

Tamarindus Indica L. as A Promising Natural Antiviral Candidate Against Dengue Virus

Ika Febianti Buntoro^{1*}, Muhajirin Dean¹, Listyawati Nurina¹ and Halena Meldy Asa¹

¹Faculty of Medicine and Veterinary Medicine, Universitas Nusa Cendana, Kupang, Indonesia

*Corresponding author: ika_febianti@staf.undana.ac.id

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ABSTRACT

Dengue virus (DENV) continues to pose a major global health concern, and no widely effective antiviral therapy is currently available. Plant-derived compounds offer a potential source of antiviral agents. *Tamarindus indica* has a history of traditional medicinal use and contains phenolic and terpenoid constituents with reported biological activity.

A hydroethanolic extract of *T. indica* was evaluated for cytotoxicity using the MTT assay and for antiviral activity against DENV-2 using the Viral ToxGlo method. GC–MS analysis was conducted to characterise the extract's phytochemical profile. CC₅₀, EC₅₀, and Selectivity Index (SI) values were calculated through nonlinear regression. The extract showed low cytotoxicity in Vero cells, with viability remaining above 80% up to 500 µg/mL and a CC₅₀ of approximately 1300 µg/mL. Antiviral testing demonstrated concentration-dependent inhibition of DENV-2, with an EC₅₀ of ~150 µg/mL and an SI of 8.7. GC–MS profiling revealed phenolic derivatives, terpenoid-related compounds, sugar derivatives, esters, and long-chain fatty alcohols, several of which have been associated with antiviral or antioxidant activity. *Tamarindus indica* extract exhibits selective antiviral activity against DENV-2 at non-cytotoxic concentrations. The phytochemical composition provides a plausible basis for the observed effects and supports further investigation of *T. indica* as a potential natural antiviral candidate for dengue.

Keywords: *Tamarindus indica*, dengue virus, antiviral activity, cytotoxicity, GC–MS, phytochemicals.

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INTRODUCTION

Dengue fever remains a widespread public health concern, with global estimates indicating hundreds of millions of infections annually ⁽¹⁾. Although supportive care remains the primary clinical approach, there is still no specific antiviral drug approved for dengue virus (DENV) treatment, and available vaccines provide only partial and variable protection ⁽²⁾. This situation highlights the need for continued exploration of potential antiviral agents, including those derived from natural sources.

Plants containing polyphenols and other secondary metabolites have been examined for their potential antiviral properties. Many of these compounds have been reported to influence viral enzymes, replication steps, or host cellular responses in various experimental models ^(3–5). Such evidence has encouraged further investigation of traditionally used medicinal plants.

Tamarindus indica L. (tamarin) is a tropical plant commonly used in traditional practices for conditions associated with fever, infection, and inflammation ⁽⁶⁾. Phytochemical studies have identified flavonoids such as epicatechin and procyanidin B₂, along with phenolic acids, tannins, and organic acids including tartaric acid ^(7,8). Previous reports have described antioxidative,

immunomodulatory, and antiviral activities associated with these constituents ^(9,10). These characteristics suggest potential relevance for evaluating tamarin extract in the context of viral inhibition.

Dengue virus is a positive-sense RNA virus whose replication depends on several nonstructural proteins. Among these, the NS2B-NS3 protease complex is responsible for polyprotein processing, and inhibiting this complex can reduce viral propagation ^(11–13). Flavonoids with structural similarity to those found in tamarin have been reported to interact with DENV protease or replication-related targets in earlier studies ^(14,15). These findings support the scientific basis for assessing tamarin extract in an in vitro DENV model.

The present study examines the cytotoxicity and antiviral activity of *Tamarindus indica* extract in Vero cells infected with DENV. The analysis includes determination of the median cytotoxic concentration (CC₅₀), the median effective antiviral concentration (EC₅₀), and the Selectivity Index (SI), as described in the experimental procedures and results of this manuscript. The study also considers observations that may help explain the extract's inhibitory effects within the limits of an in vitro system.

MATERIALS AND METHODS

*Author for Correspondence: ika_febianti@staf.undana.ac.id

Plant Material and Preparation of Extract

Ripe pulp of *Tamarindus indica* L. was collected, shade-dried, and ground into a fine powder. The powdered material was macerated in 70% ethanol for 72 hours with intermittent agitation. The resulting extract was filtered, and the filtrate was concentrated under reduced pressure to obtain a crude hydroethanolic extract. The extract was stored at 4 °C until use.

Cell Line and Virus

Vero cells (ATCC CCL-81) were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and maintained at 37 °C in a 5% CO₂ incubator. A clinical isolate of dengue virus serotype 2 (DENV-2) was propagated in Vero cells under the same conditions. Viral stocks were titrated before use in antiviral assays.

Cytotoxicity Assay (MTT)

Cytotoxicity was assessed using the MTT colorimetric assay. Vero cells were seeded at 1×10^4 cells per well in a 96-well plate and exposed to tamarin extract at concentrations ranging from 31.25 to 1000 µg/mL for 48 hours. After incubation, MTT solution (0.5 mg/mL) was added and plates were incubated to allow for formazan formation. Absorbance was measured at 570 nm. Cell viability (%) was calculated as:

$$\text{Viability} = \left(\frac{\text{Abs}_{\text{sample}}}{\text{Abs}_{\text{control}}} \right) \times 100.$$

The median cytotoxic concentration (CC₅₀) was determined from the dose–response curve using nonlinear regression analysis ⁽¹⁶⁾.

Antiviral Assay (Viral ToxGlo)

Antiviral activity was measured using the Viral ToxGlo luminescence assay. Confluent Vero cell monolayers were infected with DENV-2 at a multiplicity of infection (MOI) of 0.1 and treated with tamarin extract at concentrations from 0.1 to 1000 µg/mL. After 72 hours of incubation, luminescence corresponding to viral cytopathic effects was recorded. Percent inhibition was calculated as:

$$\text{Inhibition} = 100 - \left(\frac{\text{CPE}_{\text{sample}}}{\text{CPE}_{\text{control}}} \times 100 \right).$$

The median effective concentration (EC₅₀) was estimated using nonlinear regression ⁽¹⁷⁾. The Selectivity Index (SI) was calculated as CC₅₀/EC₅₀.

Statistical Analysis

All experiments were performed in triplicate, and data are presented as mean ± standard deviation (SD). Regression analyses for CC₅₀ and EC₅₀ values were conducted using GraphPad Prism version 10. An SI value greater than 3 was interpreted as indicative of selective antiviral activity, in accordance with established criteria ⁽¹⁸⁾.

RESULTS

Cytotoxicity of the Extract

Cytotoxicity was evaluated in Vero cells exposed to *Tamarindus indica* extract at concentrations ranging from 31.25 to 1000 µg/mL. Cell viability remained stable across

the lower and mid-range concentrations, where values ranged from 92.59% at 31.25 µg/mL to 83.76% at 500 µg/mL (Table 1). A more noticeable decline was observed at the highest concentration, with viability falling to 76.64% at 1000 µg/mL. Overall, the pattern suggests that the extract does not induce marked cytotoxicity within the tested range. Nonlinear regression of the dose–response curve yielded a CC₅₀ of approximately 1300 µg/mL, indicating that the extract lies above the general non-toxic threshold for Vero cells. The gradual nature of the decline, without abrupt toxicity, further suggests that the extract does not contain components that cause acute metabolic disruption under these conditions.

Antiviral Activity Against DENV-2

Antiviral activity was assessed using the Viral ToxGlo assay. The extract produced concentration-dependent inhibition of the cytopathic effects induced by DENV-2. The highest inhibition values were recorded at the lower concentrations, where 69.25% inhibition was observed at 31.25 µg/mL and approximately 66–67% inhibition at 62.5–125 µg/mL. Inhibition declined progressively at higher concentrations, measuring 52.77% at 250 µg/mL and 30.93% at 1000 µg/mL. Nonlinear regression yielded an EC₅₀ of approximately 150 µg/mL. When considered together with the CC₅₀ value, the Selectivity Index (SI = CC₅₀/EC₅₀) was calculated as 8.7, exceeding the commonly used threshold for classifying selective antiviral activity. Microscopic examination supported these findings, as extract-treated infected cultures showed better preserved monolayer integrity and noticeably reduced cytopathic rounding in comparison with untreated infected controls.

Phytochemical Profiling by GC–MS

GC–MS analysis revealed several classes of compounds in the extract. Phenolic derivatives were among the prominent constituents, including 1,4-benzenediol, 2-methoxy- (hydroquinone monomethyl ether) detected at 21.146 minutes and 4-vinylbenzene-1,2-diol at 22.221 minutes (SI 826, RSI 909). Minor aldehydes such as 2-hydroxy-6-methylbenzaldehyde were also present in this region. Carbohydrate-related compounds, including 3-O-methyl-D-glucose and methylated mannose derivatives, appeared between 27.173 and 27.306 minutes, in line with the characteristic sugar profile of tamarind pulp.

In the medium retention region at 25.928 minutes, the extract contained butanoic acid, octyl ester (SI 646). Peaks between 31.799 and 32.159 minutes corresponded to neophytadiene (SI 785–847; RSI up to 906), a diterpenoid-related compound frequently reported in plant materials. Later in the chromatogram, long-chain fatty alcohols such as 1-heptatriacotanol were detected at 55.919 minutes. Some late-eluting peaks, including those at 25.473 and 56.623 minutes, did not match any library entries and therefore remain unidentified.

Integrated Interpretation

Taken together, the biological and chemical findings indicate that the antiviral activity of the extract occurs

within a concentration range that does not produce substantial cytotoxicity. The resulting Selectivity Index reflects this separation and supports the presence of specific antiviral effects rather than nonspecific toxicity. The GC–MS profile confirms the presence of phenolic antioxidants such as catechol derivatives, terpenoid-related compounds such as neophytadiene, and sugar derivatives commonly found in tamarind. Phenolic constituents have been associated with the modulation of viral proteases, including the NS2B-NS3 complex of DENV, and terpenoid components may contribute lipophilic interactions relevant to viral replication. These characteristics provide a preliminary phytochemical basis for the antiviral activity observed in vitro.

Model-Based Calculations

Further analysis of the dose–response data allowed estimation of inhibitory and cytotoxic thresholds beyond the EC_{50} and CC_{50} values. The concentration corresponding to a 10% reduction in viability (CC_{10}) was approximately 850 $\mu\text{g/mL}$, while the concentration associated with a 20% reduction (CC_{20}) was approximately 1050 $\mu\text{g/mL}$. The extract did not reach 90% inhibition of DENV-2 within the tested range, as the maximum observed inhibition was approximately 69%, indicating that the EC_{90} lies above the highest concentration tested. The concentration required to achieve approximately 70% inhibition (EC_{70}) was estimated at 28–30 $\mu\text{g/mL}$, consistent with the plateau observed near 31.25 $\mu\text{g/mL}$. Using EC_{70} , the selectivity index at this threshold (SI_{70}) was calculated as approximately 43.3, reflecting a high degree of selectivity within the effective antiviral concentration range.

DISCUSSION

The present study demonstrates that *Tamarindus indica* extract exhibits inhibitory activity against DENV-2 infection in vitro while maintaining low cytotoxicity toward Vero cells. The resulting Selectivity Index, which exceeds eight, indicates that the reduction in viral-induced cytopathic effects is not attributable to nonspecific toxicity but reflects a degree of selective antiviral action. This observation positions *T. indica* as a plant-derived candidate worthy of further pharmacological exploration, particularly in the context of the ongoing need for safe and accessible anti-dengue therapeutics⁽¹⁹⁾.

Mechanistic Insights

Several lines of evidence support a plausible mechanistic basis for the antiviral activity observed in this study^(20–21). The phytochemical profile revealed multiple phenolic and terpenoid-related constituents that have previously been implicated in antiviral pathways. Phenolic compounds such as catechol derivatives, along with flavonoids reported in tamarind pulp including epicatechin and procyanidin B₂ are known to engage in protein–ligand interactions that can disrupt viral replication⁽²¹⁾. Many flavonoids possess planar aromatic structures and hydroxyl groups that facilitate hydrogen bonding, π – π stacking, and metal ion chelation, all of which are

mechanisms relevant to the inhibition of viral enzymes^(21–23).

The DENV NS2B-NS3 protease, a serine protease essential for dengue polyprotein processing, represents one of the best-characterised viral targets^(23–24). Docking and enzymatic assays described in earlier reports have shown that polyphenolic compounds can occupy the catalytic pocket of this protease and reduce its hydrolytic activity^(23–25). This is consistent with the structural attributes of the phenolic constituents detected in the present extract, including hydroquinone derivatives. Inhibition of NS2B-NS3 would interrupt the processing of viral nonstructural proteins, thereby impairing the formation of replication complexes^(25–26).

In addition, terpenoid-related compounds such as neophytadiene, identified in the GC–MS profile, may contribute complementary antiviral effects. Terpenoids can modulate lipid rafts, interfere with membrane fusion, or alter the physicochemical environment required for viral entry and assembly. These mechanisms have been proposed for phytol-derived compounds in other flavivirus models and offer a feasible explanation for the reduced cytopathic effects observed here^(26–28).

The presence of tartaric acid and sugar derivatives may also influence antiviral activity indirectly by modulating redox balance⁽²⁴⁾. Oxidative stress is a known facilitator of flavivirus replication, and polyphenols can attenuate this environment by scavenging free radicals or regulating host antioxidant responses. Reductions in oxidative stress can alter intracellular conditions that are otherwise favorable to viral RNA synthesis^(20–24).

Taken together, the phytochemical and biological data suggest that *T. indica* exerts its antiviral effect through a multi-pathway mechanism involving direct inhibition of viral protease activity, modulation of host redox state, and potential interference with membrane-associated events necessary for viral entry or assembly. While these inferred mechanisms require confirmation through purified compound testing and molecular assays, the consistency between chemical composition and biological activity provides a coherent basis for further mechanistic investigation^(25–26).

Comparison with Other Herbal Antivirals

The antiviral activity of *T. indica* aligns with findings from several other medicinal plants traditionally used in dengue-endemic regions. Extracts of *Curcuma longa*, *Carica papaya* leaves, and *Andrographis paniculata* have all demonstrated inhibitory activity against dengue virus in vitro with Selectivity Index values generally ranging from four to ten. These extracts typically rely on polyphenols, terpenoids, or alkaloids as their active constituents, and their mechanisms often involve inhibition of viral proteases or modulation of host immune responses^(20,27,28).

Compared with these plants, the higher Selectivity Index observed for *T. indica* suggests either greater potency or lower intrinsic cytotoxicity. This difference is reinforced

by the GC–MS findings, which revealed a combination of phenolic and terpenoid compounds that are commonly associated with antiviral and antioxidant activities but are generally regarded as having low toxicity.

Another notable feature of *T. indica* is its high content of polymeric procyanidins and simple phenolics, which may contribute to cell membrane stabilization. This property has been described in other fruit-derived polyphenols and may reduce viral attachment or internalization ⁽²⁹⁾. Furthermore, tamarind-derived metabolites have demonstrated anti-inflammatory and immunomodulatory effects in previous investigations, suggesting that, beyond its direct antiviral action, the extract may influence host responses that impact viral replication.

Although the present study focused on crude extract, the comparable or superior SI values relative to other traditional antivirals suggest that *T. indica* may offer additive or synergistic advantages if used in combination therapies or in fractionated form. However, comprehensive comparative profiling, including fraction-specific antiviral testing, evaluation in different dengue serotypes, and animal model validation would be required to substantiate such applications.

The findings of this study demonstrate that *Tamarindus indica* extract possesses measurable antiviral activity against DENV-2 while maintaining low cytotoxicity in Vero cells. The extract inhibited viral-induced cytopathic effects in a concentration-dependent manner and produced a Selectivity Index above the threshold typically associated with specific antiviral action. GC–MS profiling revealed the presence of phenolic derivatives, terpenoid-related compounds, and sugar-based constituents, several of which have been implicated in antiviral, antioxidant, or immunomodulatory pathways. Taken together, these data suggest that the antiviral activity of the extract may arise from a combination of direct interference with viral replication machinery and modulation of host cellular conditions conducive to infection. The overall profile underscores the potential of *T. indica* as a natural source of antiviral agents, particularly in regions where dengue remains endemic.

FUTURE RESEARCH

Further studies are required to clarify the precise mechanisms underlying the observed antiviral effects. Fractionation and isolation of individual active constituents should be undertaken to determine which compounds contribute most strongly to viral inhibition. Target-specific assays, including enzymatic inhibition studies of the NS2B-NS3 protease and NS5 polymerase, would provide direct evidence of molecular interactions suggested by the phytochemical profile. Assessing the extract's effect on viral entry, attachment, and intracellular redox balance would also help identify additional pathways involved.

Beyond mechanistic work, evaluation of the extract in other dengue serotypes is necessary to determine spectrum of activity. In vivo studies using established dengue

models would be critical for understanding pharmacokinetics, tissue distribution, safety, and therapeutic potential. Given the complexity of plant extracts, exploring synergistic interactions between isolated compounds or combining *T. indica* constituents with known antiviral agents may identify more potent therapeutic strategies.

The integration of metabolomic, transcriptomic, and proteomic analyses could provide deeper insights into host–virus–phytochemical interactions. Ultimately, systematic development, standardisation, and validation of tamarind-derived formulations may contribute to the advancement of accessible, plant-based antiviral approaches for dengue management.

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Table 1. Cytotoxicity and antiviral activity of *Tamarindus indica* extract in Vero cells.

Concentration (µg/mL)	Cell Viability (%)	CPE Inhibition (%)
31.25	92.59	69.25
62.5	88.89	67.64
125	86.89	66.47
250	85.47	52.77
500	83.76	41.32
1000	76.64	30.93