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Antihyperlipidemic Activity of Trembesi Leaf Extract (*Samanea saman*) Against Total and LDL Cholesterol Levels in Hypercholesterolemic Mice

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ABSTRACT

The potential of the trembesi plant (*Samanea saman*) which is used for traditional medicine includes antibacterial, analgesic, treating headaches and diarrhea [1]. Trembesi leaves are known to have several chemical compounds such as flavonoids, alkaloids, tannins, saponins, and steroids or triterpenoids which are thought to have activity in reducing total cholesterol levels [2]. The aim of this study was to determine the activity and optimal dose of trembesi leaf extract which can reduce total and LDL cholesterol levels in hypercholesterolemic mice. Research method: 25 test mice were divided into 5 groups consisting of group 1 given CMC-NA 1% negative control, group 2 given simvastatin 10 mg positive control, groups 3, 4 and 5 were given extract treatment trembesi leaves with doses of 35 mg/KgBW, 175 mg/KgBW and 350 mg/KgBW. The mice tested were given atherogenic feed and then examined for total and LDL cholesterol levels using the CHOD PAP enzymatic method. Data analysis used the one-way ANOVA test, followed by the post hoc test, namely Duncan's test. Results: Ethanol extract of trembesi leaves at a dose of 175 mg/KgBW can reduce total cholesterol levels by 37.07% and LDL by 50.24%. Conclusion: ethanol extract of trembesi leaves at a dose of 175 mg/KgBW provides an optimal effect on reducing total and LDL cholesterol levels ($p<0.05$).

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1. INTRODUCTION

Hypercholesterolemia is a condition where the total plasma cholesterol level exceeds normal, namely ≥ 240 mg/dL and occurs because it is influenced by several factors, namely genetic factors, gender, age, and diet [3]. The prevalence of hypercholesterolemia in the world reaches 45%, Southeast Asia reaches 30% and Indonesia reaches 35% [4]. As time goes by, humans discover the benefits of plants in the field of medicine and are passed down from generation to generation, so humans choose medicinal plants around forest areas to be used as raw materials for medicines [5]. The use of plant materials as traditional medicine has been used in medicine and is growing until now. The existence of complementary or synergistic effects in traditional ingredients to support the activities that are raised, minimal side effects and use which are considered relatively safer are the advantages of using natural ingredients. However, the low pharmacological effect due to complex active compounds and unstandardized raw materials is one of the challenges in the development of natural medicines.

One of the potential plants is the trembesi plant (*Samanea saman*) which is used as an antiseptic [6], antibacterial [7], antidiabetic [8], and antifungal [9]. The compounds known to be present in trembesi plants are flavonoids, tannins, steroids, saponins, and terpenoids [10]. In trembesi leaf extract 600 mg/kg BW it was found that there was a good analgesic activity of 63.4% [2], while research on the n-hexane fraction of trembesi leaves 500 mg/kg BW showed good analgesic activity in test animals and was declared safe in the acute toxicity test [11]. Research on hepatoprotective shows the hepatoprotective activity of methanol extract of *S. saman* against albino rats with liver damage induced by carbon tetrachloride. 400 mg/kg body weight of methanol extract was

administered to rats and it reduces the serum level of ALT, AST, and cholesterol [8] but testing for anti hyperlipidemia activity in trembesi plants has not been carried out. Flavonoid compounds have a mechanism of action that can reduce total cholesterol levels by inhibiting HMG-CoA reductase which causes a decrease in cholesterol synthesis and increases the number of LDL receptors present in the hepatic cell membranes and extrahepatic tissues so that total cholesterol levels will decrease [12]. This shows the strong potential of the trembesi plant as an antihyperlipidemic.

The community has used the trembesi plant by consuming the trembesi seeds by roasting them first, but the rest of this plant is not used more optimally, because until now there has been no research on the hyperlipidemic activity of the trembesi plant, so researchers feel interested in researching the activity of trembesi. antihyperlipidemic in ethanol extract of trembesi leaves against white rats. The purpose of this study was to determine the dose of trembesi leaf extract which has optimal anti hyperlipidemia activity, so that from this study a candidate for an antihyperlipidemic drug based on herbs was obtained which was safe for consumption by the public.

2. METHOD

Animal

Swiss albino mice (18-25g) were maintained at the Pharmacology Laboratory and then the research was carried out at the Pharmaceutical Biology Laboratory, Bhakti Wiyata Kediri Institute of Health Sciences, Madiun Healthy Pet Clinic and Persada Madiun Laboratory. For 7 days, the mice were given standard feed and drinking water, after which the mice were weighed to determine their weight before being given hypercholesterolemia feed. Atherogenic feed is prepared by mixing 100 g of goat fat, 50 g of chicken egg yolk and corn rice. Goat fat is heated first until it melts and the egg yolks that have been boiled are taken, then mixed with corn rice up to 1000 g [13].

Plant Material and Extraction

The plant material was collected and determination of the trembesi plant (*Samanea saman*) was carried out at the Materia Medica Indonesia Herbal Laboratory, Batu City, Malang. As much as 500 grams of trembesi leaf simplicia powder was macerated using 70% ethanol with a ratio of 1: 7.5 for 5 days then remaceration was carried out.

Phytochemical Screening Test

The alkaloid screening test was carried out by putting 1 mL of sample and adding 5 drops of ammonia, then shaking and filtering. The filtrate was added 2 mL of 2 N sulfuric acid and shaken. The solution is divided into 3 and added 1 drop of Wagner reagent, Mayer reagent, and Dragendorff reagent. A positive Wagner result is indicated by a brown precipitate, Mayer (white precipitate), and Dragendorff (orange precipitate). The flavonoid screening test was carried out by placing 1 mL of the sample and adding concentrated hydrochloric acid (HCl) and magnesium powder. A positive result is indicated by the formation of red, orange, or green colors. In the tannin screening test, 1 mL of the sample was added with 2 mL of distilled water and boiled, then the filtrate was added with 1% FeCl3 solution. A positive result is indicated by the formation of a black-green color.

Testing the content of saponin compounds was carried out by inserting 1 mL of the sample and adding 2 mL of distilled water, then heating it for 2-3 minutes after it was cold, shaking vigorously. The presence of stable foam for 30 seconds indicates the presence of saponins. In the steroid test, 1 mL of the sample was added with Lieberman-Burchard reagent then shaken slowly and let stand. The formation of blue or green color indicates the presence of steroids [14].

Antihyperlipidemia Test

A total of 25 mice test animals were acclimated for 7 days given food and water which were then weighed to determine their weight before being given a high-fat diet. Feeding a high-fat diet for ± 7 to 14 days then observed total cholesterol levels and LDL levels in mice. Group 1 was given CMC-NA 1% negative control, group 2 was given simvastatin 10 mg positive control, groups 3, 4 and 5 were given trembesi leaf extract treatment at doses of 35 mg/KgBW, 175 mg/KgBW and 350 mg/KgBW. On day 14 of the atherogenic feed treatment, blood samples were taken to determine total and LDL cholesterol levels in mice [13].

Data analysis

The total cholesterol and LDL levels of the test animals were analyzed using descriptive (qualitative) and quantitative data, while the percent reduction in cholesterol obtained was tested using SPSS with the One Way ANOVA method if the data is normally distributed. Meanwhile, use the Mann-Whitney method if the data is not normally distributed.

3. RESULTS AND DISCUSSION

Extraction and phytochemical screening

Trembesi leaf simplicia (500 gram) was macerated with 70% ethanol to obtain an extract weight of 65.32 g with a yield of 13.06%. The results of the phytochemical screening showed that trembesi leaf extract contained alkaloids, flavonoids, tannins, saponins, and steroids/triterpenoids. From the results of the phytochemical screening, the following results were obtained :

Table 1. Result of the phytochemical screening of trembesi leaf extract

Test	Procedure	Result	Note
Alkaloid	Extract + HCl 1% + mayer, wagner, and dragendroff reagent [15]	Red-orange precipitate	(+)
Flavonoid	Extract + Mg powder + HCl concentrated [15]	Yellow, orange or red	(+)
Tanin	Extract + boiled aquadest + 1-2 drop of $FeCl_3$ 1% [14]	Blackish green	(+)
Saponin	Extract + heated aquadest was shaken vigorously [14]	Foam was formed	(+)
Streroid or Triterpenoid	Extract + Leibermann-Burchard reagent or H_2SO_4 [15]	Blue for steroid, red pr purple for triterpenoid	(+)

Cholesterol total decrease levels were obtained from the results of the initial data (T0) and the final results (T1) using the Elisa test *in vitro* by testing the blood serum of the test animals. The results of the test for reducing total cholesterol levels of trembesi leaf extract can be seen in table 2.

Table 2. Decrease cholesterol total level

Group	$\overline{X}$ T0 (mg/dL)	$\overline{X}$ T1 (mg/dL)	$\overline{X}$ Δ Decrease cholesterol total level (mg/dL)	% Decrease cholesterol total level (mg/dL)
Positive control simvastatin 10 mg	148,6	92,6	56	37,68 %
Negative control CMC Na 1%	117,4	103,4	14	11,92 %
Group 3	153,2	101	52,2 [a]	34,07 %
Group 4	148,2	93,2	55 [a]	37,11 %
Group 5	124,4	83	41,4 [a]	33,27 %

All the value compare with control positif and did not have a significant difference [a]$P<0.05$

Information :

T0 : before treatment with extract

T1 : after treatment with extract

Group 3 : 35 mg/KgBW

Group 4 : 175 mg/KgBW

Group 5 : 350 mg/KgBW

LDL decrease levels were obtained from the results of the initial data (T0) and the final results (T1) using the Elisa test *in vitro* by testing the blood serum of the test animals. The results of the test for reducing LDL levels of trembesi leaf extract can be seen in table 3.

Table 3. Decrease LDL level

Group	$\bar{X}$ T0 (mg/dL)	$\bar{X}$ T1 (mg/dL)	$\bar{X}$ Δ Decrease LDL level (mg/dL)	% Decrease LDL level (mg/dL)
Positive control simvastatin 10 mg	99	40,6	58,4	58,98 %
Negative control CMC Na 1%	76,8	59	17,8	23,17 %
Group 3	123	61,2	61,8 [a]	50,24 %
Group 4	117,8	52,2	65,6 [a]	55,68 %
Group 5	59,4	32,8	26,6 [a]	44,78 %

All the value compare with control positif and did not have a significant difference [a]P<0.05
Information :

T0 : before treatment with extract
T1 : after treatment with extract
Group 3 : 35 mg/KgBW
Group 4 : 175 mg/KgBW
Group 5 : 350 mg/KgBW

Table 2 and 3 showed the result on the decrease in cholesterol total and LDL levels with an effective dose of 175 mg/KgBW. This condition is caused by the many components of different chemical compounds found in natural products so these components work together to create an effect. But with increasing doses, the number of chemical compounds contained increases, causing effects that are no longer linear and can reduce the expected effect or there is toxicity in the test animals because there are many compounds in the extract. Besides this, it can also be caused by a limited number of receptors, thereby limiting the effects [16].

The alleged activity of the compounds in trembesi leaf extract is flavonoid compounds with a mechanism of action that is suspected to be the same as simvastatin, which can reduce total cholesterol levels by inhibiting HMG-CoA reductase which causes a decrease in cholesterol synthesis and increases the number of LDL receptors present in cell membranes liver and extrahepatic tissue so that total cholesterol levels will decrease [12]. Alkaloid compounds work as antioxidants by donating hydrogen ions such as flavonoids. These compounds can also inhibit pancreatic lipase enzyme activity thereby increasing fat secretion through the feces; As a result, the absorption of fat by the liver is hampered so that it cannot be converted into cholesterol [17].

Tannin compounds are divided into two groups, hydrolyzable tannins and condensation tannins. These substances are used to lower blood glucose levels by metabolizing glucose and fat so that calorie deposits can be avoided [17]. Tannins inhibit the absorption of fat in the intestine by reacting with mucosal proteins and intestinal epithelial cells. In addition, tannins can precipitate protein mucosa on the surface of the small intestine thereby reducing the effectiveness of cholesterol and fat absorption. And triterpenoid/steroid compounds work as antioxidants with a primary antioxidant mechanism of action, namely being able to reduce the formation of new free radicals by breaking chain reactions and turning them into more stable products. Terpenoids can reduce cholesterol levels by inhibiting the enzyme 3-hydroxy-3-methylglutaryl (HMG-CoA) reductase which is an enzyme in cholesterol synthesis[18].

4. CONCLUSION

Based on the results of the study, it can be concluded that the most effective dose of trembesi leaf extract in reducing total cholesterol and LDL levels was a dose of 175 mg/KgBW (p<0.05). However, an increase in doses above this level carries the risk of generating a non-linear response and potential toxicity in test animals due to an excess of compounds in the extract, as well as limitations in effects possibly caused by receptor restrictions. The compound activities within trembesi leaf extract encompass flavonoid actions similar to simvastatin in inhibiting HMG-CoA reductase, alkaloids functioning as antioxidants and inhibiting pancreatic lipase enzymes, tannins impacting glucose

reduction and intestinal fat absorption, while triterpenoid/steroid compounds act as primary antioxidants and inhibit HMG-CoA reductase enzyme to lower cholesterol synthesis.

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