The Effect of Doses of Red Shoot Leaf Extract *(Syzygium myrtifolium Walp)* on Decreased Cholesterol Total Levels in Male White Wistar Rats In Vivo

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Abstract. The prevalence of cholesterol in Indonesia is 66.41%. Central Java is among the five provinces with the highest levels of consumption of fatty, cholesterol, and fried foods, at 60.3%. The purpose of this study was to determine the effect of an extract of red shoot leaves *(Syzyginium myrtifolium Walp)* on decreasing the profile value of total cholesterol levels in male Wistar strain white rats. Red sprout leaf extract was obtained using the maceration process. Red shoot leaf extract contains anthocyanin compounds that can lower cholesterol, flavonoid compounds, and tannin compounds. his research is a purely experimental design with a before-and-after test with a control group using 30 rats divided into 5 groups, negative control (rats with hypercholesterolemia and Na-CMC 0.5%), positive controls (rats with hypercholesterolemia and 10 mg simvastatin), administration of red shoot leaf extract at extract dose 1 (200 Mg/Kg BW), extract dose 2 (250 Mg/Kg BW), and extract dose 3 (300 Mg/Kg BW). The total number of test animals used was 30 animals, each group consisted of 5 rats. The results of the ANOVA repeated measures test/Friedman test analysis showed that the activity of The Red shoot leaves *(Syzyginium myrtifolium Walp)* as a cholesterol-lowering agent for reducing total cholesterol levels was demonstrated with the potential efficacy in extract group 3 (300 mg/kg BW) with a significant value of 0.647.

Keywords: Red Shoot Leaf Extract, Cholesterol Total Levels, Male White Wistar Rats

1 Introduction

As many as 7.4 million people (42.3%) died from cardiovascular causes. One of the influencing factors is high blood cholesterol levels [1] Lipids are biomolecules that play an important role in cell membranes, energy sources, and signal activation. The classification of cholesterol consists of triglycerides and lipoproteins. while lipoproteins include very low-density lipoproteins (VLDL), low-density lipoproteins (LDL), intermediate-density lipoproteins (IDL), high-density lipoproteins (HDL), and total cholesterol (TC). One of the parameters for examining the risk of cardiovascular events is total cholesterol [2].

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Globally, there are 524 million people with cardiovascular disease, of which it is claimed that more than 18 million people per year become a global burden of disease, the cause of which is hypercholesterolemia [3]. While the prevalence of hypercholesterolemia in women is equal to 13.4% and in men that is 11.4% [4]. Data in Southeast Asia, Indonesia's total cholesterol prevalence ranks second after Malaysia [5.] Meanwhile, in Indonesia, the prevalence of cholesterol is 66.41%, which is found to be more than 50% in 18 of 20 provinces in Indonesia and 23 of 27 ethnic groups [6]. Central Java Province is included in the five highest provinces with the consumption behavior of fatty, cholesterol, and fried foods, namely 60.3% [7].

The most widely prescribed class of anti-cholesterol drugs was the statin group (88.16%) with the most widely used dosage forms being caplets (72.37%) [8] The statin-class drug that is often used is simvastatin [9] However, long-term use of statin-class drugs can increase the incidence of type 2 diabetes mellitus due to a decrease in insulin sensitivity [10], [11]. The Red shoot leaves (*Syzyginium myrtifolium Walp*) contain anthocyanin compounds with the highest average levels found in the shoot leaves, namely 257.83 mg/l - 49.08 mg/l [12].The mechanism of action of anthocyanins is by inhibiting the action of HMG-Co-A reductase (an enzyme that plays a role in the formation of cholesterol) [13]Anthocyanins can lower cholesterol levels by up to 13.6% [14].

The red sprouts contain phenols (81.14 mg/g), flavonoid antioxidants (69.43 mg/g), and tannin levels (55.98 mg/g) [15] Maceration is the best extraction method for anthocyanin compounds with ethanol HCL solvent [16] Maceration can avoid damage to thermolabile compounds [17] Which compounds are thermolabile such that the extract drying process operates at low temperatures [18]. The Hypercholesterol rats and 0.5% Na-CMC as a negative control, the hypercholesterol rats and 10 mg simvastatin as a positive control and administration of red shoot leaf extract at a dose of 200 mg/kg body weight (bw), 250 mg/kg bw, 300 mg/kg (BB) has the best effects on reducing blood cholesterol levels in male white rats at a dose of 200-300 mg/kg body weight [19] [20] Based on previous research, active compounds such as flavonoids and anthocyanins contained in red sprout leaves (*Syzyginium myrtifolium Walp*) have been found to have anti-cholesterol potential. However, no studies were found on the activity of red shoot leaf extract (*Syzyginium myrtifolium Walp*) as an anticholesterol agent. Therefore, this study was designed to determine the activity of red sprout extract (*Syzyginium myrtifolium Walp*) on reducing blood cholesterol levels in male white rats of the Wistar strain. Phytochemical screening was carried out to determine the content of secondary metabolites in red shoot leaf extract. The examinations carried out included: anthocyanin, flavonoid and tannin compound tests. The extract obtained from the extraction of 400 grams of red shoots leaf powder obtained 50.32 grams of thick reddish green extract. the results obtained are better and more than other herbal leaves.

2 Method

This research is a purely experimental design with a before-and-after test with a control group conducted at Al-Irsyad Cilacap University Pharmaceutical Chemistry, Pharmaceutical Technology and Pharmaceutical Technology Laboratory, Muhammadiyah University Clinical Laboratory, Purwokerto. Environmental Laboratory at the University of Jenderal Soedirman Purwokerto. This research will be conducted from January to June 2022 for 6 months.

2.1 Mice model

The male white rats of the Wistar strain were used in this study because they tend to be easily adapted, easier to handle and care for, have a high reproductive capacity, and male rats are more hormonally stable as they do not experience estrus and gestation periods which can affect research [21]. Mice were weighed with a body weight of ± 200-250 g, rats aged 4-5 months and randomly divided into 5 groups, negative control (rats with hypercholesterolemia and Na-CMC 0.5%), positive controls (rats with hypercholesterolemia and 10 mg simvastatin). , administration of red shoot leaf extract at extract dose 1 (200 Mg/Kg BW), extract dose 2 (250 Mg/Kg BW), and extract dose 3 (300 Mg/Kg BW). The total number of test animals used was 30 animals [19], [22].

2.2 Tools and materials

This research uses is a set of glassware (Pyrex), blender (Cosmos), digital analytical balance (Matrix AJ302B), spatula, probe, mortar and pestle, water bath (B-ONC), stirring stick, aluminum foil (fresh recommendation), filter paper, tissue (Amazing), sheath Hand (latex), label paper, hematocrit tubes (Marienfeld), vacutainer tubes (Vaculeb), centrifuge, photometer (Caretium NB-201 serial number 1100308), hypodermic syringe (OneMed), and rat rearing cages. The Red shoot leaves (Syzyginium myrtifolium Walp), 95% ethanol (CV. Kimia Jaya Labora), pellet (HI-PRO-VITE A594K), sawdust, distilled water, concentrated sulfuric acid, 2M NaOH, FeCl3, 2M HCl, 0.5% Na-CMC, Silica Gel Plate (TLC Plate), Acetic Acid, Dilute Ammonia, Hypercholesterolemic Feed (Duck Egg Yolk, Butter (Blue band) and Coconut Oil), Simvastatin 10mg (Kimia Farma), CHOD-PAP Reagent Kit (Good's Buffer, Phenol, 4-Ammoniakantipyrine, cholesterol esterase, cholesterol oxidase, peroxidase) (Grory®).

2.3 The research's procedures

1. Sampling, Extract and Testing Criteria for Identifying of Red Sprout Leaves (*Syzyginium myrtifolium Walp*)

Leaf samples of red shoots (*Syzyginium myrtifolium Walp*) from Pesanggrahan village, Kesugihan district, Cilacap regency, Central Java province. The red shoot leaves used as a sample were leaves already red at the tips, the leaves were then cleaned and washed under running water to be free from impurities. The washed leaves were then

dehydrated and air-dried. A whole plant identification test was then carried out, aimed at discovering the peculiarities of the true species of the Red Shoot Leaf plant (*Syzygin-ium myrtifolium Walp*). After that, the extract is obtained from red shoots that have been picked and cut into small pieces with scissors. Then 100 g of red sprout leaves are soaked in 500 ml of 95% ethanol solution in an Erlenmeyer flask and tightly covered with plastic film. Stored at 25 °C for 3 x 24 hours in a dark place, then filtered and the filtrate collected [12].

2. Phytochemical Screening of Red Bud Leaf Extract *(Syzyginium myrtifolium Walp)*

The leaf extract obtained from red sprouts (Syzyginium myrtifolium Walp) was then qualitatively tested for the presence of flavonoids, tannins, and anthocyanins.

Test of the flavonoids

0.5 ml is taken from the solution, and 5 ml of dilute ammonia and 5 ml of concentrated sulfuric acid are added. The presence of flavonoid compounds is indicated by a color change from greenish-yellow to yellow with the addition of concentrated sulfuric acid [23].

Tannin test

Put 0.5 g of the sample in a test tube, add 5 ml of distilled water and then mix with 2 drops of 1% FeCl3 solution. If the solution shows a dark blue or greenish-black color, this indicates the presence of tannins and polyphenols [24].

Anthocyanin test

Red sprout leaf extract was added with 2M HCl and then heated at 100°C for 5 minutes. A positive result is when the color changes to red. In addition, 2M NaOH was added dropwise while observing the color changes that occurred. A positive result is when a blue-green color slowly fades [25].

3. Preparation of a suspension of simvastatin and 0.5% Na-CMC

Simvastatin was weighed out at 13.5g, then gradually dissolved with 0.5% NaCMC until homogeneous and brought to a volume of 100 ml. Na CMC can improve quality as it has a binder, stabilizer, water retention, and thickening properties in products [26] Weigh 0.5 grams of Na CMC, then add 50 ml of hot water, stir until homogeneous, and gradually add hot water to a volume of 100 ml. Given orally as a negative control.

4. Preparation of experimental mice

The rat cages were sterilized by sun drying and fitted with sawdust as the base of the cage. Once the cage was sterile, the male rats were placed in the cage and paddle fed and drank sufficient distilled water. Then the rats were acclimated for 7 days. The purpose of acclimatization is for rats to adapt to their environment [27]. Cages are cleaned and sawdust mats are replaced every 3 days [22]. Thereafter, the rats were treated for hypercholesterolemia by feeding the rats a cholesterol-raising diet of duck egg yolk, butter, and coconut oil in a 1:1:1 ratio for 12 days [22].

5. Experimental animal treatment

Hypercholesterolemic male rats were randomized and divided into 5 treatment groups, and each treatment group consisted of 5 rats. The treatment groups are:

I: rats with hypercholesterolemia and 0.5% Na-CMC (negative control).

II: Hypercholesterolemic rats and simvastatin 10 mg (positive control).

III: Hypercholesterolemic rat and red shoot leaf extract 200 mg/kg body weight (BW).

IV: rats with hypercholesterolemia and 250 mg/kg BW red twig leaf extract.

V: Rats with hypercholesterolemia and 300 mg/kg BW red sprout leaf extract [19].

Red shoot leaf extract was given to hypercholesterolemic rats once a day for 12 days, and they continued to be fed pellets and enough to drink. The activity of male rats was observed. After the end of the administration of the ethanol extract of the leaves of red sprouts, a final examination of the total cholesterol level of the rats was carried out on the 13th day. This research was conducted to see the effectiveness of red germ leaf extract in reducing total cholesterol levels in hypercholesterolemic rats at different treatment doses [22].

6. The examination of total cholesterol levels in rats

The parameters measured in this study were the total cholesterol levels in male white rats of the Wistar strain after administration of an extract of red shoot leaves (*Syzygin-ium myrtifolium Walp*).

a. The rats fasted for ± 10-12 hours before the examination. In addition, rat blood was collected through the retroorbital plexus (eyes) up to 2 ml.
b. Blood samples were stored in Vacutainer tubes and separated between blood and serum using a centrifuge. The sample obtained in the form of plasma is then mixed with the reagent according to the kit instructions. Incubation was carried out at 37°C [28]. Serum was used to check cholesterol levels.
c. The serum was drawn down to 5 µl and then added to 500 µl of the CHOD-PAP reagent kit.
d. The serum was homogenized and incubated for 5 minutes. Before the examination, the photometer was first calibrated with a standard solution of 10 µL plus 1000 µL reagent and 500 µL blank [29].
e. The examinations were carried out on days 8, 20, and 32. Hypercholesterolemia is characterized by an increase in levels Blood cholesterol is higher than normal. In Wistar rats, normal blood cholesterol levels are 10–54 mg/dl [30].

7. Data analysis

The data obtained is in the form of phytochemical screening results presented in tabular form. The total cholesterol profile value is presented in terms of cholesterol values in mg/dl units. Evaluation of antihypercholesterol activity was analyzed using repeated measures ANOVA used Friedman test to determine if there was a significant effect of administration of red shoot leaf extract (*Syzyginium myrtifolium Walp*) to male white rats of the Wistar strain.

3 Result and Discussion

Table 1. The Examination results of total cholesterol levels in rats

	Negative Control	Positive Control	Extract Dose 1 (200 Mg/Kg BW)	Extract Dose 2 (250 Mg/Kg BW)	Extract Dose 3 (300 Mg/Kg BW)
Normal	57	46	23	69	29
	73	29	31	87	35
	89	61	24	70	26
	22	55	20	76	17
	67	81	34	79	73
Mean	61.6	54.4	26.4	76.2	36.0
Hypercho-	death	71	84	110	97
lesterol In-	94	77	97	71	126
duction	136	115	88	114	88
	144	66	79	102	79
	97	126	death	110	134
Mean	398.2	91.0	87	101.4	104.8
Treatments	death	16	33	24	42
	68	27	42	42	35
	87	20	46	35	26
	77	18	28	26	24
	63	40	death	34	34
Mean	33.7	24.2	37.2	32.2	32.2
Difference	death	55	38	86	55
	26	50	72	29	91
	49	95	56	79	62
	67	48	82	76	55
	34	86	death	76	100

Description: normal total cholesterol levels in rats 10-54 mg/dl [30] death: conditions when the rats were examined died. In Table 1, the results of an examination of cholesterol levels show that in the positive control group, the extract dose group 1, the extract dose group 2, and the extract dose group 3, there was a decrease in the total blood cholesterol level in rats. While in the negative control group, there was a decrease in cholesterol levels that should not have decreased in this group or the data remained stable. This can be affected by the incubation conditions, which include incubation time and temperature. In this study, it is suspected that an incubation temperature error occurred where the temperature was unstable during the incubation process [29] that incubation time and temperature affect the level of a given substance. An increase in temperature accelerates chemical reactions according to van't Hoff's law, which states that a 10 °C increase in temperature can double the reaction rate [31]. Two rats died during the research process. The deaths of the test animals were suspected to be due to an error in taking blood from the eye. The red shoot leaf extract (*Syzyginium myrtifolium Walp*) extract can reduce cholesterol in rats because red sprout contains anthocyanin compounds, which anthocyanin compounds have an anti-cholesterol mechanism by inhibiting the action of 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGCo-A reductase) so that the HMG changes Co-A to mevalonic acid as the first step in cholesterol synthesis [13].

Table 2. Repeated-measures ANOVA test results

Total Cholesterol Levels		Mean Difference	95% Confidence Interval For Difference		Sig.
			Min	Max	
Normal	Hypercholesterol Induction	-34.87	-34.875	-34.875	.023*
	Negative control	21.000	-12.755	54.755	.142
	Positive control	20.250	-18.740	59.240	.197
	Extract Dose 1 (200 Mg/Kg BW)	17.500	-21.178	56.178	.246
	Extract Dose 2 (250 Mg/Kg BW)	17.500	-27.591	62.591	.305
	Extract Dose 3 (300 Mg/Kg BW)	17.500	-25.883	60.883	.289
Hypercholesterol Induction	Hypercholesterol Induction	44.750	21.714	67.786	.009*
	Negative control	65.750	44.745	86.755	.002*
	Positive control	55.125	12.966	97.284	.025*
	Extract Dose 1 (200 Mg/Kg BW)	52.375	7.618	97.132	.034*
	Extract Dose 2 (250 Mg/Kg BW)	62.250	16.844	107.656	.022*
	Extract Dose 3 (300 Mg/Kg BW)	52.375	9.867	94.883	.030*
Positive control	Hypercholesterol Induction	-20.250	-59.240	18.740	.197
	Negative control	-55.125	-97.284	-12.966	.025*
	Positive control	-13.500	-37.404	10.404	.170
	Extract Dose 1 (200 Mg/Kg BW)	-17.000	-27.635	-6.365	.015*
	Extract Dose 2 (250 Mg/Kg BW)	-2.750	-9.940	4.440	.311
	Extract Dose 3 (300 Mg/Kg BW)	-2.750	-20.029	14.529	.647
Negative control	Extract Dose 1 (200 Mg/Kg BW)	36.500	26.996	46.003	.001*
	Extract Dose 2 (250 Mg/Kg BW)	42.000	36.336	47.663	.000*
	Extract Dose 3 (300 Mg/Kg BW)	42.000	22.598	61.401	.006*
Extract Dose 1 (200 Mg/Kg BW)	Extract Dose 2 (250 Mg/Kg BW)	5.500	-2.969	13.969	.130
	Extract Dose 3 (300 Mg/Kg BW)	5.500	-13.439	24.439	.423
Extract Dose 3 (300 Mg/Kg BW)	Extract Dose 3 (300 Mg/Kg BW)	0.000	-19.660	19.660	1.000

Note: *Indicates significant data or there is a significant difference.

The results of the repeated measures ANOVA analysis showed that there was a significant difference between the normal group and the induced hypercholesterolemia with a significant value of 0.023 < 0.05, which means that there was an effect high cholesterol diet with an average difference of -34.87 gave. this is consistent with the research [32] that duck egg yolk supplementation can increase cholesterol levels with LDL levels > 27.2 mg/dl. [22] also conducted research using foods with a mixture of duck egg yolk: butter: and coconut oil in a 1:1:1 ratio.

In Table 2, the results of the analysis of repeated measures ANOVA also show the activity of the leaf extract of red germ (*Syzyginium myrtifolium Walp*) in reducing the level of cholesterol in the blood of the rat considering the comparison between the positive control group and extract dose 1 (200 Mg/Kg BW), extract dose 2 (250 Mg/Kg BW) and extract dose 3 (300 Mg/Kg BW) or H0 was rejected and H1 was accepted, meaning that an effect of red shoot leaf extract (*Syzyginium myrtifolium Walp*) on lowering blood cholesterol levels in rats. The positive control with extract dose 1 (200 Mg/Kg BW) showed a significant difference with a significance value of 0.197, the positive control with extract dose 2 (250 Mg/Kg BW) showed no difference with a significance value of 0.311 and the positive control with extract dose 3 (300 Mg/Kg BW) showed no difference with a significance value of 0.647. extract dose 2 (250 Mg/Kg BW) and extract dose 3 (300 Mg/Kg BW) showed no significant difference, these groups had the same cholesterol-lowering potency as the positive control group, the best potency being shown in the extract dose 3 (300 Mg/Kg BW) with a significant value of 0.647. These results are consistent with research results [19] indicating that a dose of 300 mg/dl of Bb has the most effective effect since at this dose it has the maximum effect of inhibition of the action of the enzyme 3-hydroxy-3 - a. Methylglutaryl coenzyme A reductase (HMG Co-A reductase).

The Red shoot leaf extract may lower blood cholesterol levels in rats, likely because it contains anthocyanin compounds [33] which may lower blood cholesterol levels by interfering with the mechanism of action of the coenzyme 3-hydroxy-3-methylglutaryl - A-reductase (HMGCo-A reductase).), the conversion of HMG Co-A to mevalonic acid is therefore the first step in the synthesis of cholesterol [13]. The negative control group showed significant values of 0.001, 0.000, and 0.006 compared to extract dose 1 (200 Mg/Kg BW), extract dose 2 (250 Mg/Kg BW), and extract dose 3 (300 Mg/Kg BW), which means that CMC Na-Suspension has no cholesterol-lowering effect or activity. Red sprouts contain antioxidant compounds that the body needs to neutralize free radicals and prevent free radical damage to normal cells, proteins, and fats. Antioxidants protect cells against free radical damage by donating electrons to free radicals, thereby stabilizing and stopping the chain reaction, or by accepting an unpaired electron to stabilize free radicals and prevent damage to proteins, DNA, and lipids [34]. The presence of these free radicals leads to radical cell mutations and dysfunctions that cause cancers, neuronal diseases, liver diseases, and blood vessel diseases such as coronary heart disease, diabetes, cataracts, and premature aging, and trigger other chronic diseases. Free radicals are atoms or compounds that contain one or more unpaired electrons. The most dangerous compound in free radicals is hydroxyl (OH) because it has the highest reactivity. The molecule is very reactive to finding a pair of electrons. When it forms in the body, a chain reaction occurs and produces new free radicals, which

eventually form free radicals in large quantities [35]. Natural antioxidant compounds present in red sprouts include vitamin C, vitamin E, carotenoids, phenolic compounds, and polyphenols, which can be in the form of flavonoids, cinnamic acid derivatives, coumarins, tocopherols, and organic acids. polyfunctional. Flavonoids with antioxidant activity are flavones, flavonols, isoflavones, catechins, flavonols, and chalcones, while cinnamic acid derivatives include caffeic acid, ferulic acid, and chlorogenic acid. The antioxidant effect of flavonoids is based on the -OH group and the double bond (>C=C). Red sprouts are known to be rich in flavonoids, one of which is the compound dimethyl cardamonine (2',4'-dihydroxy-6'-methoxy-3', 5'-dimethyl chalcone), a chalcone group with cytotoxic properties. Red sprouts also contain cyanidin glycoside compounds, an anthocyanin compound that has antioxidant properties and acts as an antidote to free radicals, which act as antioxidants in the body [34]. The function of anthocyanin as an antioxidant in the body can prevent and inhibit the process of atherogenesis by oxidizing the bad fats in the body, namely low-density lipoprotein or low-density lipoprotein (LDL). Anthocyanins also protect the integrity of endothelial cells that line the walls of blood vessels to prevent damage. Damage to endothelial cells is the start of atherosclerosis formation and should therefore be avoided. Moreover, anthocyanins also relax blood vessels to prevent atherosclerosis and other cardiovascular diseases [35]. Compounds derived from flavonoids, including chalcone (C15H12O) or benzylidene acetophenone, are herbal compounds derived from flavonoid and isoflavonoid compounds. Chalcone contains two aromatic rings and one unsaturated carbon atom. Ring A contains ethyl, methyl, or alkyl groups which can increase activity, and ring B contains hydrophobic groups such as halogens, nitro, and cyano which can also increase activity. It has been observed that the presence of keto groups and vinyl groups in chalcone has the function of increasing the activity as an antioxidant, chalcone has antihyperglycemic activity and plays an important role in lowering cholesterol [34].

4 Conclusion

The based on the research results, the activity of The Red shoot leaves (*Syzyginium myrtifolium Walp*) as a cholesterol-lowering agent for reducing total cholesterol levels was demonstrated with the potential efficacy in extract group 3 (300 mg/kg BW) with a significant value of 0.647.

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