



# EVALUATION OF THE POTENTIAL OF ROSELLA FLOWER ETHANOL EXTRACT (HIBISCUS SABDARIFFA) IN DEALING WITH DYSLIPIDEMIA IN MALE RATS CONSUMING A HIGH-FAT DIET

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## ABSTRACT

*Rosella (Hibiscus sabdariffa L.) is a traditional remedy for hypertension due to its flavonoids that reduce blood viscosity. Its combination with crab shell offers a safer and more effective cholesterol-lowering alternative, primarily through anthocyanins. Dyslipidemia, a lipid metabolism disorder, involves elevated TC, LDL-C, TG, and decreased HDL-C, all linked to atherosclerosis. Management includes lifestyle changes and lipid-lowering drugs. Rosella extract effectively lowers LDL-C in rats, suggesting potential anti-dyslipidemia benefits. The study, conducted in January 2024, used an experimental Pre-test and Post-test group design. Sample size calculation determined a minimum of 4 rats per treatment group, weighing 180-200 grams and aged 2-4 months. The administration of Rosella flower extract (Hibiscus sabdariffa) in rats showed diverse responses in lipid profiles and liver function. The treatment groups exhibited increased total cholesterol and HDL, reduced LDL, and elevated total cholesterol and triglycerides. Statistical analysis confirmed significant differences between treatment groups, particularly in triglycerides. These findings also indicated a significant impact on liver function, with substantial differences in SGOT and SGPT levels. Further research is needed to explore the regulatory potential of Rosella extract on lipid metabolism and liver health.*

**KEYWORDS:** *Rosella extract, Dyslipidemia management, Flavonoids and anthocyanins, Cholesterol-lowering effects, Experimental rat study*

## BACKGROUND

Rosella (*Hibiscus sabdariffa* L.) is a member of the Malvaceae family. Rosella can grow in tropical and subtropical climates. The Rosella plant's native habitat ranges from India to Malaysia. Rosella is one of the traditional medicines used to lower blood pressure. Flavonoids, which can reduce blood viscosity, are active compounds found in the calyx of the Rosella flower (Rahadian, 2017). Combining Rosella flower calyx extract with crab shell is a safer and more effective alternative. Rosella flower calyx and crab shell have cholesterol-lowering properties, and their combination is expected to provide more excellent benefits. Anthocyanins in Rosella flower calyx have been identified as the active ingredient that lowers cholesterol levels (Pratiwi, 2014).

Dyslipidemia is a lipid metabolism disorder characterized by an increase or decrease in lipid fractions in the plasma. (Wulansari, 2020). The primary abnormalities in lipid fractions include an increase in total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), triglycerides (TG), and a decrease in high-density lipoprotein cholesterol (HDL-C). In the process of atherosclerosis, all of these play important roles and are closely related, making it impossible to discuss them separately. The management of dyslipidemia patients consists of non-pharmacological and pharmacological therapies. Non-pharmacological therapy includes lifestyle changes such as physical activity, medical nutrition therapy, weight reduction, and smoking cessation. Meanwhile, pharmacological therapy involves prescribing lipid-lowering drugs (Ulilalbab and Maskanah, 2021). The single extract of Rosella flower calyx (*Hibiscus sabdariffa* L.) has an effect in lowering the levels of Low-Density Lipoprotein Cholesterol (LDL-C) in male Wistar rats (*Rattus norvegicus*) (Widyaningsih, Alif Nugroho and Ulilalbab, 2022). Therefore, researchers are interested in exploring the effectiveness of Rosella flower extract for anti-dyslipidemia (*Hibiscus sabdariffa*).

## RESEARCH METHODS

The type of research conducted is experimental, with a Pre-test and Post-test group-only control design approach. This research was conducted in January 2024. The sample size for this study was calculated using the Federer formula. Based on the calculation results, it can be concluded that at least four male Wistar rats (*Rattus norvegicus*) are needed in each treatment group. The Wistar rats in this study are males weighing 180-200 grams and aged between 2-4 months.



**Table 1. Measurement Aspects of Research Variables**

No	Variable	How to Measure	Measuring Instruments	Measurement Results	Scale Measure
1.	Ethanol extract dosage of Rosella Flower (Hibiscus sabdariffa)	Measured weight and volume of extract and vehicle extract with analytical balance and measuring flask.	Analytical balances and measuring flasks.	1. Control 2. Standard	Ordinal
2.	Profile Lipid	Lipoprotein analysis can measure blood levels from cholesterol, LDL cholesterol, HDL cholesterol, and triglycerides.	Spectroscopic	In mg/dl	Ratio
3.	Total Cholesterol Levels	A combination of the amount of LDL cholesterol, HDL cholesterol, and triglycerides in each deciliter of blood	Autocheck ®	In mg/dl	Ratio
4.	Weight	Weighed using scales	Analytical balances	In grams	Ratio

**Table 2 Overview of the Treatment of Each Group**

No	Test Group	Treatment
1.	Normal	Test animals were not given any particular treatment and were only given ad libitum food and drink.
2.	Control	Test animals were given 1 ml of 0.5% Na CMC suspension daily for 14 days. Food and drink are provided ad libitum.
3.	Standard	Test animals were given an oral suspension of simvastatin 5 ml/kgBB daily for 14 days. Food and drink are provided ad libitum.
4.	(25 mg/kgBB)	Test animals were given ethanol extract of Rosella Flowers (Hibiscus sabdariffa) dose 2.5 ml/kg body weight once a day for 14 days. Food and drink are provided ad libitum.
5.	Rosella Flower Ethanol Extract (Hibiscus sabdariffa) - I (200 mg/kgBB)	Test animals were given an ethanol extract of Rosella Flowers (Hibiscus sabdariffa) dose of 5 ml/kg body weight once a day for 14 days. Food and drink are given ad libitum.
6.	Rosella Flower Ethanol Extract (Hibiscus sabdariffa) – II (400 mg/kgBB)	Test animals were given an ethanol extract of Rosella Flowers (Hibiscus sabdariffa) dose of 10 ml/kg body weight daily for 14 days. Food and drink are given ad libitum.
7.	Rosella Flower Ethanol Extract (Hibiscus sabdariffa) – III (800 mg/kgBB)	Test animals were given an ethanol extract of Rosella Flowers (Hibiscus sabdariffa) dose of 15 ml/kg body weight daily for 14 days. Food and drink are given ad libitum.

Before the blood draw, the rats were satisfied at least 8 hours before the blood draw. Blood collection is done by direct withdrawal from the heart of mice as much as 1 ml. Put into a microtube and let stand  $\pm$  20 minutes. Then, the blood was centrifuged at a rate of 3000 rpm for 15 minutes to obtain the blood serum of the rats. The determination of lipid profiles is determined by the colorimetric method. Blood collection is done by direct withdrawal from the heart of mice as much as 1 ml. Put into a microtube and let stand  $\pm$  20 minutes. Then, the blood was centrifuged at a rate of 3000 rpm for 15 minutes to obtain the blood serum of the rats. The determination of SGOT and SGPT levels is based on enzymatic reactions using Dyasis kit reagents®. Data analysis of One-Way ANOVA Test: Is the data normally distributed with follow-up tests as a Post Hoc Tukey HSD test to see real differences between treatments? However, if the data is abnormally distributed, the Kruskal-Wallis test is used as an alternative.



**RESULTS AND DISCUSSION**

**Extract Characteristics**

**Table 3. Phytochemical Screening Results of Rosella Flowers Ethanol Extract (Hibiscus sabdariffa)**

Phytochemicals	Reagent	Result
Alkaloids	Bouchardart	+
Saponins	Mayer	+
Flavonoids	Dragondroff	-
	Wagner	+
Tannins	Aquadest + Alcohol 96%	-
	FeCl3 5%	+
Alkaloids	Mg(s) + HCl(p)	-
	NaOH 10%	-
	H2SO4 (p)	-
Saponins	FeCl3 1%	+
Flavonoids	Salkowsky	-
	Lieberman Bouchard	+

From the table data above, it can be seen that ethanol extract Rosella Flowers (Hibiscus sabdariffa) contains several phytochemical compounds, including Alkaloids, Saponins, Flavonoids, Tannins, as well as Steroids and Terpenoids.

**Table 4. Results of Data Normality Test with Shapiro-Wilk Test on All Research Parameters**

Parameters	Nilai P	Distribusi Data	
Weight	0.504	Usual	
Total cholesterol before induction	< 0.05	Abnormal	
Total cholesterol after induction	< 0.05	Abnormal	
Lipid Profile After Treatment	Total cholesterol	0.536	Usual
	Triglycerides	0.003	Abnormal
	HDL levels	< 0.05	Abnormal
	LDL levels	0.254	Usual
SGOT levels	< 0.05	Abnormal	
SGPT Rate	0.241	Usual	

The table data above shows that the body weight, total cholesterol, and LDL levels data from the lipid profile after treatment and SGPT levels have a standard data distribution. At the same time, other parameters, including total cholesterol before and after induction, triglyceride levels, HDL levels, and SGOT levels, are abnormally distributed. Based on the distribution of these data, data with standard data distribution are analyzed with parametric statistics, while abnormal data is analyzed with non-parametric statistics.

**Table 5. Comparison of Rats' Initial Body Weight in the Entire Treatment Group**

Treatment Group	Weight Loss (grams)		P Value
	Mean	SD	
Usual	230.00	28.30	0.625
Standard	232.00	26.30	
Control	230.20	26.60	
Ethanol Rosella Flower Ethanol Extract (Hibiscus sabdariffa) - I (200 mg/ kgBB)	260.00	27.30	
Ethanol Rosella Flower Ethanol Extract (Hibiscus sabdariffa) – II (400 mg/ kgBB)	230.00	28.50	
Ethanol Rosella Flower Ethanol Extract (Hibiscus sabdariffa) – III (800 mg/ kgBB)	240.00	28.50	

From the table data above, it can be seen that the P value > 0.05 (P value = 0625), which means there is no significant difference in the initial body weight of the mice used in this study. The weight range of mice used in this study ranged from 230-260 grams, evenly distributed in each treatment group.



**Table 6. Comparison of Total Cholesterol Before and After High-Fat Diet in All Treatment Groups**

Treatment Group	Total cholesterol (mg/dL)	
	Before Induction	After Induction
Usual	112.00 (112-115)	216.80 (212-221) <sup>b</sup>
Standard	110.00 (110-120)	210.00 (209-245) <sup>a</sup>
Control	115.50 (110-118)	211.70 (210-214) <sup>b</sup>
Ethanol Rosella Flower Ethanol Extract (Hibiscus sabdariffa) - I (200 mg/ kgBB)	115.50 (110-120)	210.70 (208-214) <sup>b</sup>
Ethanol Rosella Flower Ethanol Extract (Hibiscus sabdariffa) – II (400 mg/ kgBB)	112.50 (112-118)	210.00 (210-220) <sup>b</sup>
Ethanol Rosella Flower Ethanol Extract (Hibiscus sabdariffa) – III (800 mg/ kgBB)	114.00 (116-120)	209.00 (209-222) <sup>b</sup>
<b>P Value</b>	<b>0.749</b>	<b>0.015</b>

Data is displayed as Median (Range). The P value is obtained from the Kruskal-Wallis analysis. Different superscripts in the same column show significant differences.

From the table data above, it can be seen that before being given a high-fat diet, the total cholesterol of rats before giving a high-fat diet in the entire treatment group did not show a significant difference (P value = 0.749). This showed that the total cholesterol data of the rats before being given a high-fat diet were uniform. However, total cholesterol in all groups of rats after the high-fat diet showed a different distribution, where only the control group, standard ethanol extract of Rosella Flowers (Hibiscus sabdariffa)-I, II, and III, showed uniform total cholesterol.

**Table 7. Comparison of Lipid Profiles in the Entire Mouse Treatment Group**

Treatment Group	Lipid Profile			
	Total cholesterol *	Triglycerides**	LDL*	HDL**
Usual	165.50 ± 2.50 <sup>a</sup>	95.00 (95-120) <sup>a</sup>	40.50 ± 1.55 <sup>a</sup>	61.70 (61-68) <sup>a</sup>
Standard	145.00 ± 0.45 <sup>b</sup>	106.40 (101-104) <sup>b</sup>	62.40 ± 1.29 <sup>b</sup>	61.40 (60-64) <sup>a</sup>
Control	159.14 ± 6.02 <sup>c</sup>	164.00 (160-169) <sup>c</sup>	106.40 ± 6.60 <sup>c</sup>	28.00 (65-40) <sup>b</sup>
Ethanol Rosella Flower Ethanol Extract (Hibiscus sabdariffa) - I (200 mg/ kgBB)	168.20 ± 1.50 <sup>d</sup>	166.40 (166-166) <sup>d</sup>	86.25 ± 2.25 <sup>d</sup>	45.40 (46-48) <sup>b</sup>
Ethanol Rosella Flower Ethanol Extract (Hibiscus sabdariffa) – II (400 mg/ kgBB)	166.00 ± 2.20 <sup>e</sup>	120.40 (119-125) <sup>e</sup>	65.00 ± 1.20 <sup>e</sup>	61.40 (61-62) <sup>a</sup>
Ethanol Rosella Flower Ethanol Extract (Hibiscus sabdariffa) – III (800 mg/ kgBB)	145.00 ± 0.90 <sup>e</sup>	110.00 (110-115)	60.50 ± 1.25 <sup>f</sup>	60.20 (60-61) <sup>a</sup>
<b>P Value</b>	<b>&lt; 0.05</b>	<b>0.015</b>	<b>&lt; 0.05</b>	<b>0.015</b>

\*The data is displayed as Mean ± SD. P value obtained from One Way ANOVA analysis; \*\*Data is displayed as Median (Range). The P value is obtained from the Kruskal-Wallis analysis. Different superscripts in the same column show significant differences

Table 7 compares lipid profiles in rats that have been given various treatments. This lipid profile includes parameters such as Total Cholesterol, Triglycerides, LDL (Low-Density Lipoprotein), and HDL (High-Density Lipoprotein). The measurement result is expressed in terms of the average value ± standard deviation for each treatment group, and the range of values is indicated in parentheses that include the minimum and maximum values.

The first treatment group, called “Normal,” had total cholesterol values of 165.50 ± 2.50 mg/dL, triglycerides 95.00 (95-120) mg/dL, LDL 40.50 ± 1.55 mg/dL, and HDL 61.70 (61-68) mg/dL. This group is used as a basis for comparison with other treatment groups. The “Standard” treatment group showed changes with lower total cholesterol values (145.00 ± 0.45 mg/dL) compared to the standard group, while triglyceride and LDL values increased. HDL remains relatively stable. The “Control” treatment group showed significant changes with significant increases in total cholesterol, triglycerides, and LDL, while HDL experienced a considerable decrease. The treatment groups “Rosella Flower Ethanol Extract (Hibiscus sabdariffa) -I,” “Rosella Flower Ethanol Extract (Hibiscus sabdariffa) -II,” and “Rosella Flower Ethanol Extract (Hibiscus sabdariffa) -III” showed variation in lipidic response, with different changes in each lipid parameter. Statistical analysis showed that the difference between treatment groups on all lipid parameters was significant (P < 0.05), except for triglyceride parameters for the treatment groups “Rosella Flower Ethanol Extract (Hibiscus sabdariffa) -II” and “Rosella Flower Ethanol Extract (Hibiscus sabdariffa) -III” (P = 0.015).



The results showed that administering ethanol extract of Rosella flowers (*Hibiscus sabdariffa*) in rats had a mixed impact on lipid profiles. The “Rosella Flower Ethanol Extract (*Hibiscus sabdariffa*)-I” treatment group showed a significant increase in total cholesterol and HDL, while the “Rosella Flower Ethanol Extract (*Hibiscus sabdariffa*)-II” group showed a decrease in LDL. On the other hand, the group “Rosella Flower Ethanol Extract (*Hibiscus sabdariffa*)-III” showed an increase in total cholesterol and triglycerides. These effects indicate that Rosella flower extract may affect lipid metabolism in complex and varied ways, depending on the dosage or formulation used. While these results are exciting, more research is needed to confirm and understand the mechanisms behind such effects and evaluate potential applications in humans by considering factors such as proper dosage and duration of use.

**Table 8. Comparison of SGOT and SGPT Levels in All Treatment Groups**

Treatment Group	SGOT levels (U/L)	SGPT Rate (U/L)
Usual	38.50 (38-39) <sup>a</sup>	40.00 ± 1.50 <sup>a</sup>
Standard	111.00 (111-115) <sup>b</sup>	162.00 ± 1.50 <sup>b</sup>
Control	161.00 (161-165) <sup>c</sup>	97.35 ± 1.50 <sup>c</sup>
Ethanol Rosella Flower Ethanol Extract ( <i>Hibiscus sabdariffa</i> ) - I (200 mg/ kgBB)	115.00 (115-125) <sup>d</sup>	99.00 ± 3.00 <sup>d</sup>
Ethanol Rosella Flower Ethanol Extract ( <i>Hibiscus sabdariffa</i> ) – II (400 mg/ kgBB)	128.00 (128-135) <sup>e</sup>	110.00 ± 3.00 <sup>e</sup>
Ethanol Rosella Flower Ethanol Extract ( <i>Hibiscus sabdariffa</i> ) – III (800 mg/ kgBB)	130.00 (130-138) <sup>f</sup>	140.00 ± 3.00 <sup>b</sup>
<b>P Value</b>	<b>0.006</b>	<b>&lt; 0.05</b>

\*The data is displayed as Mean ± SD. P value obtained from One Way ANOVA analysis; \*\*Data is displayed as Median (Range). The P value is obtained from the Kruskal-Wallis analysis. Different superscripts in the same column show significant differences

Table 8 compares liver enzyme levels, Serum Glutamic Oxaloacetic Transaminase (SGOT), and Serum Glutamic Pyruvic Transaminase (SGPT) levels in the rat treatment group. The enzyme levels are considered an indicator of liver health, and the measurement results include average values along with a range of values for each treatment group.

The “Normal” group showed SGOT levels of 38.50 (38-39) U/L and SGPT of 40.00 ± 1.50 U/L, reflecting normal liver conditions as a comparison group. The “Standard” group showed significant increases in both enzymes, with SGOT levels of 111.00 (111-115) U/L and SGPT of 162.00 ± 1.50 U/L. This could indicate significant liver damage as a result of standard treatment that may involve substances detrimental to the organ.

The “Control” group showed increased SGOT levels to 161.00 (161-165) U/L and SGPT to 97.35 ± 1.50 U/L. This indicates the potential for liver function abnormalities in the control group, which could be affected by specific treatments or the health conditions of mice. The treatment group “Ethanol Extract of Rosella Flowers (*Hibiscus sabdariffa*)” showed variations in the enzymatic response of the liver. Group I showed an increase in SGOT and SGPT, while Group II showed an increase in SGPT. On the other hand, group III showed a rise in SGOT and SGPT.

Statistical analysis showed significant differences in SGOT levels between treatment groups (P = 0.006). Similarly, differences in SGPT levels were also important (P < 0.05), suggesting the potential effect of Rosella flowers on rat liver health. These results indicate that Rosella flowers may regulate liver function, but more research is needed to understand the mechanism and its implications thoroughly.

## DISCUSSION

The research findings indicate a significant impact of Rosella flower ethanol extract (*Hibiscus sabdariffa*) on the lipid profile of rats. Specifically, the treatment group “Rosella Flower Ethanol Extract (*Hibiscus sabdariffa*) -I” showed a significant increase in total cholesterol and HDL, while the group “Rosella Flower Ethanol Extract (*Hibiscus sabdariffa*) -II” showed a decrease in LDL levels. Conversely, the group “Rosella Flower Ethanol Extract (*Hibiscus sabdariffa*) -III” showed increased total cholesterol and triglycerides. These results reflect the potential influence of the Rosella flower in regulating lipid metabolism with varied responses, depending on the extract formulation or dosage.

When examining liver enzyme responses, the group “Rosella Flower Ethanol Extract (*Hibiscus sabdariffa*) -I” showed a significant increase in SGOT and SGPT, while the group “Rosella Flower Ethanol Extract (*Hibiscus sabdariffa*) -II” showed an increase in SGPT. The group “Rosella Flower Ethanol Extract (*Hibiscus sabdariffa*) -III” increased both enzymes. The research results suggest that administering Rosella flower extract to rats can affect liver function, especially in specific treatment groups. However, further interpretation is needed to deeply understand the causes of these enzymatic changes’ long-term impact on liver health. Phytochemistry is a category of substances found in plants that show potential for regulating human metabolism in beneficial



ways for preventing chronic and degenerative diseases. High-fat diet consumption can contribute to increased total cholesterol, LDL, and triglycerides levels while reducing HDL levels in the blood. One approach to improving lipid profiles and preventing cardiovascular complications is by consuming antioxidants. Rosella, for example, is a plant with antioxidant properties due to its content of ascorbic acid and several types of flavonoids, such as flavonol and anthocyanin pigments. Anthocyanins, the red pigments in rosella, consist of cyaniding-3-sambubroside, delphinidin-3-glucoside, and delphinidin-3-sambubroside. Additionally, there are flavonol compounds like gossypetin, hibiscetin, and quercetin in rosella, all contributing as antioxidants. These phytochemicals can provide significant benefits in maintaining lipid balance and preventing adverse impacts of excessive fat consumption.

The *Hibiscus sabdariffa* extract containing alkaloids also exhibits antimicrobial activity, combating bacteria such as *E. coli*, *E. cloacae*, *P. aeruginosa*, and *S. Aureus* (Nafisa *et al.*, 2021). Rosella (*Hibiscus sabdariffa*) has been traditionally known in West Africa as a plant with anti-hypertensive properties. Anthocyanins are compounds commonly found in *Hibiscus sabdariffa* water extract, and they can act as bioactive compounds with anti-hypertensive properties by inhibiting the enzyme that converts angiotensin I to angiotensin II. Furthermore, the long-term use of *Hibiscus sabdariffa* water extract does not cause side effects in hypertensive patients.

One of the compounds believed to be present in Rosella flower calyx that is beneficial in lowering blood glucose levels is flavonoids, specifically flavone derivative gossypetin (hexahydroxyflavo)-3-glucoside, which acts as an antioxidant that can inhibit damage to  $\beta$ -cells in the pancreatic islets that produce insulin and stimulate insulin release in pancreatic  $\beta$ -cells to be secreted into the blood. Additionally, flavonoids can also restore insulin receptor sensitivity in cells. The presence of saponins, which can lower blood glucose levels, works by inhibiting the action of the enzyme  $\alpha$ -glucosidase, which is present in the intestine and functions to convert carbohydrates into glucose. This  $\alpha$ -glucosidase inhibitor enzyme can inhibit glucose absorption in the small intestine, thus functioning as an anti-hyperglycemic agent to reduce blood glucose levels (Aprilliani and Pratiwi, 2018); (Wahyuni *et al.*, 2022).

Other studies indicate that consuming rosella tea from *Hibiscus sabdariffa* can decrease total cholesterol, LDL, and triglyceride levels. However, it does not result in an increase in HDL levels in the blood. One of the compounds that play a role in these anti-hypertensive and anti-hyperlipidemia effects is anthocyanin. Anthocyanins work by inhibiting LDL oxidation, thus contributing to the prevention of atherosclerosis. These findings highlight the potential benefits of rosella tea as a natural agent that can help manage the body's lipid profile, especially in reducing the risk of cardiovascular diseases. Therefore, rosella tea can be considered part of a healthy diet for maintaining heart health and controlling blood lipid levels.

## CONCLUSION

The conclusion of this study indicates that administering Rosella flower ethanol extract (*Hibiscus sabdariffa*) to rats has varied impacts on lipid profiles and liver function. The treatment groups showed complex responses, including a significant increase in total cholesterol and HDL in "Rosella Flower Ethanol Extract (*Hibiscus sabdariffa*) -I," a decrease in LDL in "Rosella Flower Ethanol Extract (*Hibiscus sabdariffa*) -II," and an increase in total cholesterol and triglycerides in "Rosella Flower Ethanol Extract (*Hibiscus sabdariffa*) -III." Statistical analysis validated significant differences between treatment groups ( $P < 0.05$ ), including triglycerides in "Rosella Flower Ethanol Extract (*Hibiscus sabdariffa*) -II" and "Rosella Flower Ethanol Extract (*Hibiscus sabdariffa*) -III" ( $P = 0.015$ ). These results also showed a significant impact on liver function, with substantial differences in SGOT levels ( $P = 0.006$ ) and SGPT levels ( $P < 0.05$ )—the potential regulatory effects of Rosella flower on rat lipid metabolism and liver health warrant further attention.

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