# **Evaluation of the Potential of Rosella Flower Ethanol Extract (Hibiscus Sabdariffa) In Reducing Dyslipidemia In Male Rats Induced By A High-Fat And Fructose Diet.**

Zhang Yilong<sup>1</sup>, Soehartina<sup>2</sup>, Wienaldi<sup>3</sup>

Master of Clinical Medicine Study Program Faculty of Medicine, Prima University of Indonesia, Medan

## ABSTRACT

Dyslipidemia is a condition of imbalance of blood lipid levels, which increases the risk of coronary heart disease and stroke. Causative factors include a diet high in fat and fructose, a sedentary lifestyle, obesity, and oxidative stress. Rosella flower (Hibiscus sabdariffa) is known to have potential as a natural therapy in the management of dyslipidemia due to its content of active compounds such as anthocyanins, flavonoids, and phenolic acids, which can lower total Cholesterol and triglycerides and increase HDL. This study aimed to evaluate the effect of ethanol extract of Rosella flowers on the blood lipid profile of Wistar rats fed a high-fat and fructose diet. The research method used was an experiment with the Pre-test and Post-test Group Only Control Design, involving six treatment groups consisting of a control group, a group given simvastatin, and a group given ethanol extract of Rosella flowers at doses of 100 mg/kgBB, 200 mg/kgBB, and 400 mg/kgBB. Measurements were taken on total Cholesterol, LDL, HDL, and triglyceride levels before and after treatment. The results showed that Rosella flower extract could lower total and LDL cholesterol levels and increase HDL in dyslipidemia-model mice, although higher doses tended to be more effective. Nonetheless, significant differences were only found in total and LDL cholesterol levels, with high doses of Rosella extract showing the most significant decrease. This study suggests that ethanol extract from Rosella flowers has the potential to be a supportive therapy in the management of dyslipidemia. However, more research is still needed to clarify its mechanism and long-term effectiveness.

Keywords: Dyslipidemia, Hibiscus sabdariffa, ethanol extract, lipid profile, Cholesterol, triglycerides, HDL, LDL.

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## I. Introduction

Dyslipidemia is an imbalance of blood lipid levels, characterized by an increase in total Cholesterol, LDL, and triglycerides and a decrease in HDL (Miller et al., 2021). This condition increases the risk of coronary heart disease and stroke (Schmitz & Lang, 2020). Factors that cause it include a diet high in fat and fructose, a sedentary lifestyle, obesity, genetic factors, oxidative stress, and insulin resistance (Santamarina & Medina, 2020; Kamal & Mahgoub, 2019; Ghosh & Ghosh, 2020).

An unhealthy diet worsens lipid profiles by increasing LDL and triglycerides and lowering HDL, while excessive fructose consumption exacerbates insulin resistance and inflammation (Jafari & Rahman, 2023). A sedentary lifestyle exacerbates fat accumulation, increasing the risk of dyslipidemia, Ghosh & Ghosh, 2020).

As interest in natural therapies increases, Rosella flowers (Hibiscus sabdariffa) are attracting attention due to their anthocyanins, flavonoids, and phenolic acids that have the potential to lower total Cholesterol and triglycerides and increase HDL (Ghazali et al., 2022; Zofania et al., 2020). Rosella is also known to inhibit the enzyme HMG-CoA reductase, increase bile acid excretion (Kaniawati et al., 2024), and have anti-inflammatory and antioxidant properties.

Studies in animal models with diets high in fat and fructose showed that ethanol extract from Rosella flowers improved lipid profiles, reduced LDL and triglycerides, and increased HDL (Zofania et al., 2020; Kaniawati et al., 2024). Thus, Rosella has excellent potential as an adjunct therapy in managingdyslipidemia, although further research is still needed to clarify its mechanism of action and its long-term effectiveness.

#### II. Research Methods

This experimental study uses the Pre-test and Post-test Group Only Control Design approach, using male Wistar mice as test animals. This design involved measurements of total Cholesterol, LDL, HDL, and triglyceride levels before and after treatment to assess the effects of ethanol extract of rosella flower (Hibiscus sabdariffa) compared to a control group given simvastatin. The study was conducted for six weeks in January 2025, including one week of extract-making, one week of rat acclimatization, and four weeks of treatment. Experimental animal samples were calculated using Federer's formula, obtaining a minimum of four mice per group, with the criteria of male Wistar mice aged 2–4 months and weighing 180–200 grams. The free variable was the dose of ethanol extract of rosella flower, the bound variable was the blood lipid profile of the rats, and the control variable included body weight and total cholesterol levels before treatment. Measurements were made using analytical scales, spectroscopy, and Autocheck® tools.

The tools used include standard laboratory equipment such as rotary evaporators, UV spectrophotometers, electric ovens, and surgical tools. In contrast, research materials include rosella flowers, ethanol, equates, Na-CMC, simvastatin, phytochemical reagents, food pellets, and ketamine. Rosella flower samples were obtained from traditional markets in Medan City. Rosella flower simplicia is made through washing, drying, and refining processes, then extracted using the maceration method with 96% ethanol. The extracts obtained are concentrated using a rotary evaporator. Phytochemical screening is performed to identify the content of active compounds such as phenols, flavonoids, alkaloids, terpenoids, steroids, saponins, and tannins. To test the anti-dyslipidemia, mice were fed a diet high in fat and fructose, then given extracts or controls, with Na-CMC suspension as the vehicle. The data obtained were analyzed to assess the extract's effectiveness in lowering blood lipid levels in a mouse model of dyslipidemia.

## III. Research Results and Discussion

| Table 1 Characteristics of 1  | Rosella Flower Ethanol Extract (     | (Hibiscus sabdariffa). |
|-------------------------------|--------------------------------------|------------------------|
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| Characteristics                  | Value    |
|----------------------------------|----------|
| Weight of Fresh Simplisia (gr)   | 600 gr   |
| Dry Simplisia Powder Weight (gr) | 250 gr   |
| Pelarut Volume (ml)              | 2500 ml  |
| Extract Weight(gr)               | 18.50 gr |
| Rendemen (%)                     | 7.40%    |

Table 1 shows the characteristics of the ethanol extract of the Rosella flower (Hibiscus sabdariffa). From 600 grams of fresh simplicia, 250 grams of simplicia powder are produced, which are extracted with 2500 ml of ethanol to produce 18.50 grams of extract. The extraction yield is 7.40%, which illustrates the efficiency of the extraction process in producing Rosella flower extract.

| Phytochemicals          | Reagents               | Result |  |
|-------------------------|------------------------|--------|--|
| Alkaloid                | Bouchardart            | +      |  |
|                         | Mayer                  | +      |  |
|                         | Dragondroff            | -      |  |
|                         | Wagner                 | +      |  |
| Saponin                 | Aquadest + Alcohol 96% | -      |  |
| Flavonoid               | FeC13 5%               | +      |  |
|                         | $Mg_{(s)} + HCl_{(p)}$ | -      |  |
|                         | NaOH 10%               | -      |  |
|                         | H2SO4 (p)              | -      |  |
| Tanin                   | FeC13 1%               | +      |  |
| Steroids and Terpenoids | Salkowsky              | -      |  |
| -                       | Liberman Bouchard      | +      |  |

 Table 2 Phytochemical Screening Results of Rosella Flower Ethanol Extract (Hibiscus sabdariffa)

Table 2 shows the results of the phytochemical screening of the ethanol extract of the Rosella flower (Hibiscus sabdariffa). This extract contains alkaloids (positive with Bouchardart, Mayer, and Wagner reagents), flavonoids (positive with  $FeCl_3$  5%), tannins (positive with  $FeCl_3$  1%), and terpenoids (positive with Liberman Bouchard reagents). Saponins and steroids are not detected. Overall, the extract contains alkaloids, flavonoids, tannins, and terpenoids.

Table 3 Results of Data Normality Test with Shapiro-Wilk Test on All Research Parameters

| P value | Data Distribution                        |  |
|---------|--|--|
| 0.402   | Usual                                    |  |
| < 0.05  | Abnormal                                 |  |
| < 0.05  | Abnormal                                 |  |
|         | P value           0.402           < 0.05 | P valueData Distribution0.402Usual< 0.05 |

| Evaluation Of The Pote | ential Of Rosella H | Flower Ethanol Ex | xtract (Hibiscus S | Sabdariffa) In |
|------------------------|---------------------|-------------------|--------------------|----------------|
|------------------------|---------------------|-------------------|--------------------|----------------|

|                   | P value   | Data Distribution   |
|-------------------|---|---|
| Total Cholesterol | 0.524   | Normal  |
| Trigliseride      | 0.003   | Abnormal  |
| Up to HDL         | < 0.05  | Abnormal  |
| Up to LDL         | 0.192   | Normal  |
|                   | < 0.05  | Abnormal  |
|                   | 0.156   | Normal  |
|                   | Total Cholesterol<br>Trigliseride<br>Up to HDL<br>Up to LDL | P value           Total Cholesterol         0.524           Trigliseride         0.003           Up to HDL         < 0.05 |

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

| Table 4Comparison of Initial Weight Loss of Rats in All Treatment Groups |                |       |         |  |
|--|----------------|-------|---------|--|
| Treatment Groups   | Weight (grams) |       | P value |  |
| -  | Mean           | SD    |         |  |
| Normal   | 228.00         | 27.40 |         |  |
| Standard   | 233.00         | 25.80 |         |  |
| Control  | 231.20         | 27.10 | 0.170   |  |
| Ethanol Extract of Rosella Flower (Hibiscus sabdariffa) I                | 262.00         | 28.00 | 0.179   |  |
| Ethanol Extract of Rosella Flower (Hibiscus sabdariffa) -II              | 231.00         | 28.20 |         |  |
| Ethanol Extract of Rosella Flower (Hibiscus sabdariffa) -III             | 242.90         | 29.00 |         |  |

Table 4 compares the initial weight of the mice in the entire treatment group. The normal group had an average weight of 228.00  $\pm$  27.40 grams, the standard group 233.00  $\pm$  25.80 grams, and the control group  $231.20 \pm 27.10$  grams. The Rosella extract group at a dose of 200 mg/kgBB (I) had an average of 262.00 ± 28.00 grams, a dose of 400 mg/kgBB (II) of  $231.00 \pm 28.20$  grams, and a dose of 800 mg/kgBB (III) of  $242.90 \pm$ 29.00 grams. A P value of 0.179 indicates no significant difference between groups. Thus, the initial weight of the mice was relatively similar before the treatment.

| Table 5 Comparison of Total Cholesterol Before and After Giving a High-Fat Diet in All Treatment |
|--|
| Groups   |

|  | <b>Before Induction</b>   | After Induction              |  |
|--|---------------------------|------------------------------|--|
| Treatment Groups   | Total Cholesterol (mg/dL) | Total Cholesterol<br>(mg/dL) |  |
| Normal   | 110.00 (105-115)          | 220.50 (215-225)             |  |
| Standard (Simvastatin 25 mg/kg BB)   | 108.00 (105-115)          | 210.00 (205-215)             |  |
| Kontrol (on CMC 0.5%)  | 113.00 (110-118)          | 215.00 (210-220)             |  |
| Ethanol Extract of Rosella Flower (Hibiscus<br>sabdariffa) - I (100 mg/kg BB)  | 112.50 (110-118)          | 212.50 (208-218)             |  |
| Ethanol Extract of Rosella Flower (Hibiscus<br>sabdariffa) - II (200 mg/kg BB) | 110.00 (108-115)          | 209.70 (205-215)             |  |
| Ethanol Extract of Rosella Flower (Hibiscus sabdariffa) - III (400 mg/kg BB)   | 111.00 (110-120)          | 207.50 (205-210)             |  |
| P value  | 0.736                     | 0.010                        |  |

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

Table 5 shows the comparison of total Cholesterol before and after the administration of a high-fat diet. In the normal group, total Cholesterol increased from 110.00 mg/dL (range 105-115) to 220.50 mg/dL (range 215-225). The standard group (simvastatin 25 mg/kgBB) increased from 108.00 mg/dL (range 105-115) to 210.00 mg/dL (range 205-215), and the control group from 113.00 mg/dL (range 110-118) to 215.00 mg/dL (range 210-220). The Rosella extract group at a dose of 100 mg/kgBB (I) increased from 112.50 mg/dL (range 110-118) to 212.50 mg/dL (range 208-218), a dose of 200 mg/kgBB (II) from 110.00 mg/dL (range 108-115) to 209.70 mg/dL (range 205–215), and a dose of 400 mg/kgBB (III) from 111.00 mg/dL (range 110–120) to 207.50 mg/dL (range 205–210). The Kruskal-Wallis test showed no significant difference before induction (P = 0.736) but a substantial difference after induction (P = 0.010). Different superscripts in the same column show significant differences.

| Table 6 Comparison of Lipid Profiles in All Rats Treatment Group    |                       |                   |                    |                |
|---|-----------------------|-------------------|--------------------|----------------|
| Treatment Groups  | Total<br>Cholesterol* | Trigliserida**    | LDL*               | HDL**          |
| Normal  | $160.00\pm3.00a$      | 100.00 (95-110)a  | $45.00\pm2.00a$    | 62.00 (61-64)a |
| Standard  | $148.00\pm2.50B$      | 110.00 (105-115)b | $58.00\pm2.25b$    | 60.50 (59-62)a |
| Control   | $152.00 \pm 4.50c$    | 115.00 (110-120)c | $100.00 \pm 3.50c$ | 30.00 (29-32)b |
| Ethanol Extract of Rosella<br>Flower (Hibiscus<br>sabdariffa) - I   | 170.00 ± 5.00d        | 130.00 (125-135)d | 80.00 ± 2.75d      | 48.00 (46-50)b |
| Ethanol Extract of Rosella<br>Flower (Hibiscus<br>sabdariffa) - II  | 160.00 ± 4.50e        | 120.00 (115-125)e | 65.00 ± 3.00e      | 59.00 (58-60)a |
| Ethanol Extract of Rosella<br>Flower (Hibiscus<br>sabdariffa) - III | $145.00 \pm 3.50 f$   | 110.00 (105-115)  | $60.00 \pm 2.25 f$ | 61.00 (60-62)a |
| Nilai P   | < 0.05                | 0.015             | < 0.05             | 0.015          |

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

Table 6 compares the lipid profiles between groups of treatment mice. In total Cholesterol, the normal group (160.00  $\pm$  3.00 mg/dL) was higher than the standard (148.00  $\pm$  2.50 mg/dL) and control (152.00  $\pm$  4.50 mg/dL) groups. The Rosella extract group of 100 mg/kgBB (170.00  $\pm$  5.00 mg/dL) showed the highest total Cholesterol, while the doses of 200 mg/kgBB (160.00  $\pm$  4.50 mg/dL) and 400 mg/kgBB (145.00  $\pm$  3.50 mg/dL) were lower. The difference in total Cholesterol was significant (P < 0.05). For triglycerides, the median normal group was 100.00 mg/dL, standard 110.00 mg/dL, control 115.00 mg/dL, and Rosella extract were 130.00, 120.00, and 110.00 mg/dL at doses of 100, 200, and 400 mg/kgBB, respectively. Significant difference (P = 0.015). LDL levels in the normal group (45.00  $\pm$  2.00 mg/dL) were lower than standard (58.00  $\pm$  2.25 mg/dL) and control (100.00  $\pm$  3.50 mg/dL). Rosella extract lowered LDL by 80.00, 65.00, and 60.00 mg/dL, respectively, at 100, 200, and 400 mg/kgBB (P < 0.05).

In HDL, the normal group (62.00 mg/dL) was higher than the control group (30.00 mg/dL). The standard group (60.50 mg/dL) and Rosella extract doses of 200 mg/kgBB (59.00 mg/dL) and 400 mg/kgBB (61.00 mg/dL) were close to normal, while the dose of 100 mg/kgBB was lower (48.00 mg/dL). Significant difference (P = 0.015). Overall, ethanol extract from Rosella flowers affected changes in lipid profiles in experimental mice.

| Table 7 Comparison of SGOT and SGPT Levels in All Treatment Groups            |                   |                   |  |
|---|-------------------|-------------------|--|
| Treatment Groups  | Up to SGOT (U/L)  | Up to SGPT (U/L)  |  |
| Normal  | 38.00 (37-39)a    | 42.00 ± 1.50a     |  |
| Standard  | 120.00 (118-122)b | $170.00\pm2.00B$  |  |
| Control   | 155.00 (152-158)c | $100.00\pm2.00c$  |  |
| Ethanol Extract of Rosella Flower (Hibiscus sabdariffa)<br>- I (100 mg/kgBB)  | 110.00 (108-115)d | $95.00 \pm 3.00d$ |  |
| Ethanol Extract of Rosella Flower (Hibiscus sabdariffa)<br>- II (200 mg/kgBB) | 125.00 (120-130)e | 105.00 ± 2.50e    |  |

| Treatment Groups   | Up to SGOT (U/L)  | Up to SGPT (U/L) |
|--|-------------------|------------------|
| Ethanol Extract of Rosella Flower (Hibiscus sabdariffa)<br>- III (400 mg/kgBB) | 135.00 (130-140)f | $145.00\pm2.50f$ |
| P value  | 0.006             | < 0.05           |

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

Table 7 compares SGOT and SGPT levels between treatment groups. The SGOT levels of the normal group (38.00 U/L) were lower than those of the standard (120.00 U/L) and control (155.00 U/L) groups. The Rosella extract groups of doses of 100, 200, and 400 mg/kgBB showed SGOT levels of 110.00, 125.00, and 135.00 U/L, respectively.

In SGPT, the normal group (42.00  $\pm$  1.50 U/L) was also lower than the standard (170.00  $\pm$  2.00 U/L) and control (100.00  $\pm$  2.00 U/L) groups. The Rosella extract groups of doses of 100, 200, and 400 mg/kgBB showed SGPT levels of 95.00  $\pm$  3.00, 105.00  $\pm$  2.50, and 145.00  $\pm$  2.50 U/L. Differences in SGPT levels between groups were significant (P < 0.05). These results show that ethanol extract from Rosella flowers affects liver enzyme levels in experimental mice.

The findings of this study show a significant impact of ethanol extract of Rosella flower (Hibiscus sabdariffa) on the lipid profile of mice. The "Rosella Flower Ethanol Extract (Hibiscus sabdariffa)-I" treatment group that received a dose of 100 mg/kg BB showed a significant improvement in total Cholesterol and HDL, while the "Rosella Flower Ethanol Extract (Hibiscus sabdariffa)-II" treatment group (dose 200 mg/kg BB) showed a significant decrease in LDL levels. In contrast, the group "Rosella Flower Ethanol Extract (Hibiscus sabdariffa)-III" (dose 400 mg/kg BB) showed an increase in total Cholesterol and triglycerides. However, HDL levels did not show significant changes. These results reflect that Rosella flower extract has the potential to regulate lipid metabolism, with effects that vary depending on the dosage administered.

The group receiving a lower dose (100 mg/kg BB) of Rosella flower extract showed significant improvements in SGOT and SGPT levels. The 200 mg/kg BB dose increased SGPT levels, while the highest dose (400 mg/kg BB) raised both enzyme levels. This suggests that Rosella extract affects liver function, potentially reflecting the body's response to lipid metabolism.Previous studies confirm that Hibiscus sabdariffa's phytochemicals, such as anthocyanins, flavonoids, and ascorbic acid, influence liver enzymes and offer hepatoprotective benefits. Research by Bahadoran et al. (2013) and Durojaiye et al. (2015) indicates that Rosella extract reduces liver damage from oxidative stress and inflammation. Chandan et al. (2018) also found that Rosella extract could lower SGPT and SGOT levels in toxin-induced mice, showing its liver-protective potential. The antioxidants in Rosella flower extract help regulate lipid balance and prevent the negative effects of excessive fat consumption. Studies show that Rosella extracts lower total cholesterol, LDL, and triglyceride levels, though effects on HDL are more limited. Bioactive compounds like flavonoids and anthocyanins help prevent LDL oxidation, reducing the risk of atherosclerosis and cardiovascular diseases. Abubakar et al. (2015) also found that Rosella extract lowers cholesterol and triglyceride levels in high-fat diet rats, supporting its role in regulating lipid profiles and reducing cardiovascular risks.

Rosella flowers also have significant antibacterial activity, especially concerning their alkaloid compounds. Research by Abdallah (2016) and Djeussi et al. (2013) shows that Hibiscus sabdariffa extract has quite good antimicrobial potential against various types of bacteria, which further confirms that Rosella flowers are not only beneficial in managing lipid profiles but can also provide protection against bacterial infections. In addition, Rosella is also known to have potential as an antihypertensive agent. Research by Mojiminiyi et al. (2007) showed that Rosella extract can lower blood pressure in NaCl-induced mice. The use of anthocyanins in Rosella extract is believed to inhibit the conversion of angiotensin I to angiotensin II, essential in regulating blood pressure and improving vascular function.

Overall, this study indicates that Rosella flower extract has the potential to regulate lipid profiles and support heart health in a multifaceted way, including its effects on lipid metabolism, LDL oxidation-reduction, and antihypertensive potential. Nonetheless, further interpretation is needed to understand the long-term impact and mechanisms underlying enzymatic changes on the liver and evaluate the potential of Rosella flower extract in the prevention of cardiovascular disease in humans.

### IV. Conclusions and Suggestions

Ethanol extract from Rosella flower (Hibiscus sabdariffa) significantly affectedrats' lipid profile and liver function, with an increase in SGOT and SGPT at all doses. Its active compounds act as antioxidants and can potentially prevent cardiovascular disease. Further research and clinical trials are needed to ascertain its mechanism of action, safety, and effectiveness in managingdyslipidemia.

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