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Anticholesterolemia Efficacy of *Sawi Dayak* (*Elephantopus mollis* Kunth.) against the Hypercholesterolemia in Male White Mice

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Abstract

This study aims to determine the effect of ethanolic extract from *Sawi Dayak* on lipid profile modulation, specifically on reducing total cholesterol, triglycerides, and LDL levels, while increasing HDL levels in mice. A total of 25 male white mice were divided into five groups. Hypercholesterolemia was induced by administering pure cholesterol at a dose of 56 mg/20 g body weight for 7 days, followed by a 7-day treatment period. The groups included a normal control, a negative control treated with 0.5 % Na CMC, a positive control given Simvastatin, and two groups receiving *Sawi Dayak* extract at doses of 5.2 mg/20 g and 10.4 mg/20 g body weight. The results indicated that *Sawi Dayak* extract at both doses exhibited significant anti-hypercholesterolemic effects, with higher doses demonstrating more pronounced improvements in lipid profiles. It is concluded that *Sawi Dayak* extract possesses potential as an anticholesterolemic agent, with a dose-dependent effect on cholesterol reduction and HDL elevation.

Major Findings: The most significant findings of this research is giving the *Sawi Dayak* extract demonstrated antihypercholesterolemic properties in a dose-dependent manner, effectively reducing total cholesterol, triglycerides, and LDL levels, while increasing HDL levels.

Keywords: Anticholesterol Agents, Cholesterol, Extract of *Sawi Dayak*

1. Introduction

Cholesterol is a major lipid and sterol group in body tissue which in normal amounts can help the body's metabolic processes[1]. Naturally, cholesterol is a building block for cell walls, making bile acids for emulsifying fats and producing steroid hormones and some vitamins[2]. The average cholesterol needed in adults, which is 1,100 mg/day for cell wall maintenance with recommendations for consuming cholesterol should not exceed 300 mg/day[3]. As much as 25 - 40 % of cholesterol is produced through food while the rest is synthesized by the liver in the body[4].

Cholesterol is a compound that does not dissolve in water; therefore, it binds to proteins called lipoproteins, allowing it to circulate through the blood vessels. Cholesterol and triglycerides in the body are transported in the form of lipoproteins which are classified into four types, namely chylomicrons (0.06 % phospholipids, 1.7 % protein, 1.75 % cholesterol, 96 % triglycerides), VLDL (Very Low Density Lipoprotein) (10 % protein, 15 % phospholipids 15 % cholesterol, 60 % triglycerides),

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LDL (Low Density Lipoprotein) (10 % triglycerides, 45 % cholesterol, 25 % protein, 20 % phospholipids), and HDL (High Density Lipoprotein) (3 % triglycerides, 18 % cholesterol, 30 % phospholipid, 50 % protein)[5-7].

Chylomicrons have a function to transport triglycerides throughout the body[2,8]. VLDL serves to store cholesterol that has been secreted by the liver to muscle tissue as an energy reserve and transports triglycerides throughout the body[2,8]. LDL has the function of transporting cholesterol to peripheral tissues to break down membranes, form sterol hormones and steroid hormones. HDL has the function of transporting excess cholesterol from the blood vessels to be secreted by bile and then reabsorbed by the intestine to be recycled in the liver[8]. Cholesterol that is not absorbed by the intestines is then excreted from the body through the feces. Normal levels of total cholesterol in the blood are <240 mg/dl, normal levels of HDL amount of >60 mg/dl, normal levels of LDL <100 mg/dl, and normal levels of triglyceride levels <150 mg/dl[2,9].

Cholesterol is a source of energy for the body that contains very high calories. Excess calories in the body are converted into triglycerides and stored in adipose tissue. If this condition is allowed to continue, it will cause hypercholesterolemia which has the potential to lead to cardiovascular disease, especially the heart and blood vessels. A metabolic disorder condition characterized by cholesterol levels in the blood exceeding normal levels is hypercholesterolemia[2,9]. Hypercholesterolemia is defined by an increase in cholesterol levels, especially LDL and a decrease in HDL in the bloodstream[9,10]. High levels of LDL and triglycerides, combined with low HDL, can raise the risk of coronary heart disease. This condition is caused by the buildup of fat in the arteries, causing arteriosclerosis[10,12].

The treatment of hypercholesterolemia with synthetic drugs typically requires long-term therapy, which can be costly[13]. The synthetic drug commonly used in the treatment of hypercholesterolemia is Simvastatin[4,10]. However, long-term use of this drug can cause hepatotoxicity, malaise, rhabdomyolysis, and myopathy[4]. One way to overcome the negative impact of synthetic drugs is the use of herbal plants as an alternative treatment for hypercholesterolemia. Several studies have shown a variety of herbal plants that are proven to be successful in reducing blood's cholesterol levels, such as bay leaves (*Syzygium polyanthum*)[9], peel of red dragon fruit (*Hylocereus polyrhizus*)[10,13], soybean germ oil[14], *Elephantopus scaber*[15], leaves of cerme (*Phyllanthus acidus* (L.) Skeels)[16], and rambutan (*Nephelium lappaceum*)[17].

West Borneo has quite a lot of herbal plants that are believed to be passed down from generation to generation, one of which is *Sawi Dayak*. *Sawi Dayak* is a perennial herbaceous plant that typically grows between 0.5 and 1 meter tall, although it can occasionally reach up to 2 meters. Initially, it forms a rosette of leaves measuring up to 30 cm in length, from which stems covered with soft hairs emerge. The stem leaves are alternately arranged, ranging from ovate to lanceolate in shape, with a rough texture on the upper surface and a hairy, gland-dotted, resinous underside. These leaves measure between 5 and 25 cm in length and 3 to 10 cm in width, featuring shallow to sharply toothed edges. The leaf stalk is winged and extends along the stem. The plant produces flower heads up to 2 cm wide, containing small florets that are either white or purple and approximately 4 mm long, surrounded by three leaf-like bracts about 1 cm in length. The seeds are black, roughly 3 mm long, densely covered with fine, short hairs, and topped with five white bristles measuring 3 – 4.5 mm long, each with a broad base. The receptacle is devoid of scales.

Traditionally, various Chinese ethnic groups and the Dayak community in West Borneo have utilized *Sawi Dayak* as both a food source and a traditional remedy for hypercholesterolemia. Phytochemical analyses of *Sawi Dayak* have revealed a rich composition of bioactive compounds, including sesquiterpene lactones, flavonoids, steroids, and alkaloids[18-20]. Recent scientific investigations have confirmed the therapeutic potential of *Sawi Dayak*. Extracts from *Sawi Dayak* have demonstrated cytotoxic effects against various cancer cell lines. For instance, ethyl acetate extracts have been shown to induce apoptosis in human lung cancer and myeloid leukemia cells[21].

Traditional uses of *Sawi Dayak* include treatments for inflammation-related conditions, and scientific studies have supported its anti-inflammatory potential[22]. The compound[3,4]-di-O-caffeoyl quinic acid from the methanolic extract of this plant was found to exhibit concentration-dependent α-glucosidase inhibitory activity, with an EC_{50} value of 241.80 ± 14.29 µg/ml. Inhibition of the α-glucosidase enzyme can help reduce postprandial blood glucose levels, which is crucial for managing type 2 diabetes[23].

Extracts of *Sawi Dayak* exhibit broad-spectrum antimicrobial properties, effectively inhibiting Gram-positive and Gram-negative bacteria as well as pathogenic

fungi. This activity positions *Sawi Dayak* as a potential candidate for addressing antibiotic-resistant infections[23]. The phenolic and flavonoid content of *Sawi Dayak* confers strong free radical-scavenging activity, which could mitigate oxidative stress, a contributor to aging and degenerative diseases[22,23].

Previous research has shown that *Elephantopus mollis* Kunth. has high antioxidant activity compared to *Elephantopus tomentosus*[23]. In addition, based on activity tests on plants of the same genus, such as *Elephantopus scaber* (commonly known as *Tapak Liman*) showed hypolipidemic activity[15], so, it is assumed that *Sawi Dayak* also has the same potential as anticholesterolemic as *Tapak Liman* because both are in the same genus, namely *Elephantopus*. As in previous studies, it was proved that plants in one genus *Elephantopus*, namely *Elephantopus mollis* Kunth., *Elephantopus tomentosus* L., *Elephantopus scaber* L., and *Elephantopus carolinianus Raeusch* were known to have antioxidant activity[23].

2. Materials and Methods

2.1 Extraction

Sawi Dayak is collected from a traditional market on Ampera, Sungai Jawi Village, Pontianak City, West Borneo, Indonesia. *Sawi Dayak* with a wet weight of 1.03 kg was taken from the leaves and stems then cleaned in running water and shade dried. The leaves and stems of the *Sawi Dayak* were then air-dried to a dry weight of 93 g of the sample. *Sawi Dayak* was extracted using the maceration method. The dried *Sawi Dayak* was soaked in 96 % distilled ethanol. The extraction process was carried out 3 x 24 hours with the replacement of the filter fluid every 24 hours. Then the results of the immersion were filtered with filter paper to obtain macerates I, II, and III. The three macerates were combined and concentrated with a rotary evaporator, after which they were weighed, and the extract weight was 6.619 g with a yield of 7.12 %.

2.2 Experimental Animal

Twenty-five male white mice, aged 2 to 2.5 months and weighing 25 – 30 grams, were obtained from the laboratory of the Center for Food and Nutrition Studies at Universitas Gadjah Mada, Yogyakarta, Indonesia. Before the start of experiment, all mice were acclimatized for 7 days, during which they were fed a standard AD-II diet (comfeed-brand from PT JAPFA) and given drink freely (ad libitum). After acclimatization, all mice were observed for their health condition and weighed. The research's implementation received approval from the Health Research Ethics Commission of the University of Respati Yogyakarta, with Ethical Clearance number 196.3/FIKES/PL/VIII/2021.

2.3 Preparation of Na CMC 0,5 % Solution

As much as 0.5 g Na CMC17 (Sigma-Aldrich) was added to a beaker and dissolved in approximately ±30 ml of warm distilled water (aquades), then stirred until fully mixed and uniform.

2.4 Preparation of Simvastatin Solution

The dose of Simvastatin (PT Hexpharm Jaya) for adults is 10 mg. The dose was converted for mice, so that the dose in mice was 0.026 mg/20 grams of body weight[10]. One Simvastatin tablet was crushed using a mortar and pestle, then dissolved in 5 ml of 0.5 % Na CMC solution.

2.5 Extract Dose of Sawi Dayak

The dose of *Sawi Dayak* extract used is based on the use of the dose by the community, which is as much as 300 g for one time use. Furthermore, the dose in humans was converted to mice so that the dose used was 5.2 mg/20 g body weight.

2.6 Hypercholesterolemic Mice Model

White male mice were induced with 95 % pure cholesterol (PT Sigma-Aldrich) the dose used was 56 mg/20-gram body weight[16]. Mice were fed with pure cholesterol for 7 days so that cholesterol levels exceeded the normal range[9]. If cholesterol levels have reached 82.4 mg/dl, it can be said that mice have hypercholesterolemia.

2.7 Testing the Anticholesterol Activity of Sawi Dayak

Testing of the anticholesterolemia activity of *Sawi Dayak* was carried out with 25 male mice adapted for 7 days by being fed and drinking ad libitum. After adaptation, 25 mice were measured for initial total cholesterol, triglyceride, HDL, and LDL levels. The first group (normal control) received no treatment, while the second group (negative control) was given 0.5 % Na CMC at a dose of 0.5 ml per 20 g body weight. The third group (positive control) was administered Simvastatin at a dose

of 0.026 mg per 20 g body weight. The fourth and fifth groups were treated with *Sawi Dayak* extract at doses of 5.2 mg and 10.4 mg per 20 g body weight, respectively. After 7 days of treatment, blood samples were collected from all mice on day 16 to measure total cholesterol, HDL, triglycerides, and LDL levels to determine the effects of the treatments. Blood samples were tajen on day 1 from mice ofvall groups through retroorbital pelxuses. Day 1, taking mice blood samples via the orbital sinus. Five animals were separated and became a normal control group, while the other 20 animals were induced with pure cholesterol once a day starting on day 1 to day 7 at a dose of 56 mg/20 g body weight orally. Furthermore, all experimental animals were provided with standard food and drinking water. Following the administration of pure cholesterol suspension for 7 days, blood cholesterol levels were measured on day 8 for each mouse to assess the increase in total cholesterol, triglycerides, HDL, and LDL. If the blood cholesterol level of mice has reached 82.4 mg/dl, then the mice can be categorized as having hypercholesterolemia and can be continued with the treatment according to the group, the preparation is given orally and the test preparation is given once a day for 7 consecutive days 9. The treatment phase began on day 9 and lasted until day 15.

2.8 Cholesterol Level Measurement

The measurement of cholesterol levels of mice was carried out 3 different days, on the 1st (D-1), 8th (D-8), and 16th (D-16) day. Total cholesterol, triglycerides, LDL and HDL levels were measured using the Diasys enzymatic method. Taking blood's mice using a microhematocrit from the orbital sinus as much as 100 µl was put in a test tube, added 1000 µl of cholesterol reagent solution then mixed and left for 10 minutes at 37˚C. Measured absorption wavelength was at 500nm against the blank using a photometer 5010V5+. As a blank, 1000 µl of cholesterol, triglyceride, LDL, and HDL reagents were used for each test and distilled water was added. The standard absorption measurement is the same as the total cholesterol absorption measurement, but the blood serum is replaced with the cholesterol standard.

2.9 Data Analysis

The data obtained from the measurements of total cholesterol, triglycerides, HDL, and LDL levels were analyzed using a one-way ANOVA test, followed by Duncan's test. The analysis was performed using SPSS 26 for Windows with a significance level of $\alpha = 0.05$.

3. Result

Cholesterol levels, including triglycerides, total cholesterol, HDL, and LDL, were measured at three distinct time points: baseline prior to induction with pure cholesterol (D-1), after hypercholesterolemia induction (D-8), and following seven days of treatment (D-16).

At baseline, all mice exhibited normal lipid profiles, with total cholesterol, triglycerides, LDL, and HDL values within the ranges of 62.88–75.00 mg/dL, 79.46–91.96 mg/dL, 21.32–29.41 mg/dL, and 60.78–71.57 mg/dL, respectively. These values were consistent with the normal ranges for mice: total cholesterol 26.0–82.4 mg/dL, LDL 7–27.2 mg/dL, HDL >35 mg/dL, and triglycerides 58.5–91.0 mg/dL.

After administration of pure cholesterol to induce hypercholesterolemic condition, significant changes were observed in all groups except for the normal control group. There was a marked increase in total cholesterol, triglycerides, and LDL levels, with corresponding ranges of 177.48–186.09 mg/dL, 120.14–130.04 mg/dL, and 68.51–79.58 mg/dL, respectively. Conversely, HDL levels decreased to 24.49–30.61 mg/dL, falling below the normal threshold.

After seven days of treatment, both the positive control group and the extract-treated group demonstrated improvement in lipid profiles. HDL levels increased to 102.77–139.13 mg/dL, while total cholesterol, triglycerides, and LDL levels decreased to 100.00–124.62 mg/dL, 31.73–56.83 mg/dL, and 42.14–58.57 mg/dL, respectively. These results indicate a reversal of the hypercholesterolemic condition. The normal range for total cholesterol levels in mice is 26.0–82.4 mg/dl with normal LDL 7–27.2 mg/dl, normal HDL >35 mg/dL, and normal triglyceride levels 58.5–91.0 mg/dL[9].

A substance's ability to reduce total cholesterol, triglycerides, and LDL levels while simultaneously increasing HDL levels is indicative of its antihyperlipidemic potential. The observed lipid-lowering effect of the tested preparation highlights its promise as a therapeutic agent for managing hypercholesterolemia. Detailed profile data are presented in Table 1.

Table 1. The mean and Standard Deviation (SD) values for total cholesterol, triglyceride, LDL, and HDL levels in the normal group, Negative Control (NC), Positive Control (PC), and *Sawi Dayak* extract (Dose 1 and Dose 2)

Parameter	Measurement Time	Group				
		Normal	NC	PC	D1	D2
Total Cholesterol	D-1	68.18a ± 2.39	69.24a ± 3.74	67.57a ± 2.85	70.45a± 2.39	70.76a ± 2.76
	D-8	71.79a ± 2.42	181.59b ± 3.73	183.17b ± 2.18	181.19b ± 4.21	182.65b ± 2.86
	D-16	72.59a ± 2.28	182.66e ± 4.01	105.93b ± 2.49	130.27d ± 5.63	115.10c ± 2.77
Triglycerides	D-1	85.89a ± 5.18	83.93a ± 1.41	82.68a ± 3.19	85.89a ± 1.72	85.00a ± 3.18
	D-8	87.18a ± 5.38	126.92b ± 2.16	126.64b ± 2.85	124.66b ± 2.98	125.23b ± 2.98
	D-16	88.22a ± 5.52	128.53d ± 2.41	104.46b ± 3.32	120.15c ± 3.58	115.07c ± 4.37
HDL	D-1	64.51a ± 1.89	64.71a ± 2.86	65.29a ± 2.83	64.71a ± 3.61	65.69a ± 3.98
	D-8	63.27b ± 1.52	27.89a ± 1.07	26.80a ± 2.01	28.16a ± 1.84	28.71a± 1.47
	D-16	62.14e ± 2.35	26.57a ± 1.16	54.14d ± 2.69	44.29b ± 1.82	49.00c ± 1.48
LDL	D-1	25.59a ± 3.26	26.18a ± 4.12	23.82a ± 1.98	25.94a ± 1.61	26.18a ± 0.84
	D-8	26.38a ± 2.52	76.83c ± 1.92	74.88c ± 1.33	71.83b ± 2.37	72.01b ± 2.08
	D-16	27.74a ± 2.38	77.85e ± 1.84	35.42b ± 3.08	52.10d ± 2.98	42.06c ± 1.65

Note: *) The data are presented as mean ± SD, with different letters within the same column indicating significant differences ($p<0.05$).

Information:

Normal (Normal Control) = No treatment

NC (Negative control) = A dose of 0.5 ml/20 g body weight of 0.5 % Na CMC

PC (Positive control) = A dose of 0.026 mg/20 g body weight of Simvastatin

D1 (Dose 1) = A dose of 5.2 mg/20 g body weight of extract

D2 (Dose 2) = A dose of 10.4 mg/20 g body weight of extract

4. Discussion

Cholesterol is a lipid molecule classified as a sterol and functions as a crucial biochemical component in living organisms. It appears as a yellowish, fatty substance synthesized predominantly in the liver and transported through the blood vessels[1,4]. Cholesterol serves multiple physiological roles, including acting as an essential structural component of cellular membranes, where it contributes to membrane fluidity and integrity. Furthermore, it plays a pivotal role in the biosynthesis of steroid hormones (e.g., glucocorticoids, mineralocorticoids, and sex hormones), vitamin D, and bile acids, which are critical for lipid digestion and absorption[2].

Dietary sources of cholesterol are predominantly derived from animal-based products. Foods such as whole milk, cheese, butter, and other dairy products are recognized for their high cholesterol content. Similarly, egg yolks and organ meats (e.g., liver, kidneys, and brain) are particularly rich in cholesterol[24,25]. Notably, the brain contains the highest concentration of cholesterol in the human body, where it is essential for the formation and maintenance of myelin sheaths and neuronal function.

In contrast, plant-based foods are inherently cholesterol-free[24]. Unlike animals, plants synthesize structurally similar compounds, such as phytosterols, which are not absorbed efficiently by the human gastrointestinal tract and can even inhibit dietary cholesterol absorption. These differences highlight the distinctive metabolic and dietary roles of cholesterol in animal and plant systems.

Hyperlipidemia is a clinical condition characterized by an abnormal elevation of lipoprotein (lipid) levels in the blood. This condition may result from metabolic dysfunctions or abnormalities in lipid processing mechanisms[26]. Hypercholesterolemia, a common subtype of hyperlipidemia, is defined by total cholesterol levels exceeding 240 mg/dL and LDL levels greater than 160 mg/dL[2,9,10]. This condition is of particular concern because LDL, often referred to as "bad cholesterol," contributes to the formation of atherosclerotic plaques in arterial walls, thereby increasing the risk of cardiovascular diseases, including atherosclerosis, myocardial infarction, and stroke[26].

Moreover, elevated cholesterol levels are frequently associated with a reduction in HDL, known as "good cholesterol." HDL plays a crucial role in reverse cholesterol

transport, whereby cholesterol is carried from peripheral tissues back to the liver for excretion. Characterized by increased LDL and triglyceride levels and decreased HDL levels[9,10]. This pattern is characteristic of dyslipidemia and indicates an imbalance in lipid homeostasis.

Triglycerides and cholesterol are key lipid components commonly found in fatty foods. The administration of pure cholesterol is hypothesized to elevate both cholesterol and triglyceride levels in the bloodstream due to its direct contribution to lipid metabolism and circulation[2]. In this study, the induction of hypercholesterolemia was achieved through the daily administration of pure cholesterol over a period of seven consecutive days.

The accumulation of cholesterol in the bloodstream following the administration of pure cholesterol leads to the development of hypercholesterolemia, a condition marked by elevated serum lipid levels[2]. The process begins with the absorption of pure cholesterol in the small intestine, where it is incorporated into chylomicrons, a type of lipoprotein responsible for transporting dietary lipids through the lymphatic system into circulation[8].

Chylomicrons, along with Very-Low-Density Lipoproteins (VLDL), deliver triglycerides to adipose tissue, where the enzyme Lipo-Protein Lipase (LPL) hydrolyzes triglycerides into free fatty acids for storage or energy use. Following triglyceride release, the remaining chylomicrons transport cholesterol to the liver for metabolism and excretion via bile[27,28]. However, not all cholesterol is excreted; a portion of it is repackaged into VLDL particles for redistribution.

In circulation, lipoprotein lipase, located on the endothelial surfaces of blood vessels, hydrolyzes the triglycerides present in VLDL. This enzymatic activity results in the formation of Intermediate-Density Lipoproteins (IDL). IDL particles, which have a short half-life of approximately 2–6 hours, are either cleared by the liver or further metabolized into LDL[28].

LDL, which is rich in cholesterol, plays a central role in lipid homeostasis but is also implicated in atherogenesis. The prolonged presence of LDL in the bloodstream increases the risk of cholesterol deposition in arterial walls, leading to atherosclerosis[27,28]. The dynamic process of lipoprotein metabolism demonstrates the interconnected pathways of lipid transport and the critical role of enzymes like lipoprotein lipase in modulating lipid profiles.

The data presented in Table 1 highlights the effects of *Sawi Dayak* extract (at two dosages) compared to normal control (Normal), Negative Control (NC), and Positive Control (PC) groups on lipid profile parameters, including total cholesterol, triglycerides, HDL, and LDL on days 1 (D-1), days 8 (D-8), and days 16 (D-16) of the study.

In Table 1, the results of the average total cholesterol, triglycerides, and HDL in D-8 which are post-induced pure cholesterol show a decrease in HDL, an increase in cholesterol and triglyceride levels in all mice ($p<0.05$). Despite the increase, LDL levels were statistically different among the positive control group, the negative control group, and the extract treatment group. Based on the results of previous studies, it was stated that variations in LDL levels could be caused by uncontrollable factors such as differences in body weight of mice, age differences, and unknown factors.

After the mice experienced hypercholesterolemia, the mice were then given a test preparation according to the treatment group for 7 days, namely first group (the normal control) without treatment, the second group (negative control) with 0.5 % Na CMC at a dose of 0.5 ml/20 g of body weight of the mice, the third group (positive control) with the administration of Simvastatin dose 0.026 mg/20 g body weight of mice, the fourth group was given a dose of 5.2 mg/20 g of the body weight of the mice and the fifth group was given a dose of 10.4 mg/20 g of the body weight of the mice.

4.1 Effects on Total Cholesterol

The post-treatment (D-16) results indicated a reduction in total cholesterol levels in the groups treated with *Sawi Dayak* extract (D1 and D2) and the positive control (Simvastatin). The group receiving the higher dose of *Sawi Dayak* extract (D2, 10.4 mg/20 g body weight) exhibited a more pronounced reduction in total cholesterol levels (115.10 ± 2.77 mg/dL) compared to the group receiving the lower dose (D1, 5.2 mg/20 g body weight; 130.27 ± 3.63 mg/dL). Although both doses effectively decreased cholesterol levels, the reduction achieved by the D2 group approached the values observed in the positive control group treated with Simvastatin (105.93 ± 2.49 mg/dL).

Despite the significant reductions, the total cholesterol levels in the test groups treated with *Sawi Dayak* extract did not return to the normal levels recorded in the normal control group (72.59 ± 2.28 mg/dL). These findings suggest a dose-dependent anticholesterol effect of *Sawi Dayak* extract, with the higher dose (D2) demonstrating superior efficacy. However, further research is required to investigate the potential for achieving normal cholesterol

levels and to explore the underlying mechanisms responsible for this effect.

4.2 Effects on Triglycerides

Post-treatment (D-16) analysis revealed a significant reduction in triglyceride levels across the treated groups. The group receiving the higher dose of *Sawi Dayak* extract (D2, 10.4 mg/20 g body weight) demonstrated triglyceride levels of 115.07 ± 4.37 mg/dL, which were closer to those observed in the positive control group treated with Simvastatin (104.46 ± 3.32 mg/dL). In comparison, the group receiving the lower dose of *Sawi Dayak* extract (D1, 5.2 mg/20 g body weight) achieved triglyceride levels of 120.15 ± 3.58 mg/dL.

These findings suggest a dose-dependent effect of *Sawi Dayak* extract in lowering triglyceride levels, with the higher dose exhibiting greater efficacy. While the reductions observed in the D2 group approach those of the positive control, further investigation is warranted to confirm the extract's full potential and to evaluate its long-term effects on triglyceride metabolism.

4.3 Effects on HDL

The analysis of HDL levels, which serve as a key marker of protective lipoproteins, revealed an increase in both groups treated with *Sawi Dayak* extract. The group receiving the lower dose (D1, 5.2 mg/20 g body weight) showed HDL levels of 44.29 ± 1.82 mg/dL, while the higher-dose group (D2, 10.4 mg/20 g body weight) achieved an elevated HDL level of 49.00 ± 1.48 mg/dL. Notably, the HDL levels in the D2 group were closer to those observed in the positive control group treated with Simvastatin (54.14 ± 2.69 mg/dL).

Despite these improvements, the HDL levels in both treatment groups remained below the levels recorded in the normal control group (62.14 ± 2.35 mg/dL). These results indicate a dose-dependent effect of *Sawi Dayak* extract in enhancing HDL levels, suggesting its potential to improve lipoprotein profiles. However, further studies are necessary to explore whether prolonged treatment or higher doses can normalize HDL levels and enhance cardiovascular protective effects.

4.4 Effects on LDL

Post-treatment evaluation showed a significant reduction in LDL levels, a critical marker for "bad cholesterol," across all treatment groups. The group receiving the higher dose of *Sawi Dayak* extract (D2, 10.4 mg/20 g body weight) exhibited LDL levels of 42.06 ± 1.65 mg/dL, which approached the reduction observed in the positive control group treated with Simvastatin (35.42 ± 2.98 mg/dL). In contrast, the lower-dose group (D1, 5.2 mg/20 g body weight) had LDL levels of 52.10 ± 2.98 mg/dL, reflecting a smaller reduction compared to D2.

These results underscore the dose-dependent efficacy of *Sawi Dayak* extract in lowering LDL levels. The observed trend aligns with the extract's potential to mitigate hyperlipidemia, highlighting its therapeutic promise in managing cholesterol profiles. Further investigations are recommended to elucidate the molecular mechanisms underlying its lipid-lowering effects.

The administration of *Sawi Dayak* extract demonstrated significant improvements in lipid profiles, characterized by reduced total cholesterol, triglycerides, and LDL levels, alongside an increase in HDL levels. However, the lipid parameters in both the lower-dose group (D1, 5.2 mg/20 g body weight) and higher-dose group (D2, 10.4 mg/20 g body weight) remained above those observed in the normal control group, indicating that full normalization of lipid levels was not achieved with the treatment.

Compared to the negative control group, which exhibited a hyperlipidemic condition, both D1 and D2 groups showed marked improvements in all lipid parameters, reinforcing the anticholesterol potential of *Sawi Dayak* extract. Notably, the lipid-lowering effects in the D2 group were closer to those observed in the positive control group treated with Simvastatin. While these findings highlight the anticholesterol activity of *Sawi Dayak* extract, the effects at the highest tested dose (D2) were slightly less potent than those achieved with Simvastatin. This suggests that while *Sawi Dayak* extract holds promise as a natural alternative for managing dyslipidemia, further optimization of its dosage or combination with other treatments may be necessary to enhance its therapeutic efficacy.

Therefore, it is assumed that the higher the dose of *Sawi Dayak* extract given, the higher the ability to lower cholesterol levels. It's just that when contrast with the normal control group, the decrease has not reached normal cholesterol levels. In contrast, compared to the negative control group, the group treated with the extract showed a decrease in LDL, triglycerides, and total cholesterol, along with an increase in HDL. Furthermore, the study's findings indicate that the *Sawi Dayak* extract

has anticholesterol activity although it is not as good as the positive control group.

Simvastatin, a member of the statin drug class, was employed as the positive control in this study to evaluate its efficacy in cholesterol regulation[10,11]. Statins, including Simvastatin, are widely recognized for their ability to lower cholesterol levels by targeting the rate-limiting enzyme in cholesterol biosynthesis, 3-Hydroxy-3-Methylglutaryl-Coenzyme A (HMG-CoA) reductase. Specifically, Simvastatin exerts its effects by inhibiting the conversion of HMG-CoA to mevalonate, a precursor in the cholesterol synthesis pathway. This mechanism results in a significant reduction in total cholesterol and LDL levels while simultaneously increasing HDL concentrations by approximately 10 %[10,11].

Further investigations have demonstrated additional biochemical actions of Simvastatin. It has been reported to inhibit the activity of triglyceride lipase, thereby reducing the hydrolysis of triglycerides[29]. Moreover, Simvastatin decreases the binding of free fatty acids to albumin, which in turn reduces the activity of lipoproteins, including pre-lipoproteins[29,30]. These combined effects make Simvastatin a highly effective therapeutic agent in managing hyperlipidemia, particularly in conditions characterized by elevated cholesterol and triglyceride levels. By serving as a positive control, Simvastatin provides a benchmark for comparing the cholesterol-lowering potential of alternative treatments, including experimental therapies or natural extracts under investigation.

Based on the data presented in Table 1, *Sawi Dayak* has demonstrated the ability to reduce LDL, triglycerides, and total cholesterol levels while increasing HDL levels in treated subjects. These findings align with previous studies highlighting the high antioxidant activity of *Sawi Dayak*, attributed to the presence of caffeoylquinic acid, a derivative of chlorogenic acid[19,20]. In addition to its antioxidant properties, *Sawi Dayak* exhibits significant anti-inflammatory activity, as evidenced by the identification of seven sesquiterpene lactones, including *8-O-methacryloylelephanpane*, *2,4-bis-O-methyl-8-O-methacryloylelephanpane*, *4-O-ethyl-8-O-methacryloylelephanpane,8-O-methacryloylisoelephanpane*, *2-O-demethyltomenphantopin C*, *molephantin A*, and *molephantin B*18.

The roots, stems, and leaves of *Sawi Dayak* are also rich in phytochemical compounds such as isochlorogenic acid, 1,5-dicaffeoylquinic acid, and 4,5-dicaffeoylquinic acid, along with flavonoids such as apigenin, luteolin, and tricin[31]. Additionally, other studies have identified the presence of flavonoids, steroids, and alkaloids in *Sawi Dayak*[18-20]. These bioactive compounds are believed to act synergistically to reduce cholesterol levels through various mechanisms.

Flavonoids, as potent antioxidants, exhibit antiatherogenic properties by preventing LDL oxidation[10], which is a critical factor in the development of atherosclerosis. Steroids, on the other hand, reduce cholesterol absorption in the intestinal lumen, thereby lowering circulating cholesterol levels. Meanwhile, alkaloids play a role in enhancing the excretion of cholesterol through feces, further contributing to cholesterol reduction[10,14].

This combination of bioactive components and their diverse mechanisms of action underscores the therapeutic potential of *Sawi Dayak* as a natural anticholesterol agent. Further studies are warranted to explore these mechanisms in greater detail and to establish its efficacy and safety in clinical applications.

5. Conclusions

It was concluded that administration of *Sawi Dayak* extract at doses of 5.2 mg/20 g and 10.4 mg/20 g body weight improved lipid profiles in mice, with the higher dose showing more pronounced effects. The extract demonstrated antihypercholesterolemic properties in a dose-dependent manner, effectively reducing total cholesterol, triglycerides, and LDL levels, while increasing HDL levels. These findings suggest that *Sawi Dayak* extract has potential as a therapeutic agent for managing hypercholesterolemia. Further studies are needed to optimize the dosage for more effective and consistent results.

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7. References

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