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Short Communication

# Antiviral Activity of *Sterculia Quadrifida* R.Br Extract Against Dengue Serotype 2 Virus

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ABSTRACT

There are four serotypes of dengue viruses (DENV1, 2, 3 and 4). Dengue is typically transmitted in a cycle involving humans and mosquito vectors. There has been a significant increase in the prevalence of dengue virus worldwide in recent decades. At present, there is no specific drug available to control the infection, and the most effective preventive measures are the control of the mosquito population. The objective of this study was to assess the antiviral properties of *Sterculia quadrifida* ethanol extract against dengue infection. The stem bark of *Sterculia quadrifida* R.Br was extracted using three different solvents: n-hexane, water, and 70% ethanol. Dengue serotype 2 (DENV-2) virus was propagated in C6/36 cells. MTT reagents were used to perform cytotoxicity tests, and the antiviral activity of the extract was evaluated in Vero cells. The result of the cytotoxicity test showed that the extracts of *Sterculia quadrifida* caused the inhibition of DENV-2 with inhibitory potential of 19.156 µmg/mL, 3.592 µmg/ml, and 6.028 µmg/ml at concentrations of 36.296 µmg/mL, >200 µmg/mL, and 99.743 µmg/mL, for n-hexane, water, and 70% ethanol, respectively. The cytotoxicity of *Sterculia quadrifida* suggests it might be a valuable source of antiviral agents.

***Keywords*:** Dengue, antiviral, DENV-2, Flaviviridae, *Sterculia*

## Introduction

The virus causing dengue fever is transmitted by the Aedes mosquito, and this disease is rapidly spreading worldwide, particularly in subtropical and tropical nations. Most cases, estimated at 96 million per year, are asymptomatic, posing a life-threatening risk for many individuals. Dengue virus (DENV) belongs to the Flaviviridae family, characterized by an enclosed, single-stranded RNA structure. Comprising four serologically distinct serotypes, namely DENV-1, DENV-2, DENV-3, and DENV-4, these viruses contribute to various dengue epidemics in different regions. Infection with multiple serotypes increases the likelihood of the disease progressing to severe forms such as Dengue Hemorrhagic Fever (DHF) or Dengue Shock Syndrome (DSS). Despite the global threat of the pandemic, there is currently no specific therapy for dengue illness. Although several antiviral drugs have been evaluated, none have demonstrated sufficient efficacy to be recommended for treating dengue infection.[2-3]

Efforts have been made to assess the antiviral activity of various natural products, including plants, to identify and characterize novel chemicals limiting virus replication or treating viral illness. Developing antiviral medications for dengue encounters substantial hurdles stemming from the virus's complex nature. Dengue virus manifests in four diverse serotypes, each presenting distinct genetic traits. Crafting a universal antiviral solution becomes intricate due to the need to account for these genetic variations. The virus's rapid mutation rate further complicates drug development, necessitating continuous adaptation of antiviral strategies to keep pace with viral evolution.

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Additionally, the intricate interactions between the virus and the host immune system pose challenges, as the virus employs mechanisms to evade the host's defenses, hindering the development of drugs that can effectively target viral replication without compromising the host's immune response. As a result, plant extracts and constituents are gaining significance as possible antiviral drugs. The screening of plant extracts has led to the discovery of efficient *in vitro* viral replication inhibitors.[4-5] This study investigated *Sterculia quadrifida* R.Br extracts *in vitro* against DENV-2 to discover new antiviral medicines to combat dengue using different extracting solvents.

## Materials and Methods

*Collection and preparation of plant materials*
*Plant collection*

The stem bark samples of *Sterculia quadrifida* R.Br., family, Malvaceae, were collected in the month of June, 2022, from Kupang City, Indonesia. The plant sample was identified at the Department of Agriculture, Universitas Nusa Cendana. The herbarium specimen was collected with voucher number FA:023-MACHUNG-2022.

*Preparation of plant samples*

The stem bark sample was dried under shade for two weeks and ground to powder using a mechanical blender. The powdered stem bark sample of *Sterculia quadrifida R.BR* was extracted separately in n-hexane, water, and 70% ethanol. First, the plant sample was boiled at 95°C for 30 minutes, filtered with Whatman no. 1 filter paper, and concentrated to dryness over a hot water bath to obtain the water extract. Again, the powdered sample was extracted with 70% ethanol for 2x24 hours with periodic stirring, filtered, and dried with a rotavapor at 45°C to yield a dry extract. The n-hexane extract was derived from the powdered stem bark of *Sterculia quadrifida*. The process involved immersing the powder in n-hexane for two cycles of 24 hours each, with intermittent stirring. Subsequently, the mixture was filtered using filter paper and dried using a rotavapor at a temperature of 40°C.

*Cell Lines and Virus Preparation*

The C6/36 cell line, a clone originating from Aedes albopictus larvae, was supplied by the Laboratorium Institute Tropical Disease at Universitas Airlangga in Indonesia. The cell lines obtained from The Global Bioresource Center in the United States were cultured in a medium consisting of 2% penicillin-streptomycin and 10% FBS (Gibco, United States). The cultures were then incubated at 37°C with 5% CO2. Vero cells, forming a single confluent layer, were detached using trypsin-EDTA, incubated for 5 minutes at 37°C, and then the medium was added slowly. Hemocytometer readings (Paul Marienfeld, Germany) were taken. The cells were grown at a density of 1x106 cells/mL in a 96-well plate and further incubated at 37°C with 5% CO2. For this study, DENV-2, initially isolated in Surabaya, Indonesia, and DENV-2 clinical isolates discovered by the Dengue Study Group at the Institute of Tropical Diseases, Universitas Airlangga (NCBI access number: KT012513), were employed. As per prior research, all DENV-2 strains discovered in Surabaya were classified as cosmopolitan genotypes. Clinical isolates were propagated and maintained following the methodology described by Kotaki et al. Once the virus isolates were titrated, stock files were preserved at -80°C for future investigations.[6]

*Cytotoxicity Assay*

As previously outlined by Sucipto et al., a singular dense layer of Vero cells at a concentration of 1x105 cells/mL was cultivated in a 96-well plate. The quantity of cell cultures was controlled by assessing the ATP levels utilizing the Luminescent CellTiter-Glo® Cell Viability Test from Promega, USA. Moreover, the CellTiter-Glo® Luminescent Cell Viability Test was specifically employed for cytotoxicity assessments, following the instructions provided by the manufacturer.[7]

*Antiviral Assay*

In this study, the Viral ToxGloTM Assay from Promega, USA, was employed to detect the cytopathic effects of viral infections. A confluent layer of Vero cells (1x106 cells/mL) was cultivated on a 96-well plate, and DENV-2 titers were introduced at a concentration of 2x104 FFU/well. The Viral ToxGloTM Assay reagents were applied following the instructions provided by the manufacturer. Additionally, the selectivity index (SI) value was computed by comparing the ratio of the 50% cytotoxic concentration (CC50) to the 50% antiviral concentration (IC50), as previously elucidated by Zandi *et a*l.[8]

## Results and Discussions

Using the MTT assay, the cytotoxic effects of *Sterculia quadrifida R.BR* extracts were quantified by their $CC_{50}$ values. The highest concentration (200 g/mL) of the three extracts (water, n-hexane, and 70% ethanol) did not produce 100 percent mortality in cytotoxicity tests. Table 1 and Figure 1 illustrate the cell viability rate induced by *Sterculia quadrifida R.BR* extracts. Before conducting antiviral testing, the cytotoxicity of the extract against C6/36 cells was examined, and the result showed that it was non-toxic.

In Figure 1, the three regression equations $y=-0.3321x+62.054$, $y=-0.059x+92.093$, and $y=-0.4819x+98.006$ were employed to model the relationship between the cytotoxicity concentration ($CC_{50}$) of *Sterculia quadrifida* extracts and the live cells in the experiment. These equations yielded corresponding $R^2$ values of 0.6601, 0.294, and 0.9125, respectively. The analysis suggests that the 70% ethanol extract provides the most robust and comprehensive model, as it demonstrates the highest $R^2$ value, indicating that around 91.25% of the variability in $CC_{50}$ can be explained by the linear relationship with the independent variable. There may be several mechanisms involved in the antiviral activity of the extracts. The viral suppression mechanism inhibits the activity of non-structural protein (NS) viruses. However, protein analysis enables more precise observations of cellular apparatuses. Another mechanism may be due to the inhibition of virion assembly. The penultimate stage of virus replication in host cells is virion assembly. Recently, emphasis has been placed on inhibiting viral assembly during Envelope and PRM structural protein synthesis. On day four, viral replication was identified. In this study, antiviral assays were conducted on the fifth day of infection, optimizing the research approach. The extracts from *Sterculia quadrifida* R.BR were found to suppress DENV-2 reproduction in C6/36 cells by 52.82%, 61.81%, and 61.00%, as illustrated in Figure 2 and Table 2.

The use of active plant extracts in pretreatment may alter host components, such as receptors, to restrict virus entry and/or replication. Extracts active under cotreatment conditions may bind to the virus, preventing it from attaching to cellular receptors necessary for entry. Those showing activity under posttreatment conditions may impact viral replication and/or assembly by interacting with viral or host proteins involved in these processes. Further investigation is necessary to elucidate the mechanisms of action of these plant extracts.

While many trials reported antiviral activity as a decrease in infectious virus particles (FFU), discrepancies were observed where viral RNA titers did not decrease proportionally. This suggests that certain plant extracts causing a reduction in FFU may not influence viral RNA replication but could limit virus particle formation. Quantitative real-time RT-PCR, being more sensitive than FFU, can detect RNA even from non-infectious particles.

Hence, slight variations in viral RNA titers may not be reflected in quantitative real-time RT-PCR assay results until the RNA titer is significantly different. Extracts affecting both FFU and viral RNA titers may hinder the replication of viral RNA. The concentration of extracts exhibiting antiviral activities varied, with those active at lower concentrations potentially containing higher amounts of active antiviral phytoconstituents. Extracts with antiviral activity at lower concentrations could be explored for further formulation as phytopharmaceutical medicine. However, for extracts with antiviral activity at higher concentrations, identifying the active ingredient is crucial before considering them as antiviral drugs.

Identifying active fractions and compounds from extracts with anti-dengue and anti-chikungunya activity will aid in developing formulations for further investigation in preclinical and clinical research. These findings pave the way for future research on plant-based antivirals against DENV to discover effective therapeutics for this severe virus.

## Conclusion

The ethanol extract of *Sterculia quadrifida* R.BR demonstrated promising antiviral activity against Dengue serotype 2 (DENV-2). Cytotoxicity tests revealed low toxicity, suggesting potential safety. While these findings suggest *Sterculia quadrifida* R.BR as a candidate for further antiviral research, additional studies, including *in vivo* and clinical trials, are needed for validation and practical application.

## Conflict of Interest

The authors declare no conflict of interest.

**Table 1:** The rate of cell death due to the treatment of *Sterculia quadrifida R.BR* extracts

| No. | Type of extract | Cells viability (mg/mL) |
|---|---|---|
| **1** | n-hexane extract | 36.296 |
| **2** | Water extract | >200 |
| **3** | 70% ethanol extract | 99.743 |

**Table 2:** The Inhibitory Potential ($IC_{50}$) of *Sterculia quadrifida R.BR* extracts

| NO. | Type of extract | Inhibitory Potential (mg/mL) |
|---|---|---|
| **1** | n-hexane extract | 19.156 |
| **2** | Water extract | 3.592 |
| **3** | 70% ethanol extract | 6.028 |

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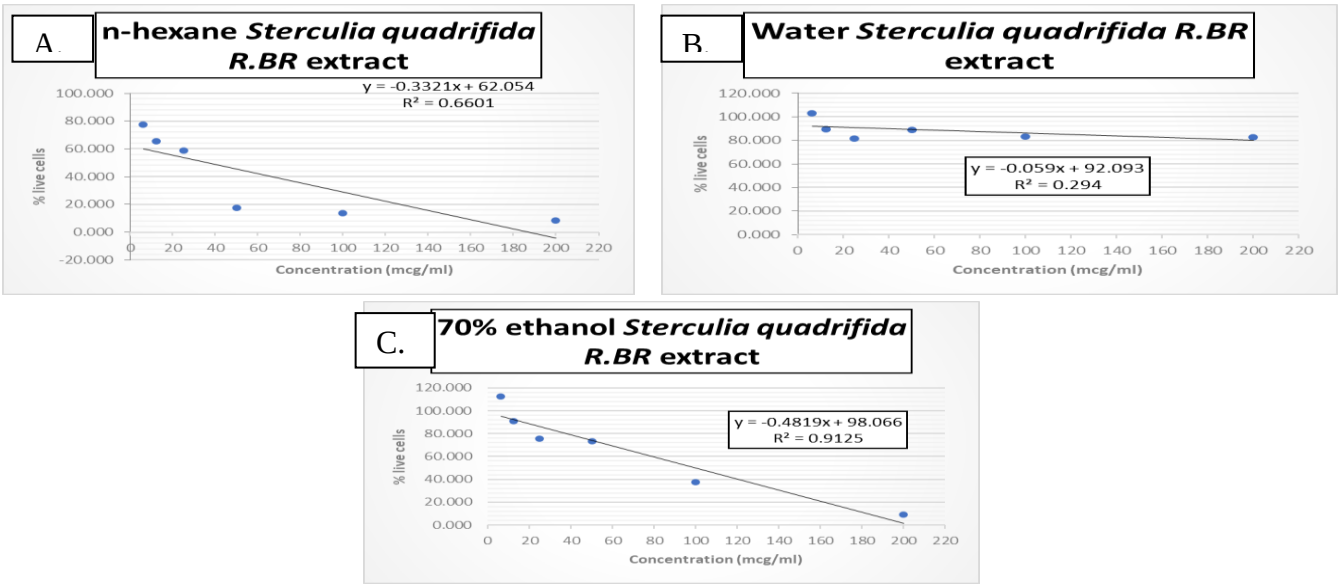


**Figure 1:** The regression equation of the Cytotoxicity Concentration ($CC_{50}$) of *Sterculia quadrifida R.BR* extracts.

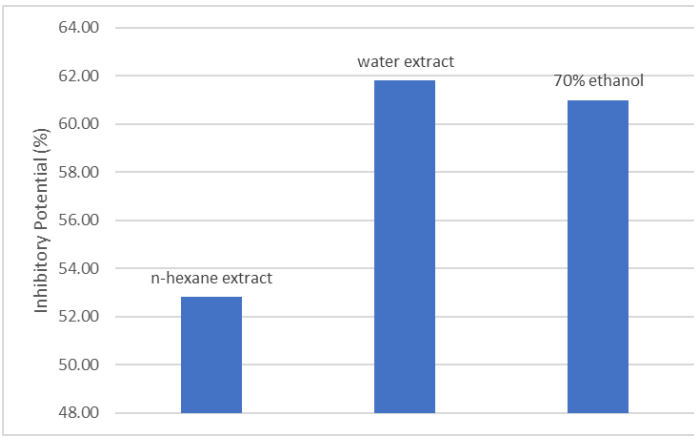


**Figure 2:** The inhibitory potential of *Sterculia quadrifida* extracts

## Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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