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Research Article

Effectiveness of Ethanol Extract Dosage of Arabian Bidara Leaf (*Zizphus spina-christi* (L.) Desf.) on Reduction of Hypercholesterolemia in *Mus musculus*

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ABSTRACT

Hypercholesterolemia is one of the main risk factors for cardiovascular disease, which is characterized by increased cholesterol levels in the blood. The leaves *of Ziziphus spina-christi* (Arabian bidara) are known to contain flavonoids, saponins, tannins, and alkaloids that have the potential to provide antihypercholesterolemia effects. This study aimed to evaluate the effectiveness of ethanol extract of *Z. spina-christi* leaves on the reduction of total cholesterol in hypercholesterolemia mice. Extraction is carried out by the maceration method using 96% ethanol and produces a yield of 3.8%. A total of 36 male mice were divided into six groups (n=6): negative control, positive control (propylthiouracil induction), drug control (simvastatin), and three extract treatment groups with doses of 14, 28, and 56 mg/20 g BB. The treatment was given orally for four days, then cholesterol levels were analyzed using the enzymatic method CHOD-PAP. The results showed that the positive control cholesterol levels increased significantly (189.3 ± 6.2 mg/dL) compared to the negative control (112.7 ± 10.3 mg/dL). The administration of the extract lowered cholesterol levels gradually: 170.4 ± 6.3 mg/dL (10%), 159.6 ± 7.6 mg/dL (15.7%), and 147.3 ± 11.7 mg/dL (22.2%). Simvastatin decreased more (117.7 ± 12.5 mg/dL; 37.8%). It was concluded that bidara arabic leaf extract has antihypercholesterolemia potential with a multifactorial mechanism, although its effectiveness is lower than that of simvastatin.

Keywords: *Ziziphus spina-christi*; hypercolesterolemia; flavonoid; saponin; antihypercolesterolemia

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INTRODUCTION

Hypercholesterolemia, also known as hyperlipidemia, is a medical condition characterized by an increase in Low-Density Lipoprotein (LDL) (normal value < 100 mg/dl), triglycerides (normal value < 150 mg/dl), phospholipids, plasma lipids, and cholesterol levels in the blood exceeding normal limits (normal value < 200 mg/dl) or significantly increasing (Ayunda and Malita 2024). 69.6% of people in Indonesia who are 15 years old or older have high cholesterol levels. This condition can occur even at a young age, where from the age of 20 years and above, cholesterol levels in both men and women tend to increase. The increase is influenced by various risk factors, including lack of physical activity, unhealthy diets such as consumption of high-fat fast food, excessive cholesterol intake accompanied by low fiber, smoking habits, excessive alcohol consumption, stress, and hypertension. These risk factors can contribute to the occurrence of hypercholesterolemia (Yuningrum et al. 2022).

Several previous studies have shown that the leaves of *Ziziphus spina-christi* L. are detected to contain all five secondary metabolite compounds, namely alkaloids, saponins, steroids, flavonoids, and tannins Hastiana et al. (2022), which have the potential to provide antihypercholesterolemia effects. According to Ngibad et al. (2025), *Ziziphus spina-christi* L. leaf extract can provide a significant reduction in cholesterol due to its flavonoid content. In line with research Abdulrahman et al. (2022) showed that administration of *Z. spina-christi* leaf extract can improve liver and kidney function and decrease lipid peroxidation in male rats with hypercholesterolemia.

Although preliminary data show the potential of anticholesterolemia, research on the effectiveness of *Z. spina-christi* extract doses against hypercholesterolemia is still very limited. Many studies only consider one dose without comparing effective dose variations. In addition, the use of animal models induced with propylthiouracil has not been widely applied in the evaluation of antihypercholesterolemia. This creates an important gap in determining the optimal, effective, and safe dosage.

This study aims to evaluate the effectiveness of various doses of ethanol extract of *Ziziphus spina-christi* (L.) Desf. leaves in reducing hypercholesterolemia in mice (*Mus musculus*). The novelty of the study lies in the use of several comparatively compared doses and the application of an animal model of propylthiouracil-induced hypercholesterolemia, a step that was rarely done before. Thus, this study is expected to make a scientific contribution to the development of safe and effective herbal therapy as an alternative to the treatment of hypercholesterolemia.

MATERIALS AND METHODS

Materials

The materials used in this study included *Ziziphus spina-christi* L. (Arabic bidara) leaf extract obtained from Situbondo, East Java, Indonesia; 96% ethanol as the extraction solvent; simvastatin as the reference antihypercholesterolemic drug; propylthiouracil as the hypercholesterolemia-inducing agent; distilled water (aquadest); whole blood samples without anticoagulant; cholesterol reagent kits (Glory Diagnostic); and male mice (*Mus musculus*) used as experimental animals..

Methods

Extraction Ziziphus spina-christi L.

The extraction process of bidara arabic leaves is carried out using the maceration method with 96% ethanol solvent. A total of 500 grams of Arabic bidara leaf powder is put in a closed container for the maceration process. Next, 2,500 ml of 96% ethanol is added until the entire powder is submerged (Nabella et al. 2025). The maceration process is carried out for three days (72 hours) at room temperature, with a material-to-solvent ratio of 1:5, and stirred periodically to ensure optimal extraction. After the maceration process is complete, the mixture is filtered to obtain filtrate, which is then evaporated to produce a concentrated extract (Hamka and Muis 2025). The thickened extract is then weighed using an analytical scale.

Test Animal Preparation

The experimental animals used were male mice (*Mus musculus*) weighing 20–30 grams and 2–3 months old (Wahyuni et al. 2023), as many as 36 heads. Mice are kept in cages of adequate size to provide room for movement and prevent stress. The cage is made of safe and non-toxic materials and is equipped with husks as a base that is cleaned regularly to prevent the spread of disease. The ventilation of the cage is maintained so that air circulation is good so that there is no accumulation of heat or excess humidity. Drinking water is available ad libitum, while feed is given according to nutritional needs, which is about 5 grams per head per meal (Muharni et al. 2023). The cage is also equipped with a secure and sturdy cover to prevent accidents and keep mice from escaping.

Hypercholesterolemia Activity Testing

Before treatment, male mice are acclimatized for 7 days to adapt to the environment in an animal facility with ad libitum access to food and drinking water (Bobaya et al. 2023), and then they are randomized into six groups (n = 6/group) consisting of negative controls (only fed and drank), a positive control (propyltiouracil induction), a drug control group (simvastatin), and three groups of extract treatments with doses of 14, 28, and 56 mg/20 g BB suspended in Na-CMC. The extract is administered orally once a day in a volume of 0.5 mL. After induction, the rats were fasted for 12 hours, and then blood was taken through the coccygeus vein of the tail to confirm an increase in total cholesterol levels. Furthermore, treatment was continued in the simvastatin group and the extract dose group from day 23 to day 26 to assess the reduction in cholesterol levels. At the end of treatment, blood is taken back through the caudal vein of the tail, centrifuged at 3000 rpm for 10 minutes to obtain serum, and total cholesterol levels are analyzed using the Cholesterol Oxidase-Para Aminophenazone (CHOD-PAP) enzymatic method with a spectrophotometer according to the kit instructions.

Animal Cholesterol Level Measurement Test

The CHOD-PAP method is used to measure the blood cholesterol levels of mice after the process of hydrolysis and oxidation. The inspection procedure begins with the preparation of tools and materials. Blood sampling is then carried out by massaging and disinfecting the tail of the mouse. After that, a cut along the 0.2 cm mark is carried out to accommodate the blood in the serum tube, which is then centrifuged for 15 minutes at 3,500 rpm to form serum. Next, the reagents and samples are homogenized and incubated for 10 minutes at a temperature of 20–25°C, according to the predetermined volume for blank, standard, and test samples. Total cholesterol levels were measured using a spectrophotometer at a wavelength of 546 nm. Interpretation of results refers to normal cholesterol levels in mice, which range from 40 to 130 mg/dL.

RESULTS

Extraction *Ziziphus spina-christi* L.

Table 1. Extraction results *Ziziphus spina-christi* L.

Sample (g)	Extract Weight (g)	Result (%)
500	19.1	3.8

Table 1 shows the results of the extraction of *Z. spina-christi* leaves by the maceration method in the form of the weight of dry extract from 500 grams of leaf powder. The yield value is calculated based on the comparison between the weight of the extract and the initial sample.

Cholesterol Reduction in Male Mice Given *Ziziphus spina-christi* L Leaf Extract.

Table 2. Cholesterol level test of male mice given ethanol extract of *Z.spina-christi* L leaves.

Test Animals	Negative Control	Positive Control	Drug Control	Dose 14 mg/20g BB	Dose 28 mg/20g BB	Dose 56 mg/20g BB
1	120.9	191.2	130.6	165.4	162.1	159.3
2	125.5	189.3	128.5	167.2	160.5	143.7
3	102.4	196.5	120.6	171.8	153.2	133.5
4	99.3	194.7	117.5	178.4	152.7	138.3
5	116.8	183.7	96.2	176.9	173.1	163.1
6	111.5	180.4	112.5	162.8	155.8	146.0
Average	112.7	189.3	117.7	170.4	159.6	147.3

The values in Table 2 are presented as the average ± standard deviation (SD) in mg/dL with the number of test animals six per group (n = 6). The negative control group was normal mice without hypercholesterolemia induction. Meanwhile, the positive control group was mice induced by hypercholesterolemia without being given treatment. The drug control group consisted of mice induced hypercholesterolemia and then given standard drugs (e.g. simvastatin with a specific dose and route of administration), while dose groups 1, 2, and 3 were mice that were induced and subsequently given ethanol extract of *Z. spina-christi* leaves with consecutive doses of 14 mg/20 g BB, 28 mg/20 g BB, and 56 mg/20 g BB.

Table 3. Percentage Decrease in Cholesterol Levels in Male Mice Compared to Positive Control

Group	% Decrease vs Positive Control
Negative Control	40.4
Drug Control	37.8
Dose 14 mg/20g BB	10.0
Dose 28 mg/20g BB	15.7
Dose 56 mg/20g BB	22.2

Table 3 of the data shows the average percentage decrease in cholesterol levels compared to the positive control group. Percentages are calculated using the formula (Chaerunnisa and U.S 2021). The number of test animals in each group was six (n = 6). Values in the negative control group were only shown as physiological comparisons, not as a result of treatment-induced decline.

Table 4. Total Cholesterol Levels of Male Mice After Treatment of *Ziziphus spina-christi* L Leaf Ethanol Extract.

Group	Mean ± SD (mg/dL)
Negative Control	112.7 ± 10.3
Positive Control	189.3 ± 6.2
Drug Control	117.7 ± 12.5
Dose 14 mg/20g BB	170.4 ± 6.3
Dose 28 mg/20g BB	159.6 ± 7.6
Dose 56 mg/20g BB	147.3 ±11.7

The values in Table 4 are presented as the average ± standard deviation (SD) in mg/dL with the number of test animals of six per group (n = 6). Measurements were taken at three time points, namely baseline (before the induction of hypercholesterolemia), post-induction (after the administration of the inducing agent), and post-treatment (after the administration of extracts or medications). Inter-group comparisons were analyzed using the standard deviation (SD) calculation formula (Rahmanto 2024).

DISCUSSION

The extraction process using the maceration method with ethanol solvent produced a yield of 3.8% from 500 grams of dry leaf powder. This value indicates that from several initial samples, only a small fraction of the components can be extracted in the form of dry extracts. Yield is affected by a variety of factors, including solvent type, compound polarity, extraction time, temperature, and simplicia particle size (Wijaya et al. 2022). Ethanol was chosen because it has semipolar properties Nofita et al. (2022), so that it is able to attract bioactive compounds such as flavonoids, saponins, tannins, and alkaloids that are suspected to play a role in antihypercholesterolemia activities. According to Sakalaty et al. (2024), the process of dissolving chemical compounds from the sample using an appropriate solvent. The yield value of 3.8% can still be categorized as reasonable compared to the research of Putri et al. (2023), regarding leaf extraction by a similar method. The higher yield generally indicates good extraction efficiency, but more importantly, it is the phytochemical content in it that determines biological activity.

The data in Tables 2 and 4 show that the positive control group had an average total cholesterol level of 189.3 ± 6.2 mg/dL, higher than negative controls (112.7 ± 10.3 mg/dL). This difference proves that the induction of hypercholesterolemia was successful and that animal models are suitable for assessing cholesterol-lowering activity. The variation in standard deviation (SD) values in the positive control group was relatively small, which indicates induction had a consistent effect across test animals. Thus, the difference in cholesterol levels in the treatment group can be interpreted as an effect of the administration of drugs or extracts, not as a failure of the animal model.

Administration of *Z. spina-christi* leaf extract showed a dose-dependent trend of decreasing cholesterol levels. At a dose of 14 mg/20 g BB, cholesterol levels were still relatively high (170.4 ± 6.3 mg/dL) with a percentage reduction of only 10% compared to positive controls. Increasing the dose to 28 mg/20 g BB resulted in a greater reduction (159.6 ± 7.6 mg/dL, a 15.7% decrease), and the highest dose of 56 mg/20 g BB had the most noticeable effect (147.3 ± 11.7 mg/dL, a 22.2% decrease). These results show a dose-response relationship, where the higher the dose of the extract, the greater the reduction in cholesterol levels achieved (Ulfiah et al. 2020).

When compared to control drugs (e.g., simvastatin), the effect of the extract is still lower. Drug control lowered cholesterol levels to 117.7 ± 12.5 mg/dL (37.8% decrease), almost equivalent to normal group cholesterol levels. This confirms that although *Z. spina-christi* leaf extract has the potential to lower cholesterol, its effectiveness still does not match that of standard drugs that have been clinically proven.

The cholesterol-lowering effect of *Z. spina-christi* leaf extract is most likely due to its phytochemical content (Abdulrahman et al. 2022). Flavonoids act as antioxidants that can inhibit LDL oxidation, thereby preventing atherosclerosis (Ciumărnean et al. 2020; Sun et al. 2022). Saponins are known to bind bile acids in the intestines, thereby increasing their excretion, which in turn forces the liver to use cholesterol (Cao et al. 2024), for the synthesis of new bile acids so that plasma cholesterol levels decrease. Tannins can inhibit the absorption of lipids in the gastrointestinal tract (Cosme et al. 2025). While alkaloids have the potential to modulate lipid metabolism. The combination of the activity of these compounds allows the extract to provide a multifactorial cholesterol-lowering effect (Zhang et al. 2024). These findings are in line with several previous studies that reported hypolipidemic activity in *Z. spina-christi* extract and other herbal plants rich in flavonoids and saponins.

The results of this study are in line with the report by Chaerunnisa & U.S. (2021), which states that the administration of saponin-rich leaf extract can reduce total cholesterol levels in hypercholesterolemia animal models. However, the percentage reduction in this study was still lower than in some other studies that used larger doses or different extraction methods. This shows that the factors of extraction method, dose, and duration of administration affect the effectiveness of the extract. Thus, although this study has succeeded in proving the potential of *Z. spina-christi* leaf extract as an antihypercholesterolemia, optimization of dosage and extraction method needs to be done to obtain more optimal results.

CONCLUSIONS

Z. spina-christi leaf ethanol extract has been shown to be able to reduce total cholesterol levels in hypercholesterolemia mice in a dose-dependent manner. The highest dose (56 mg/20 g BB) provided a 22.2% reduction, although the effect was still lower than that of simvastatin. This antihypercholesterolemia activity is suspected to come from the synergy of flavonoids, saponins, tannins, and alkaloids. The results of this study support the potential use *of Z. spina-christi* as an alternative herbal therapy for hypercholesterolemia, but more research is needed for dose optimization, safety, and effectiveness.

ETHICAL ISSUES

The UNUSA Health Research Ethics Committee approved the animal testing protocol with approval number 0063/EC/KEPK/UNUSA/2024.

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