Contents lists available atIOCS

Science Midwifery

journal homepage:www.midwifery.iocspublisher.org

In vitro study the effect of flavonoid routine in cassava leaves (manihot esculenta c.) on the replication of dengue virus serotype 1 strain new guinea C

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ARTICLEINFO

Article history:

Received Jun 1, 2026
Revised Jun 7, 2026
Accepted Jun 16, 2026

Keywords:

Dengue Virus
Manihot Esculenta C
Rutin Flavonoids

ABSTRACT

Dengue hemorrhagic fever (DHF) is an infectious disease caused by the dengue virus with clinical manifestations of fever, muscle pain and joint pain accompanied by leukopenia, rash, lymphadenopathy and thrombocytopenia. According to WHO, DHF is still a major health problem in Indonesia, while dengue antiviral therapy does not yet exist, but several studies have found the potential of cassava leaves because they contain routine flavonoid compounds that function as antivirals. Objective: To determine the effect of routine flavonoids from cassava leaves (Manihot esculenta C.) on the replication of Dengue virus serotype 1 strain New Guinea C in Vitro by analyzing the 50% inhibitory concentration (IC50), 50% cytotoxic concentration (CC50), and selectivity index. Method: The research was experimental with a post-test control group design. This study used 7 groups, namely 1 control group and 6 treatment groups. The treatment group consisted of routine administration of Flavonoids from cassava leaves (Manihot esculenta C.) with concentrations of 50 µg/ml, 25 µg/ml, 12.5 µg/ml, 6.25 µg/ml, 3.125 µg/ml and 1.5 µg/ml. The control group (K) was given 0.2% Dimethyl sulfoxide (DMSO). Data were tested using the Kruskal Wallis test. Results: In the Kruskal Wallis test, there was an effect of routine Flavonoids on dengue virus replication (p value = 0.006). The IC50 value obtained at a concentration of 3.36 µg/ml was declared very strong inhibition, and the CC50 at a concentration of 17.48 µg/ml was declared toxic, for the Selectivity Index obtained a value of 5.2 declared safe in its use. Conclusion: Routine flavonoids in cassava leaves (Manihot esculenta C.) have an effect on the replication of Dengue virus Serotype 1 Strain New Guinea C in Vitro.

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INTRODUCTION

Dengue hemorrhagic fever (DHF) is an infectious disease caused by the dengue virus with clinical manifestations of fever, muscle and joint pain accompanied by leukopenia, rash, lymphadenopathy and thrombocytopenia. According to WHO, dengue hemorrhagic fever is still a major health problem in Indonesia where according to the World Health Organization (WHO) also stated that the number of dengue fever cases reported worldwide has increased more than 8-fold over the past 4 years, from 505,000 cases to 4.2 million in 2019. In data obtained from the Indonesian Ministry of Health in 2021, the highest number of dengue fever cases occurred in West Java province with 18,608 cases, in addition to that, the district and city of Cirebon were ranked third as the city and district with the most dengue fever patient deaths in 2020 (Ministry of Health, 2021). Based on data from the Directorate General of Disease Prevention and Control, at the end of 2022, the number of dengue cases in Indonesia reached 143,000 cases with (IR: 52.7), in 2020 (103,509 with IR: 38.15), in 2019 (138,127 with IR: 51.48), and in 2018 (65,602 with IR: 24.73) (Samad I, Handito A, Sugiarto A, Setiani E, Gunawan D, Silalahi FSM, 2022). Compared with data from research on the global distribution and burden of dengue, the number of dengue cases reached 7,590,213 cases or 50 times higher than the number of cases reported in Indonesia.

Dengue fever transmission can be caused by 4 types of dengue virus, namely DENV-1, DENV-2, DENV-3, and DENV-4. Symptoms of dengue fever only appear when someone who has been infected by one of the four types of dengue virus experiences an infection by a different type of dengue virus, causing the immune system that has formed in the body after the first infection to actually result in the emergence of more severe symptoms of the disease when infected for the second time, but the same type of virus can only infect once due to the body's formed immune system (Siswanto & Usnawati, 2019). The dengue virus is a pathogen transmitted through mosquito vectors and after infection the immune system will be activated, in fact, treatment to prevent dengue virus replication must wait for the immune system to be activated first and show symptoms (Saptawati et al., 2019). According to the Handbook for Clinical Management of Dengue by WHO, specific antiviral therapy is still being developed to not only treat symptoms symptomatically, such as administering fluids and antipyretics, but also to seek out new therapies, one of which is the use of herbal medicines (Alamsyah, 2022).

Indonesia itself is a country rich in herbal plants, one of which is cassava (Manihot eculenta C.). The most beneficial part is the cassava leaves, which contain various compounds, including flavonoids. One of the flavonoids in cassava leaves is rutin, which has antioxidant potential (Azizah et al., 2020). *Antioxidants*It is a substance that can inhibit free radical reactions caused by the body's defense system's ability to fight viral replication, such as dengue fever. Because cassava leaves have antiviral potential, they contain the flavonoid rutin, which has antioxidant potential to inhibit viral replication.

However, research exploring the potential of cassava leaves in dengue virus therapy is still limited. Therefore, researchers are interested in conducting an in vitro test of the flavonoid rutin from cassava leaves on dengue virus replication, which is expected to be a potential antiviral therapy for dengue.

RESEARCH METHOD

Scope of Research

This research covers the scope of clinical microbiology, clinical pharmacology and tropical medicine.

Place and Time of Research

This research was conducted at the Department of Microbiology, Faculty of Medicine, University of Indonesia from May to June 2024.

Types and Design of Research

This study used an experimental research method and used a post-test only research design with a control group design. By using the dengue virus in vitro as the object of research. This study used 7 groups, namely 1 control group and 6 treatment groups. The treatment group consisted of P (1), P (2), P (3), P (4), P (5) and P (6) with interventions namely routine administration of Flavonoids from cassava leaves (Manihot esculenta C.) with concentrations of 50 µg/ml, 25 µg/ml, 12.5 µg/ml, 6.25 µg/ml, 3.125 µg/ml and 1.5 µg/ml. The control group (K) was given 0.2% Dimethyl sulfoxide (DMSO). The treatment group was immediately given the intervention and then simultaneously carried out a post-test. Then the results of the intervention will be seen the difference between the treatment group and the control group to indicate that there is an effect on the treatment group.

Population and Sample

a. Inclusion Criteria, dengue virus serotype 1 strain New Guinea C grown in cell growth medium. Dengue virus multiple of infection (MOI) 0.2. Determining an MOI of 0.2 in the virus infection process is carried out to maintain a balance between adequate infection levels and maintained cell viability. This rationale allows more accurate observation of the dynamics of virus replication without causing excessive cytotoxic effects, so that the research results are more representative of physiological conditions.
b. Exclusion Criteria, contaminated cell growth medium.

Data Collection Method

The materials used in this study were, Flavonoid rutin from cassava leaves (Manihot esculenta C.) obtained from MarkHerb Manufacturing, besides that other materials were vero cells, MEM (Modified Eagle Medium), DMSO 0.2%, PBS (Phosphate-buffered saline), Complete medium (FBS 10% (Fetal Bovine Serum) + MEM), Formaldehyde 3.7%, BSA 0.2%, Tryton-X 0.5%, a-human dengue serum (1st antibody), a-human IgG-HRP (2nd antibody), Horseradish Peroxidase (HRP), DAB (diaminobenzidine peroxidase staining kit), trypsin EDTA 0.25% and dengue virus serotype 1 strain New Guinea C (DENV-2 NGC).

The tools used in the research are CO2 incubator, sterilizer, microscope, washing place and tools, centrifuge, cell freezing, Biosafety cabinet class II, cell counter, micropipette, serology pipette, hemocytometer chamber, inverted microscope, flash T-25, microwell, water bath, ice tray.

Research Procedures

a. Preparation stage of routine Flavonoids, in this study, the routine flavonoid compound was obtained through a purification process of cassava leaves using the Ultra High Performance Liquid Chromatograph method, namely the process of separating components specifically, which was carried out by MarkHerb manufacturing and the final result was routine flavonoids in the form of pure powder (Annissa, Musfiroh, & Indriati, 2019).
b. Preparation stage of dengue virus serotype-1, the dengue virus used for this study was obtained from the dengue virus collection from the Department of Microbiology, Faculty of Medicine, University of Indonesia.
c. Cell preparation stage, in this study, the cells used are vero cells from the collection of the FK UI Microbiology lab in the form of frozen cells stored in cell freezing at a temperature of -80°C. The cells to be used must be thawed first by soaking them in a water bath at a temperature of 37°C until the remaining frozen layer is thin and then put into a 5ml tube that has previously been added with MEM medium, then centrifuged at a speed of 1000 rpm for 5 minutes, remove the supernatant with a micropipette and the resulting cell pellet is added with complete medium then homogenized with a vortex and incubated for 3 days at a temperature of 37°C and a CO2 concentration of 5%, the results will be used in the cell culture process (Jihannisa N, Ahmad .FM, 2022)(Umam AC, Apriyanto DR, 2020)(Dewi, Ratningpoeti, Desti, & Angelina, 2019).

Research Ethics

This study used dengue virus serotype 1 strain New Guinea C from the Department of Microbiology, Faculty of Medicine, Ul. Vero cells were used as a medium for virus growth. This study has received ethical approval from the Research Ethics Committee of the Faculty of Medicine, Swadaya Gunung Jati University, under No. 20/EC/FKUGJ/IV/2024, and adhered to ethical principles for research involving experimental animals.

RESULTS AND DISCUSSIONS

Results of the inhibition and cytotoxicity test of routine flavonoids from cassava leaves (Manihot esculenta C.) against the replication of Dengue virus Serotype 1 New Guinea C strain in vitro

This study aims to demonstrate the influence of the flavonoid rutin on the replication of Dengue virus Serotype 1 New Guinea C strain in vitro. The inhibitory concentration of the flavonoid rutin was assessed by subtracting 100% from the percentage of infectivity. The test was conducted using the Focus Forming Unit Assay method, and the test results are presented in Table 1.

Table 1. Focus forming unit assay test results

Treatment (µg/ml)	DMSO control (ffu/well)	Average (ffu/well)	Infectivity Rate (%)	Large Barrier (%)	*IC50*(µg/ml)
P1(50)	253	5	1.98	98.02	3.36
P2(25)	253	6	2.37	97.63	
P3(12.5)	253	7	2.77	97.23	
P4(6.25)	253	75	29.64	70.36	
P5(3,125)	253	136	53.75	46.25	
P6(1.5)	253	161	63.64	36.36	

Information:
K : DMSO 0,2% (Control)
P1 : Routine flavonoids from cassava leaves (Manihot esculenta C.) with a concentration of 50 µg/ml.
P2 : Flavonoids from cassava leaves (Manihot esculenta C.) with a concentration of 25 µg/ml.
P3 : Flavonoids from cassava leaves (Manihot esculenta C.) with a concentration of 12,5 µg/ml.
P4 ; Routine flavonoids from cassava leaves (Manihot esculenta C.) with a concentration of 6,25 µg/ml.
P5 : Routine flavonoids from cassava leaves (Manihot esculenta C.) with a concentration of 3.125 µg/ml.
P6 : Flavonoids from cassava leaves (Manihot esculenta C.) with a concentration of 1,5 µg/ml.

Based on table 4, it can be seen that the magnitude of infectivity at each concentration has a different percentage value, where the smallest infectivity value is at the highest concentration, namely 50 µg/ml at 1.98% and the largest infectivity value is at the lowest concentration, namely 1.5 µg/ml with an infectivity percentage value of 63.64%. Then the magnitude of inhibition will have the opposite value to the infectivity value, namely, the smallest concentration will produce a low inhibition value of 36.36% and the highest concentration has the largest value reaching 98.02%. The effective inhibition value is at a concentration of 3.36 µg/ml.

Table 1. Microculture tetrazolium technique assay (MTT-Assay) test results

Treatment (µg/ml)	DMSO control (abs)	Average (abs)	Viability Rate (%)	Large Cytotoxic (%)	*CC50*(µg/ml)
P1(80)	0.642	0.429	34.89	65.11	17.48

P2(40)	0.642	0.349	37.33	62.67
P3(20)	0.642	0.373	41.90	58.10
P4(10)	0.642	0.419	61.94	38.06
P5(5)	0.642	0.619	76.22	23.78
P6(2.5)	0.642	0.762	100.00	0.00

Information:
K : DMSO 0,2% (Control)
P1 : Flavonoids from cassava leaves (Manihot esculenta C.) with a concentration of 80 µg/ml.
P2 : Flavonoids from cassava leaves (Manihot esculenta C.) with a concentration of 40 µg/ml.
P3 : Routine flavonoids from cassava leaves (Manihot esculenta C.) with a concentration of 20 µg/ml.
P4 ; Routine flavonoids from cassava leaves (Manihot esculenta C.) with a concentration of 10 µg/ml.
P5 : Routine flavonoids from cassava leaves (Manihot esculenta C.) with a concentration of 5 µg/ml.
P6 : Flavonoids from cassava leaves (Manihot esculenta C.) with a concentration of 2,5 µg/ml.

Based on Table 5, it can be seen that the viability at each concentration has a different percentage value, where the smallest viability is at a concentration of 80 µg/ml at 34.89% and the highest viability concentration is at a concentration of 2.5 µg/ml at 100%. For the cytotoxic concentration (CC50) of routine flavonoids, it occurs at a concentration of 17.48 µg/ml.

Data Analysis Results

a. Normality Test, a normality test was conducted to determine whether the data was normally distributed. This test was intended to determine the use of further tests. In this study, the Shapiro-Wilk test was used because the sample size was <50. After conducting the test, a p-value (0.001) or probability value <0.05 was obtained, indicating that the data were not normally distributed. Therefore, a non-parametric test was conducted based on these results.
b. Homogeneity Test, the Homogeneity Test aims to show that the research data comes from the same population. After testing, a p-value (0.082) or probability value ≥ 0.05 is obtained, so the data is declared homogeneous, so the assumption in the analysis of variance is fulfilled, that the data comes from the same population.
c. Post Hoc Test, a post hoc test was conducted to determine differences in inhibitory power between variables based on their significance. The post hoc LSD test used in the data analysis.

Table 1. Microculture tetrazolium technique assay (MTT-Assay) test results

Group		*Mean difference*	*Sig.*
P1	P2	-.333	.957
	P3	-.667	.914
	P4	27,333*	<,001
	P5	51,333*	<,001
	P6	61,333*	<,001
	K	-155,667*	<,001
P2	P1	-.333	.957
	P3	-.333	.957
	P4	27,000*	<,001
	P5	51,000*	<,001
	P6	61,000*	<,001
	K	-156,000*	<,001
P3	P1	-.667	.914
	P2	-.333	.957
	P4	26,667*	<,001
	P5	50,667*	<,001
	P6	60,667*	<,001

Group		*Mean difference*	*Sig.*
	K	-156,333*	<,001
P4	P1	-27,333*	<,001
	P2	-27,000*	<,001
	P3	-26,667*	<,001
	P5	24,000*	.001
	P6	34,000*	<,001
	K	-183,000*	<,001
P5	P1	-51,333*	<,001
	P2	-51,000*	<,001
	P3	-50,667*	<,001
	P4	-24,000*	.001
	P6	-10,000	.121
	K	-207,000*	<,001
P6	P1	-61,333*	<,001
	P2	-61,000*	<,001
	P3	-60,667*	<,001
	P4	-34,000*	<,001
	P5	-10,000	.121
	K	-217,000*	<,001
K	P1	155,667*	<,001
	P2	156,000*	<,001
	P3	156,333*	<,001
	P4	183,000*	<,001
	P5	207,000*	<,001
	P6	217,000*	<,001

Information:
K : DMSO 0,2% (Control)
P1 : Routine flavonoids from cassava leaves (Manihot esculenta C.) with a concentration of 50 µg/ml.
P2 : Flavonoids from cassava leaves (Manihot esculenta C.) with a concentration of 25 µg/ml.
P3 : Flavonoids from cassava leaves (Manihot esculenta C.) with a concentration of 12,5 µg/ml.
P4 ; Routine flavonoids from cassava leaves (Manihot esculenta C.) with a concentration of 6,25 µg/ml.
P5 : Routine flavonoids from cassava leaves (Manihot esculenta C.) with a concentration of 3.125 µg/ml.
P6 : Flavonoids from cassava leaves (Manihot esculenta C.) with a concentration of 1,5 µg/ml.

The Post Hoc LSD test is used to determine whether a group has a significant difference from another group. The results of the Post Hoc LSD test analysis show an asterisk (*) indicating that the group has a significant difference from the other groups or a Sig. value <0.05, while groups without an asterisk indicate that the group has no significant difference from the other groups.

Discussion

a. Inhibitory concentration test of routine flavonoids from cassava leaves (Manihot esculenta C.) against the replication of dengue virus serotype 1 strain New Guinea C in vitro.

The results of the routine Flavonoid inhibition concentration test from cassava leaves (Manihot esculenta C.) with concentrations of 50 µg/ml, 25 µg/ml, 12.5 µg/ml, 6.25 µg/ml, 3.125 µg/ml and 1.5 µg/ml were carried out using the Focus Forming Unit-Assay method, which is a method for determining virus titers to calculate the number of viruses that infect Vero cells (Jihannisa N, Ahmad .FM, 2022; Umam AC, Apriyanto DR, 2020).

Based on the results of the routine Flavonoid inhibition concentration test in table 4, it shows that all concentrations have an effect in inhibiting dengue virus replication with the highest percentage of inhibition at a concentration of 50 µg/ml of 98.02% and the lowest percentage of

inhibition at a concentration of 1.5 µg/ml of 36.36%. These results state that the greater the concentration, the greater the inhibition obtained and vice versa. However, to analyze the effective inhibition concentration of routine Flavonoid concentration, the IC50 value is calculated using a trend-line in Excel software, IC50 is the effective concentration of routine Flavonoid needed to inhibit 50% of the number of Vero cells infected with the virus. The results of the routine Flavonoid IC50 value were obtained at a concentration of 3.36 µg/ml, where these results are in accordance with the data on the results of the large inhibition in table 4, namely between a concentration of 3.125 µg/ml and a concentration of 6.25 µg/ml. In addition, research by Jihannisa et al stated that the IC50 value was almost similar to this research, namely at a concentration of 2.7 µg/ml (Jihannisa N, Ahmad .FM, 2022; Umam AC, Apriyanto DR, 2020).

In this study, there was an inhibition of DENV replication by routine Flavonoids, this is because routine Flavonoids are compounds that inhibit dengue virus protein protease, specifically inhibiting the serine protease enzyme, namely NS2B-NS3pro (non-structural protein 2B-non-structural protein 3), which is a structural component of the virus that functions as a resource for the virus's life cycle so that it can replicate. This has been proven by Dwivendi et al, who conducted bioflavonoid testing on DENV replication, bringing the results that routine Flavonoids have the best effect compared to other bioflavoid compounds such as quercetin, although quercetin has a greater inhibition rate according to Umam CA et al's research, but the results of Dwivendi et al's research stated that routine Flavonoids are better because from the results of the density functional theory and ADMET (Absorption, Distribution, Metabolism, Excretion and Toxicity) tests, it states that routine Flavonoids are safer to use because they have a low toxicity rate but have a fairly good inhibition rate (Dwivedi et al., 2021; Umam AC, Apriyanto DR, 2020).

The results of calculating the inhibition value of routine Flavonoids state that the greatest inhibition value is at the highest concentration. This shows that the higher the concentration, the greater the inhibition produced. This is because the higher the concentration, the more routine Flavonoids in the solution, making the effect of routine Flavonoids on inhibition greater. This is in accordance with the research results of Umam AC et al and Jihannisa et al who presented similar results in their research as well as several factors that influence the solubility of a concentration such as the type of solvent, temperature, and stirring tool (Jihannisa N, Ahmad .FM, 2022; Umam AC, Apriyanto DR, 2020).

The large value of inhibition will be sought for the IC50 value, namely finding the effective concentration of routine Flavonoids in inhibiting DENV replication, the results data show that the IC50 value is at a concentration of 3.36 µg/ml, this value is stated to be very strong in inhibiting DENV replication, in accordance with the statement of Souhoka et al, that the concentration is said to be very strong if the IC50 value is <50 ppm/50 µg/ml (Souhoka, Hattu, & Huliselan, 2019).

From the results of the concentration inhibition test of routine flavonoids, good inhibition results were obtained for DENV-1 replication, these results are in line with the research of Akwayamai JJ et al which stated that routine flavonoids have an effect in inhibiting DENV by inhibiting the NS2B-NS3 protein, also supported by the research statement of Renantha RR et al in the results of their research stating that routine flavonoids have a good effect in inhibiting DENV replication (Akwayamai et al., 2022; Renantha et al., 2022).

The inhibitory mechanism of routine flavonoids on DENV replication occurs before and after viral infection of host cells. The inhibitory mechanism before infection occurs when routine flavonoids interfere with the interaction between the virus and host cells by inhibiting the binding of the virus to host cell receptors, namely DC-SIGN and TIM-1, routine flavonoids reduce the ability of the virus to enter cells and start its replication cycle, this can reduce the viral load in infected cells or increase the number of viruses that successfully infect cells. In addition, routine flavonoids can increase the body's immune response by preparing the immune system to face infections including increased cytokine production and activation of immune cells.

The inhibitory mechanism after infection when the dengue virus enters the host cell and the viral RNA is released into the cell cytoplasm, at this phase the flavonoid rutin inhibits the production of NS2B-NS3 proteins that play a role in processing viral proteins required for replication. NS3 functions as a protease that cuts viral polypeptides into smaller functional proteins, while NS2B functions as a cofactor that increases the activity of NS3 protease, this process produces structural and non-structural proteins required for the assembly of new virions. In addition to its role in protein processing, the NS2B-NS3 complex is also involved in viral genome replication. NS3 has helicase activity that helps in the separation of RNA chains during the transcription initiation phase. Therefore, flavonoid rutin can have a synergistic effect, which not only functions as a preventative before infection but also as a therapeutic agent or after infection occurs. This makes flavonoid rutin a potentially useful compound in the treatment and prevention of viral infections.

Several studies using routine Flavonoids to inhibit replication in the Flaviviridae virus family, the same as DENV, also obtained similar results, such as in the Zika virus in the studies of Lima SC et al and Cataneo et al, which stated that routine Flavonoids have an inhibitory effect on Zika virus replication in the NS2B-NS3 protease protein in vitro. In addition, in the study of Agrawal KP et al, it was stated that routine Flavonoids have an effect as an inhibitor of Sars-COV2 virus replication in the Main Protease (Mpro) 6GLU7 binding pocket protein, which makes it a potential therapy for the anti-virus. In addition, routine flavonoids have a role as antioxidants, one of the potential applications is as a chemotherapy antioxidant in cancer patients, according to research by Sateri A et al., it states that routine flavonoids act as antioxidants that inhibit the proliferation of cancer cells in breast, lung and prostate cancer. Routine flavonoids work by regulating several cancer marker signaling pathways such as Ras/Raf and PI3K/Akt, MAPK and TGF-β2/Smad2/3Akt/PTEN which are related to the process of carcinogenesis and apoptosis induction which makes routine flavonoids a potential cancer chemotherapy (Agrawal, Agrawal, & Blunden, 2021; Al-Zahrani, 2020; Cataneo et al., 2021; Lima et al., 2021; Satari, Ghasemi, Habtemariam, Asgharian, & Lorigooini, 2021).

b. Cytotoxic concentration test of routine flavonoids from cassava leaves (Manihot esculenta C.) against Vero cells.

The toxicity test of routine Flavonoids from cassava leaves (Manihot esculenta C.) against Vero cells was carried out using the MTT Assay method. MTT Assay is a method for calculating living cells based on the cell's mitochondrial absorbance activity to determine the cytotoxicity of routine Flavonoids against Vero cells. Determination of the cytotoxic concentration was carried out by calculating cell viability in percentage units read using an ELISA reader, where the cytotoxic concentration was obtained from subtracting 100% of the concentration from the viability (Jihannisa N, Ahmad .FM, 2022; Umam AC, Apriyanto DR, 2020).

To analyze the cytotoxic concentration that can reduce cell viability by 50%, the CC50 (Cytotoxic Concentration 50%) value was calculated using a trend-line in Excel software. Based on Table 5, the CC50 value was obtained at a concentration of 17.48 µg/ml, indicating that the concentration of routine Flavonoids has cytotoxic activity against Vero cells by 50% at that concentration.(Jihannisa N, Ahmad .FM, 2022; Umam AC, Apriyanto DR, 2020)This value is stated to be toxic to Vero cells, in accordance with the statement by Rachmatiah T et al, that the concentration is said to be toxic if the CC50 value is < 30 ppm/30 µg/ml (Rachmatiah, Daud, & Artanti, 2022).

In the research of Tingting VA et al, routine flavonoids can be toxic to Vero cells because routine flavonoids include phenol compounds that can damage cell membranes because they have -OH groups that tend to bind to cell membrane proteins and stop the active transport of Na+ and K+ ions that function as cell nutrition and intracellular signaling, resulting in uncontrolled entry of these ions into cells and causing rupture of the cell membrane to cell death (apoptosis) (Tinting, Kamu, & Aritonang, 2024).

In other research results such as Umam AC et al who tested cassava leaf extract (Manihot Esculenta C) against DENV-2 showed that the compounds contained were not toxic to vero cells, these results are contrary to the results of this study, because the study of Umam AC et al only tested cassava leaf extract without analyzing which compounds were not toxic to vero cells. In addition, research on the same virus family as DENV such as Lima SC et al who tested routine Flavonoids on Zika virus, showed results in line with this study, where there was cytotoxicity to cells that had been infected with Zika virus (AR, Burhan, Awaluddin, & Mustidar, 2021; Lima et al., 2021; Umam AC, Apriyanto DR, 2020).

c. Analysis of the results of the Selectivity Index of cytotoxic concentration against inhibitory concentration

*Selectivity Index*is a value that indicates the safety level of routine flavonoid use against the magnitude of inhibition of dengue virus replication and stotoxicity to Vero cells. Based on the results of the inhibition and cytotoxic concentrations, the selectivity index value of routine flavonoids can be calculated using the formula, SI = CC50 / IC50 (Jihannisa N, Ahmad .FM, 2022; Umam AC, Apriyanto DR, 2020).

The calculation results obtained the SI value of routine flavonoids on the magnitude of the inhibition of dengue virus replication and cytotoxicity against Vero cells is 5.2, this value can be said to be good, where the SI value is said to be good if the value is ≥ 3, in addition, the higher the SI value, the more effective the inhibition and the smaller the toxicity according to the research statement of Indrayanto G et al, in addition, this value is stated to be able to be used as herbal therapy (Indrayanto, Putra, & Suhud, 2021; Jihannisa N, Ahmad .FM, 2022).

The Selectivity Index value is influenced by the IC50 and CC50 values, where the greater the difference in values, the greater the SI value obtained or the greater the inhibition and the smaller the cytotoxicity of routine Flavonoids, the safer the compound is to use (Indrayanto et al., 2021; Jihannisa N, Ahmad .FM, 2022).

d. Kruskal-Wallis Hypothesis Test

In testing the research hypothesis, testing was carried out using the Kruskal-Wallis method, where the method taken was in accordance with the research data criteria, the results of the analysis showed a p value (0.006) or a probability value <0.05, so it was stated that the hypothesis was accepted, namely that routine flavonoids in cassava leaves (Manihot esculenta C.) have an effect on the replication of the Dengue virus Serotype 1 Strain New Guinea C in Vitro.

The test results are in accordance with the results of the Focus Forming Unit Assay (FFU) test, where the results of the FFU test observations obtained brown foci that indicate how many Vero cells were successfully infected by the dengue virus serotype 1 at each concentration. The results compared to the control group showed a difference in the number of brown foci, where the brown foci of the concentration were fewer, which indicates that the concentration of routine Flavonoids has an inhibitory effect on the replication of the dengue virus serotype 1.

Research Limitations

The first limitation of this study is that it did not conduct routine Flavonoid inhibition mechanism testing before and after dengue virus infection to determine the mechanism of inhibition that occurred.In this study, only routine Flavonoid testing was carried out on dengue virus serotype-1, it is necessary to carry out testing on other dengue virus serotypes.This study only tested routine flavonoids, not other flavonoid derivatives such as quercetin and nicotiflorin.

This research only uses routine flavonoids from cassava leaves, research needs to be conducted with routine flavonoids which comes from other plants.

CONCLUSION

The results of the hypothesis test analysis showed that routine flavonoids in cassava leaves (Manihot esculenta C.) have an effect on the replication of Dengue virus Serotype 1 Strain New

Guinea C in Vitro. The results of the inhibitory concentration analysis (IC50) of routine flavonoids on the replication of Dengue virus Serotype 1 Strain New Guinea C, were obtained at a concentration of 3.36 µg/ml and stated that the IC50 results were very strong. The results of the cytotoxic concentration analysis (CC50) of routine flavonoids on Vero cells were obtained at a concentration of 17,48 µg/ml and the CC50 result was declared toxic. The results of the Selectivity Index (SI) analysis of routine flavonoids from cassava leaves (Manihot esculenta C.), obtained an SI value of 5,2, so that routine Flavonoids are declared safe for use.

This research makes a significant contribution to the development of nature-based therapies for dengue by demonstrating the potential of natural ingredients to inhibit viral replication. These findings open the door to further exploration of natural compounds as safer, more sustainable therapeutic candidates, potentially reducing dependence on synthetic drugs.

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