**Mangifera foetida** L. (Macang) Source of Potent Antiviral Activity of Against Dengue Virus Serotype 2 (Anti DENV2)

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**Abstract** The new discovery about the potential of *Mangifera foetida* L. as an antiviral will help conservation efforts in nature while maintaining and increasing its biodiversity value. This study aims to characterize the in vitro potential of three varieties of *M. foetida* L. against the dengue virus. Dengue virus is infected in Vero cells, viral replication was measured using the Viral ToxGlo Assay method. The selectivity ability of *Mangifera foetida* L. stem bark extract to inhibit the dengue virus was seen from the Selectivity Index (SI) value. The standard curve between the concentration of the compound (µg/mL) and % cell viability was analyzed by linear regression using Microsoft Excel 2010 software. The results showed that the selectivity index (SI) value of *M. foetida* L stem bark extract of Limus, Manis and Batu varieties were 7.58, 6.82 and 16.43, respectively. It was concluded that the extract of Macang stem bark of the Limus, Manis and Batu varieties had the potential to be used as an antiviral for dengue.

**1. Introduction**

Dengue fever is a major cause of childhood mortality worldwide, especially in the tropics and subtropics. In Indonesia, there were 95,893 cases of dengue fever throughout 2020 [1]. Consequently, the search for drugs as anti-dengue agents becomes an urgent matter to be carried out to solve major vector diseases such as malaria. Plants as drugs are in demand by the public because they are considered safer, less harmful when compared to synthetic drugs.

Macang (*Mangifera foetida* L.) is a plant that grows wild and has the potential to be used as herbal medicine. However, it is rarely cultivated by the community and has low economic value due to the sour taste of the fruit, coarser fiber, and more sap than other species of mango. People often use wood as boards for building materials [2]. The existence of this species is increasingly threatened with extinction due to the rapid rate of deforestation in Indonesia so that the forest area as the Macang natural habitat continues to decrease. In nature, Macang has ecological value as the main food for primates and other wildlife.
The sour taste in Macang fruit is an indication of the high antioxidant content. Quantitative analysis of antioxidants on wild mangoes from Sumatra by Fitmawati et al.[3], reported that Macang contains gallic acid and quercetin which have the potential as antioxidants. In addition, Macang is the type that contains the largest mangiferin content, about 2.56% higher than other species of mango [2].

Many studies have shown that Macang has the ability to pharmacological activities such as antibacterial, antifungal, anti-inflammatory, toxic, antioxidant, and immunomodulatory [4-7]. Based on this research, testing of Macang's antiviral activity has never been informed so that this research needs to be carried out and become a pioneer of research for Macang. These advanced antivirus reviews can provide information about the sources of new antiviruses. This research was conducted in vitro using samples of three varieties of Macang, namely Limus, Manis and Batu. Characterization of the potential of the three varieties of Macang aims to obtain Macang that have the potential as dengue antiviral by testing the antiviral activity and cytotoxicity.

2. Methodology

2.1. Materials and extract preparation
The materials used in this study were 50 g of stem bark three varieties of M. foetida L. The samples of Macang were made into powder using blander. The powder was used for extraction. The process of making Macang stem bark extract was done by preparing 50 g of powder, then macerated with methanol and added until submerged. All macerates were collected and evaporated using a vacuum rotary evaporator at 50°C until thick extracts were obtained.

2.2. DENV-2 Virus Preparation
The virus used in this study was dengue virus serotype 2 (DENV2). The virus was obtained from the Dengue laboratory, Tropical Disease Institute, Airlangga University.

2.3. Vero Cell Preparation
Preparation of vero cells (African green monkey kidney) followed the method of Sucipto and Martak [8]. Cells were propagated in Minimum Essential Eagle Medium (MEM) containing 10% fetal bovine serum (FBS). Vero cell cultures were incubated at 37 °C in 5% CO2. Single layers of vero cells were separated using trypsin-EDTA and incubated at 37 °C for 5 minutes. After that, the Minimum Essential Eagle Medium (MEM) containing 10% fetal bovine serum (FBS) was added, pipetted gently to break up the clumps of cells and counted using a hemocytometer. Cells were added to a 96 well microplate as much as 1x10^6 cells/10 mL and incubated at 37 °C in 5% CO2. Cells were monitored every day or every other day until cell density reached > 90%.

2.4 Dengue Serotype 2 (Anti-DENV2) Antivirus Test
Antiviral activity testing was carried out with the Viral ToxGlo Assay following the Maharani et al method [9], by modifying the virus serotype and the concentration of the extract used. Vero cells with a concentration of 1x10^6 cells/10 mL were sown into 96 well microplates and incubated at 37 °C in 5% CO2 for at least 4 hours (up to 24 hours). A total of 100 L of dengue virus serotype 2 (DENV2) with a concentration of 4x10^5 FFU/ml were combined with various extract concentrations (100, 50, 25, 12.5, 6.25, 3.13, 1.56 g/mL). After 1 h of exposure to the extract at room temperature, 100 L of extract-treated virus was added to vero cells in individual wells. Cells were infected for 1 hour. After 48 hours post-infection, 100 L of ToxGlo reagent was added (Promega, Madison, WI). Then 100 L of ATP detection reagent was added to each well and waited 10 min before calculating the luminescence value. Relative Luminescence Units (RLU) using a luminometer.

2.5 Cytotoxicity Test Against Vero Cells
Cytotoxicity testing was carried out using the MTT assay following the Laysa method [10], by modifying the cell type and the concentration of the extract used. This study uses vero cells. Vero cells
were added to 96 well microplate extract samples with each concentration of 200, 100, 50, 25, 12.5, 6.25, 3.13, 1.56 g/mL and incubated for 46 hours. Then the medium was discarded and replaced with 150 L/well MTT 10% using a Multichannel pipette and incubated for 4 hours at 370 C and 5% CO2. Then 100 L/well 100% DMSO was added to dissolve the precipitate caused by the MTT reaction. After that the absorbance was measured at a wavelength of 560 nm and 750 nm.

2.6 Data analysis
The data obtained in this study in the form of 50% inhibitory concentration (IC50) and 50% cytotoxicity concentration (CC50), analyzed by linear regression Microsoft Excel 2010 software (10.11) Selectivity of Macang stem bark extract in inhibiting dengue virus seen from the value of Selectivity Index (SI) Selectivity Index (SI) values were obtained using the formula [12]:

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SI = \frac{\text{Half Maximal Cytotoxicity Concentration (CC50)}}{\text{Half Maximal Inhibitory Concentration (IC50)}}
\]

3. Results and Discussion

3.1 IC50 Value (Half Maximal Inhibitory Concentration)
The IC50 value (Half maximal inhibitory concentration) is the amount of concentration capable of providing a 50% inhibitory effect on viral infectivity in cells [11]. The IC50 value was obtained from linear regression analysis using cell viability data in the dengue antiviral activity test. Following are the results of the analysis of the IC50 value of the stem bark extract of the three Macang varieties (Table 1).

Based on the analysis of the IC50 value of the stem bark extract of three Macang varieties, the Batu variety showed the strongest antiviral activity, followed by the Manis variety and the Limus variety. Laysa [10] stated that the smaller the IC50 value of the sample obtained, the higher the antiviral activity of the sample. The three varieties of Macang are included in the category of Significant or strong cytotoxicity because they have an IC50 value of < 40 µg/mL [13].

| Varieties | IC50 (µg/mL) | IC50 Category                  |
|-----------|--------------|--------------------------------|
| Limus     | 18.69        | Significant or strong cytotoxicity |
| Manis     | 10.46        | Significant or strong cytotoxicity |
| Batu      | 8.14         | Significant or strong cytotoxicity |

Table 1. IC50 Value of stem bark Mangifera foetida L.

The difference in the ability of Macang stem bark extract in inhibiting dengue virus activity (IC50) is thought to be due to the presence of certain phytochemicals that have the potential to inhibit dengue virus activity. One of the most abundant phytochemical content in Macang is flavonoids. Giving flavonoids can reduce dengue virus infection, through the mechanism of inhibiting the polymerase enzyme owned by the virus so that the replication process in the host is disrupted [14]. In addition, flavonoid compounds can inhibit protease enzymes in the dengue virus and prevent the entry of the virus into host cells through the mechanism of inhibiting virus adhesion to the host cell surface [15-16]. Therefore, the flavonoid compounds contained in Macang stem bark extract are thought to have an effect on IC50 inhibition.

Varieties of Limus, Manis and Batu have higher flavonoid compounds in the stem bark than in the leaves [3]. The main flavonoids contained in mango are quercetin and catechins [17]. Batu variety has the highest flavonoid content compared to the other two varieties, so it has the strongest antiviral activity. Macang is known to contain secondary metabolites in the form of mangiferin which is a flavonoid derivative. Mangiferin can be found in various mango organs such as leaves, roots and...
stems [8-19]. Purwaningsih [2] stated that Macang has the highest mangiferin content, which is 2.56% higher than other types of mango. Mangiferin has potential as an antioxidant, anti-inflammatory, antitumor, immunomodulatory and anti-HIV. In addition, based on Herman [20] using in silico analysis, it was found that mangiferin compounds had the best potential for inhibiting all dengue virus proteins compared to other compounds.

Research Fitmawati et al. [3] showed that the stem bark of Macang contains quercetin and gallic acid compounds, these two compounds play an important role as a source of antioxidants. Antioxidants play an important role in increasing immunity, overcoming several types of degenerative diseases, and responding to external attacks such as bacteria, fungi, and viruses. In research testing several types of bioflavonoids, it was reported that quercetin has antiviral activity against the dengue virus, but it is not yet known how the mechanism of quercetin provides an antiviral effect against the dengue virus [14]. Another study by Bachmetov et al. [21] reported that quercetin has antiviral activity against the hepatitis C virus (HCV) by binding to and inactivating the viral NS3 protease.

Dengue antiviral activity may also be derived from gallic acid compounds. Gallic acid is the main polyphenolic compound in mango [22]. Belur and Pallabhanvi [23] stated that gallic acid has antiviral, antibacterial, analgesic, and antioxidant abilities. Nutan et al. [24] reported that gallic acid can act as anti-HIV, gallic acid inhibits protease activity and reverses transcriptase of HIV-1.

3.2 CC50 Value (Half Maximal Cytotoxicity Concentration)

The value of CC50 (half-maximal cytotoxicity concentration) is the amount of extract concentration needed to kill 50% of total cells [11]. The CC50 value was obtained from linear regression analysis using Vero cell viability data in the cytotoxicity test. Following are the results of the analysis of the CC50 value of the stem bark extract of the three Macang varieties (Table 2).

| Varieties | CC50 (µg/mL) |
|-----------|-------------|
| Limus     | 141.68      |
| Manis     | 71.36       |
| Batu      | 133.78      |

Based on the analysis of the CC50 value of the stem bark extract of three Macang varieties, the Manis variety showed the strongest cytotoxicity, followed by the Batu variety and the Limus variety. The difference in the ability of Macang stem bark extract to kill Vero cells (CC50) is thought to be due to differences in the amount of phytochemical content in each variety. The higher the phytochemical content, the higher the potential for the presence of phytochemicals that have the ability to kill Vero cells.

According to research of Fitmawati et al. [25] showed that the Limus, Manis and Batu Macang varieties contained 250, 269, and 235 chemical compounds in the methanol solvent, respectively, while the Manis variety contained 11 chemical compounds in the dichloromethane solvent, while the Limus and Batu varieties both contains 9 chemical compounds. The compounds contained in the three varieties of Macang are alkaloids, phenolics, flavonoids, amino acids, aromatic compounds, carboxylic acids, sesquiterpenoids, essential oils, medium-chain fatty acids, and nucleic acids. The most common group of compounds found in the study were flavonoids and amino acids.

Research Fitmawati et al. [26] revealed that the Limus and Batu varieties were in one group based on kinship analysis using metabolite compounds, while the Manis variety formed a separate group. Fitmawati et al. [25] informed that the Limus and Batu varieties had the highest similarity of compounds, namely 257 compounds so that the Limus and Batu varieties had a CC50 value that was not much different.
3.3 Selectivity Index (SI) Value
The selectivity of the extract in inhibiting the dengue virus was seen from the value of the Selectivity Index (SI). The SI value was obtained from the CC50 divided by the IC50 of each Macang variety. According to Sutejo [27] (2016), an extract can be said to have high selectivity if the SI value is > 3 and it is said to be less selective if it has an SI value < 3.

Based on the Selectivity Index (SI) value (Table 3), the Batu variety had the highest SI value compared to the other Macang samples, which was 16.43. The three Macang varieties have SI values > 3, so it can be said that the stem bark extract of the three Macang varieties namely Limus, Manis, and Batu is selective in inhibiting dengue virus activity. This shows that extracts of Macang stem bark varieties Limus, Manis and Batu can be developed and used as dengue antiviral in the future.

Table 3. Selectivity Index (SI) Value of Mangifera foetida L.

| Varieties | SI (µg/mL) |
|-----------|------------|
| Limus     | 7.58       |
| Manis     | 6.82       |
| Batu      | 16.43      |

Research on plants that have the potential as dengue antiviral has been carried out by Saptawati et al. [28] revealed that Psidium guajava and Carica papaya were proven can inhibit dengue virus activity with SI values of 21.28 and 37.25, respectively. The SI values of Psidium guajava and Carica papaya were higher than the SI values of the three Macang varieties.

4. Conclusions
The stem bark extract of the three Macang varieties showed antiviral activity and cytotoxicity effects. Stem bark extract of Limus, Manis, and Batu varieties showed IC50 values of 18.69, 10.46 and 8.14, respectively. The CC50 values were 141.68, 70.36, and 133.78, respectively. The selectivity index (SI) values were 7.58, 6.82, and 16.43, respectively. The variety of Batu has the most potential to be used as dengue antiviral, followed by Limus and Manis varieties.

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