NI, Delfira A, Asty ZF, Riawan AZ, Sudaryati NLG, et al. Insect-Specific Flaviviruses: Novel Strategies for Indonesian Vector-Borne Disease Control. J Vector Borne Dis. 2026 Jun 29;363_25. doi:10.4103/jvbd.jvbd_363_25

2. Wijayanti N, Kautsar R, Podhi FM, Sofyantoro F, Sucipto TH, Adnyana IMDM, et al. A systematic review of dengue epidemiology in Indonesia: Ten-year trends in case distribution and viral serotype dynamics. Asian Pac J Trop Med. 2026 Apr;19(4):148–64. doi:10.4103/apjtm.apjtm_23_26

3. Dinas Kesehatan Provinsi Jambi. Profil Kesehatan Provinsi Jambi tahun 2024. Dinas Kesehatan Provinsi Jambi. 1st ed. Jambi: Dinas Kesehatan Provinsi Jambi; 2024. p. 291.

4. Adnyana IMDM, Utomo B, Sulistiawati S, Adiwinoto RP, Sudaryati NLG. Climate Matters: Integrating Bioclimatology into Dengue Vector Control within the One Health Framework. J Arthropod Borne Dis. 2025 Aug 29;19(1):1729.

5. Adnyana IMDM, Sudaryati NLG, Eljatin DS, Adiwinoto RP, da Cruz ZV. One Health approach worldwide and challenges in collaboration. In: Gaikwad PS, Shukla VH, Choudhary P, editors. One Health Integration: Global Perspectives on Animal Health and Sustainable Agriculture. 1st ed. United States: John Wiley & Sons, Inc.; 2026. p. 33–62. doi:10.1002/9781394295982.ch02

6. Cruz ZV da, Adnyana IMDM. Spatio-temporal analysis of dengue virus infection in the Bobonaro Municipality (2022-2024): insight from demographics and risk. Berita Kedokteran Masyarakat. 2026 Jul 29;e28313. doi:10.22146/bkm.v42i07.28313

7. Parra-González M, Nájera-Maldonado L, Peralta-Cuevas E, Gutierrez-Onofre AJ, Garcia-Atutxa I, Villanueva-Flores F. Overcoming dengue vaccine challenges through next-generation virus-like particle immunization strategies. Front Cell Infect Microbiol. 2025 Jun 16;15:1614805. doi:10.3389/fcimb.2025.1614805

8. Anumanthan G, Sahay B, Mergia A. Current Dengue Virus Vaccine Developments and Future Directions. Viruses. 2025 Jan 31;17(2):212. doi:10.3390/v17020212

9. Sreekanth GP. Perspectives on the current antiviral developments towards RNA-dependent RNA polymerase (RdRp) and methyltransferase (MTase) domains of dengue virus non-structural protein 5 (DENV-NS5). Eur J Med Chem. 2023 Aug;256:115416. doi:10.1016/j.ejmech.2023.115416

10. Ugbaja SC, Mushebenge AGA, Kumalo H, Ngcobo M, Gqaleni N. Potential Benefits of In Silico Methods: A Promising Alternative in Natural Compound's Drug Discovery and Repurposing for HBV Therapy. Pharmaceuticals. 2025 Mar 16;18(3):419. doi:10.3390/ph18030419

11. Maharani MP, Salsabila MT, Fajriyah DR, Sucipto TH, Tukiran, Wardana AP. Potential of nano Phyllanthus niruri L. as anti-DENV candidate. Tropical Journal of Natural Product Research. 2025 Sep 30;9(9):24. doi:10.26538/tjnpr/v9i9.24

12. Wang Y, Mao J, Zhang M, Liu L, Zhu Y, Gu M, et al. An Umbrella Insight into the Phytochemistry Features and Biological Activities of Corn Silk: A Narrative Review. Molecules. 2024 Feb 18;29(4):891. doi:10.3390/molecules29040891

13. Adyana IMDM, Pratama MR, Jayaputra AM, Sudaryati NLG, Paiva MJM de, Suryani EM. The multi-target potential, mechanisms, and clinical evidence of Zea mays L. for health: A narrative review. Widya Kesehatan. 2026;8(1):45–54. doi:10.32795/bm04nz54

14. Sudaryati NLG, Utomo B, Adnyana IMDM, Fauziyah S, Sucipto TH, Cahyaningrum PL, et al. Phytochemical profiling, dengue antiviral properties, and cytotoxicity of novel Baper tea polyherbal infusion: Insights from in silico and in vitro studies. Salud, Ciencia y Tecnología. 2025 Jun 19;5:1791. doi:10.56294/saludcyt20251791

15. Papaefthimiou M, Kontou PI, Bagos PG, Braliou GG. Antioxidant Activity of Leaf Extracts from Stevia rebaudiana Bertoni Exerts Attenuating Effect on Diseased Experimental Rats: A Systematic Review and

Meta-Analysis. Nutrients. 2023 Jul 26;15(15):3325. doi:10.3390/nu15153325

16. Aladejana EB. Biological Properties of Polyherbal Formulations: A Review of their Antimicrobial, Anti-Inflammatory, Antioxidant, and Toxicological Activities. Pharmacognosy Journal. 2023 Nov 2;15(5):933–63. doi:10.5530/pj.2023.15.178

17. Blanco VP, Lombardo M, Da Silva TO, De Souza MRE, Eserian JK. Avanços nas diretrizes de boas práticas de fabricação de produtos farmacêuticos estéreis. Infarma - Ciências Farmacêuticas. 2023 Oct 4;35(3):364–74. doi:10.14450/2318-9312.v35.e3.a2023.pp364-374

18. Gomes VM, Silva VM de L e, Silva PGSA, Quemel GKC, Rivera JGB. Caracterização física, bromatológica e fitoquímica do fruto da euterpe oleracea mart. Infarma - Ciências Farmacêuticas. 2025 Dec 19;36(1):6–18. doi:10.14450/2318-9312.v36.e1.a2024.pp6-18

19. Tang J, Dunshea FR, Suleria HAR. LC-ESI-QTOF/MS Characterization of Phenolic Compounds from Medicinal Plants (Hops and Juniper Berries) and Their Antioxidant Activity. Foods. 2020 Dec 20;9(1):7. doi:10.3390/foods9010007

20. Magno dos Santos Ferreira A, Portela Radtke AC, Silva Carlotto Herrera SD, Bezerra D'Alessandro W, Martins de Paiva MJ. Análise da atividade antioxidante de plantas do cerrado do tocantins. Infarma - Ciências Farmacêuticas. 2026 Jul 21;38:e202609. doi:10.14450/infarma.e202609

21. Hariyono H, Sucipto TH, Kautsar R, Oktamia RH, Damayanti M, Dahliyanti SP. Anti-Dengue Serotype 2 Virus Activity of 2,4,5-Triphenylimidazole Hariyono. Advanced Journal of Chemistry, Section A. 2025;8(12):2128–40. doi:10.48309/ajca.2025.532927.1876

22. Menis Candela F, Soria EA, Moliva MV, Suárez Perrone A, Reinoso EB, Giordano W, et al. Anti-DENV-2 Activity of Ethanolic Extracts from Arachis hypogaea L.: Peanut Skin as a Relevant Resource of Bioactive Compounds against Dengue Virus. Plants. 2024 Oct 15;13(20):2881. doi:10.3390/plants13202881

23. Jayasekara KG, Suresh S, Goonasekara C, Soyza P, Perera N, Gunasekera K. Anti-dengue viral activity of Glycyrrhiza glabra roots in Vero cells. Sci Rep. 2024 Oct 29;14(1):25922. doi:10.1038/s41598-024-76184-5 PubMed PMID: 39472523.

24. Alagarasu K, Patil P, Kaushik M, Chowdhury D, Joshi RK, Hegde H V., et al. In Vitro Antiviral Activity of Potential Medicinal Plant Extracts Against Dengue and Chikungunya Viruses. Front Cell Infect Microbiol. 2022 Apr 7;12:866452. doi:10.3389/fcimb.2022.866452

25. Saqallah FG, Abbas MA, Wahab HA. Recent advances in natural products as potential inhibitors of dengue virus with a special emphasis on NS2b/NS3 protease. Phytochemistry. 2022 Oct;202:113362. doi:10.1016/j.phytochem.2022.113362

26. Vázquez-Calvo Á, Jiménez de Oya N, Martín-Acebes MA, Garcia-Moruno E, Saiz JC. Antiviral Properties of the Natural Polyphenols Delphinidin and Epigallocatechin Gallate against the Flaviviruses West Nile Virus, Zika Virus, and Dengue Virus. Front Microbiol. 2017 Jul 11;8:01314. doi:10.3389/fmicb.2017.01314

27. Adnyana IMDM, Utomo B, Fauziyah S, Eljatin DS, Setyawan MF, Sumah LHM, et al. Activity and potential of Phyllantus niruri L. and Phyllantus urinaria L. as Hepatitis B virus inhibitors: A narrative review of the SANRA protocol. Journal of Research in Pharmacy. 2024;28(1):335–50. doi:10.29228/jrp.700

28. Joković N, Matejić J, Zvezdanović J, Stojanović-Radić Z, Stanković N, Mihajilov-Krstev T, et al. Onion Peel as a Potential Source of Antioxidants and Antimicrobial Agents. Agronomy. 2024 Feb 24;14(3):453. doi:10.3390/agronomy14030453

29. Adnyana IMDM, Sudaryati NLG, Sucipto TH, Kautsar R, Nastiti NI, Delfira A, et al. Revealing the Dengue Antiviral Activity of Baper Tea Polyherbal: In Silico and In Vitro Approaches for the NS2B/NS3 Protease Inhibitors. Advanced Journal of Chemistry, Section A. 2026;9(7):1377–90. doi:10.48309/ajca.2026.569340.2017

30. Ghazali NAH, Balan T, Selvarajoo PD, Mohd Abd Razak MR, Ruzamri NB, Leong SW, et al. Effects of Apigenin Isolated From Christia vespertilionis Leaves on Anti-SARS-CoV-2 Activity: In Silico and In Vitro Studies. Chem Biodivers. 2025 Dec 8;22(12):202500478. doi:10.1002/cbdv.202500478