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Anticholesterol Activity of Combined Binahong and Keji Beling Leaf Extracts in Mice (*Mus musculus*)

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ABSTRACT

Cholesterol is a type of lipid found in the human body, with approximately 80% produced endogenously by the liver and the remaining 20% obtained from dietary sources. Elevated total cholesterol levels in the blood increase the risk of various health conditions such as cardiovascular disease, obesity, hypertension, and even stroke. Prolonged elevation can lead to plaque formation in blood vessels, resulting in impaired circulation and potentially life-threatening complications. The use of synthetic cholesterol-lowering drugs is often associated with unwanted side effects, including liver toxicity, muscle pain, and gastrointestinal disturbances. This has prompted increasing interest in safer, natural alternatives such as herbal medicine, which is perceived to provide fewer side effects and additional health benefits. This study aimed to investigate the anti-cholesterol activity of a combination of Binahong (*Anredera cordifolia*) and Keji Beling (*Strobilanthes crispus*) leaf extracts in mice (*Mus musculus*) induced with a high-cholesterol diet. The extraction method employed was maceration, using 96% ethanol as the solvent to ensure optimal phytochemical yield. The experimental design consisted of five groups: a negative control group, a positive control group, and three treatment groups receiving combinations of Binahong and Keji Beling extracts in ratios of 1:1, 1:3, and 3:1. Total cholesterol levels were measured using the Point of Care Test (POCT) method. The results showed that all treatment groups experienced a significant reduction in total cholesterol levels ($p = 0.001 < 0.05$). The most effective reduction was observed in the 1:1 ratio group, with a decrease of 45.39%, followed by the 3:1 group (27.80%) and the 1:3 group (14.17%). These findings indicate strong potential for development as an effective and safe herbal alternative for lowering cholesterol levels.

Keywords: Cholesterol, Binahong, Keji Beling, Herbal Medicine

Introduction

Cholesterol is a lipophilic molecule that contributes to the proper functioning of cells. Cholesterol is transported through the blood, along with triglycerides, inside lipoprotein particles (HDL, LDL, VLDL, and chylomicrons) (Huff *et al.*, 2025). Primary sources of cholesterol in foods include meat, poultry, eggs, dairy, and seafood (Dinh *et.*, 2016). Cholesterol is a fat-like substance found in the body. 80% of cholesterol is produced by the liver, while the remaining 20% comes from outside the body, through the food we eat (Utama & Indasah, 2021). The prevalence of hypercholesterolemia remains quite high. Currently, about 45% of people worldwide have hypercholesterolemia, including 30% in Southeast Asia and 35% in Indonesia. Hypercholesterolemia causes around 2.6 million deaths and 29.7 million disabilities each year (Uda'a *et al*., 2023). In Indonesia, 47.1% of people aged 3 years and older consume fatty foods more than once a day. High cholesterol intake can affect lipid profiles, increasing total cholesterol, triglycerides, and LDL, while decreasing HDL. Nearly 28.8% of Indonesian adolescents have abnormal cholesterol levels (Badan Litbangkes, 2018). This rising trend emphasizes the importance of effective management strategies to control cholesterol levels and prevent long-term complications.

Various treatment approaches can be used, including synthetic or herbal medications. Commonly used cholesterol-lowering synthetic drugs include statins, terbinafine, bile acid-binding resins, ezetimibe, and lapaquistat acetate (Basak, 2025). However, medical treatment with synthetic drugs has many side effects, so careful consideration is necessary before using them. To reduce the side effects of synthetic drugs, herbal medicines can be an alternative. Some plants that can be used to treat cholesterol include binahong leaves (*Anredera cordifolia*) and keji beling leaves (*Strobilantes crispus*). The Binahong plant contains flavonoids, saponins, steroids, and alkaloids. Flavonoid compounds can help reduce cholesterol deposits on the walls of blood vessels in the heart (Ramadhani *et al*., 2021). The Keji Beling plant is a plant that is empirically used by the community to help treat (Riyantika, 2020). *S. crispa* exhibits a broad spectrum of pharmacological activities both in vitro and in vivo. These include antihyperglycemic, antioxidant, antimicrobial, wound healing, anticancer, anti-trypanosomal, anti-inflammatory, anti-obesity, anti-urolithiatic, anti-angiogenic, and vasorelaxant effects (Chen *et al.,* 2023). This plant contains high levels of minerals and vitamins C, B1, and B2. The keji beling plant contains phytochemical compounds such as polyphenols, flavonoids, catechins, alkaloids, caffeine, and tannins (Ramadhani *et al.*, 2021). Both binahong and keji beling contain saponins that can bind to bile acids and increase their excretion along with sterols, which helps reduce cholesterol levels in blood plasma (Alfauzi *et al*., 2021).

According to Rachman & Ardiansyah's (2019) research, the keji beling plant can reduce cholesterol levels in test animals, mice (*Rattus novergicus*), and according to Taslim's (2021) research, the binahong plant can also reduce cholesterol levels by 35 % in male white rats (*Rattus novergicus*). The combination of the two plants is expected to be synergistic, increasing the anticholesterol effect. Therefore, a study will be conducted on the Anticholesterol Activity of Binahong Leaf Extract Combination (*Anredera cordifolia*) and Keji Beling Leaf (*Strobilanthes crispus*) in mice (*Mus musculus*).

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Methodology

The type of research conducted in this study is true experimental, which involves examining the existence of a causal relationship by controlling for variables. The extraction method utilized is maceration. Binahong leaf and Keji Beling leaf simplicia were weighed separately at 500 grams each and placed in a closed vessel. Then, 96% ethanol solvent was added with a simplicia to solvent ratio of 1:7.5 until the simplicia was completely submerged. After 24 hours, the solution was filtered using a cloth and filter paper to separate the residue from the filtrate. The maceration process was repeated three times. The resulting liquid extract was concentrated using a rotary evaporator at 70°C until a concentrated extract was obtained.

Simvastatin suspension was used as a positive control in test animals. The suspension was prepared by weighing 1.3 mg of simvastatin and suspending it in 1 ml of 0.5% sodium chloride (CMC) solution. The mice were housed in cages with rice husk bedding and a closed top to prevent escape, but with ventilation holes. They adapted over 7 days and were supplied with food and water. After acclimatization, the mice were fed a high-fat diet for three days to increase their total blood cholesterol levels. Mice were considered hypercholesterolemic if their total cholesterol levels were >130 mg/dl.

Treatment was administered orally to the test mice using a 0.5 ml dose tube. Treatment was administered once daily, and total blood cholesterol levels were measured on days 0, 5, 10, and 15 with POCT. The sample used to measure total cholesterol levels was a capillary blood sample from the tail of a mouse.

The analysis method used is bivariate analysis, conducted on two variables (independent and dependent). Data on differences in cholesterol levels were tested for normality using the *Shapiro-Wilk* test (P>0.05). The process continued with the *Levene* test to assess the homogeneity of the data. If the data is not normally distributed, the *Kruskal-Wallis* test is performed, followed by the Pairwise Comparison test to compare the average values across all groups or to identify the most significant differences between groups.

Result and Discussion

Giving a combination of ethanol extract of Binahong leaves and Keji Beling leaves for 15 days had different effects on cholesterol levels in mice.

Table 1. Results of Cholesterol Level Reduction in Mice (Primary Data, 2025)

	Cholesterol level (mg/dl)			
Treatment	Day 0	Day 5	Day 10	Day 15
Control (-)	208.4	275.4	284.4	299.8
Control (+)	274.8	227.2	207.6	185.4
Extract 1:1	249.8	223.4	198.6	136.4
Extract 1:3	285	218.8	251.4	244.6
Extract 3:1	254.6	189.6	250	183.8

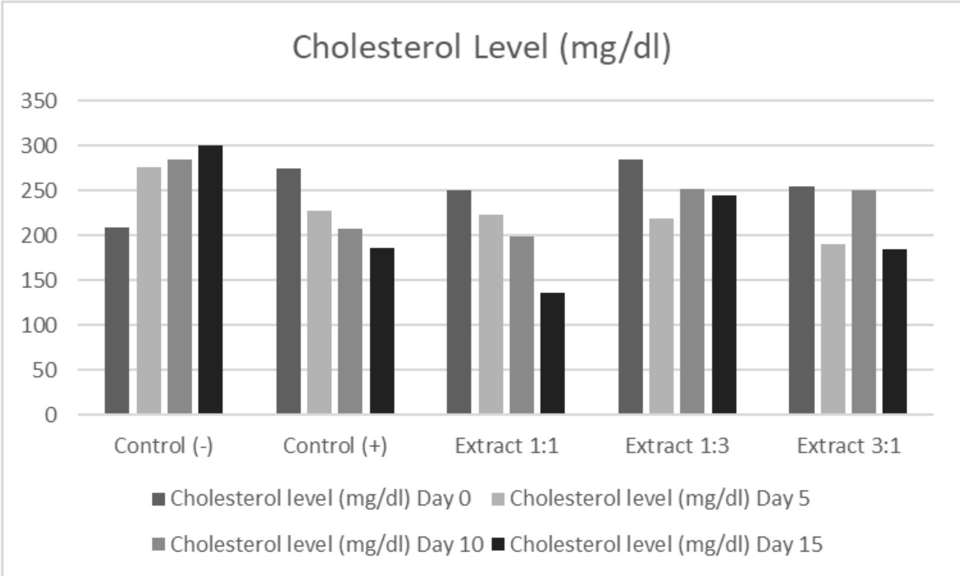

Figure 2. Graph of cholesterol levels in mice (Primary Data, 2025)

The calculation of the reduction in total blood cholesterol levels was done by comparing the results before and after treatment. The examination results of total blood cholesterol levels in mice over 15 days of treatment showed an increase from 208.4 mg/dl to 299.8 mg/dl, or an increase of 91.4 mg/dl, in the negative group. This was caused by the administration of Na CMC, which did not affect total cholesterol levels, and no treatment was given to mice indicating hypercholesterolemia. Additionally, the high-fat diet contributed to the increase in total cholesterol levels in mice.

The group given simvastatin experienced a reduction in cholesterol levels from 274.8 mg/dl to 185.4 mg/dl, a decrease of 89.4 mg/dl, as shown in table 1. This can occur due to the administration of the cholesterol-lowering drug simvastatin at a dose of 1.3 mg/20 g BW. The decrease was 32.54%. This aligns with research conducted by Dwicahyo Priambodo (2023), where the positive control group given simvastatin at a dose of 10 mg/Kg BW also experienced a 30% reduction. Simvastatin is an oral HMG-CoA reductase inhibitor, a semi-synthetic derivative of lovastatin. Simvastatin helps reduce cholesterol production and reduce complications related to dyslipidemia (Talreja & Cassagnol, 2023). While there was a decrease in cholesterol levels, they did not return to normal and could still be considered hypercholesterolemic. This is due to the influence of a high-fat diet, which affects the drug's effectiveness in lowering cholesterol.

A high-fat diet can alter liver structure, causing cells to enlarge because of triglyceride buildup. Impaired β-oxidation of fatty acids in mitochondria results in lipid accumulation in the liver. Excess triglycerides cause membrane permeability to become unbalanced, permitting free radicals to enter cells and damage internal structures and organelles. Such liver damage and functional impairment can increase lipoprotein release, directly affecting cholesterol levels (Sijabat *et al.*, 2024). The results of administering the extract combination in a 1:1 ratio showed a decrease in total cholesterol levels. Before treatment, the average total cholesterol level in mice was 249.8 mg/dl. After 15 days of treatment, the average total cholesterol level in mice dropped to 136.4 mg/dl. This represents a decrease of 113.4 mg/dl, or 45.39%. The results of administering a combination of extracts with a ratio of 1:3 showed a decrease and also an increase in total cholesterol levels in mice. Before being treated, the average total cholesterol level in mice was 285 mg/dl. After being treated for 5 days, the average total cholesterol level in mice decreased to 218.8 mg/dl, or a decrease of 66.2 mg/dl. After being treated for 10 days, there was an increase in total cholesterol levels in mice to 251.4 mg/dl or by 32.6 mg/dl. The treatment was continued for 15 days and showed a decrease in total cholesterol levels in mice to 244.6 mg/dl or by 6.8 mg/dl.

The results of administering a combination of extracts with a ratio of 3:1 showed a decrease and also an increase in total cholesterol levels in mice. Before being treated, the average total cholesterol level in mice was 254 mg/dl. After being treated for 5 days, the average total cholesterol level in mice decreased to 189.6 mg/dl or a decrease of 65 mg/dl. After being treated for 10 days, there was an increase in total cholesterol levels in mice to 250 mg/dl or 60.4 mg/dl. The treatment was continued for 15 days and showed a decrease in total cholesterol levels in mice to 183.8 mg/dl or 66.2 mg/dl.

The 1:3 and 3:1 extract combination groups failed to maintain their cholesterol-lowering effects on day 10. In the 1:3 and 3:1 combination groups, the effects were not as stable as those observed in the 1:1 group. The observed increase may also occur because the mice experienced physiological adaptation to the compounds present in the administered extracts. This adaptation is the body's effort to adjust and maintain balance, for example by altering metabolic activity or liver function. In addition, the increase may result from antagonistic effects between the active compounds in the combined extracts. One possible antagonistic interaction occurs through the hydrogen bonding interaction between the carbonyl group of ellagic acid and the o-dihydroxyl group of catechin, which may limit the hydrogen-donating ability of catechin. This interaction could therefore reduce its overall antioxidant potential and affect its pharmacological effectiveness, especially its cholesterol-lowering activity (Uduwana *et al*., 2023). In this study, total cholesterol levels in mice given keji beling leaf extract increased on the 14th day of treatment, with an average rise of 54 mg/dl, and then decreased again on the 28th day, with an average drop of 18 mg/dl. Previous research conducted by Taslim in 2021 on the administration of ethanol extract of binahong leaves to total blood cholesterol levels in mice showed a significant decrease in cholesterol levels at a dose of 1000 mg/kgBW or 35%.

The reduction in total cholesterol levels may occur due to the synergistic effect between the antioxidant compounds contained in binahong leaves and keji beling leaves. Binahong leaves contain antioxidant compounds, including flavonoids, saponins, terpenoids, steroids, and alkaloids (Astuti *et al.*, 2011). Keji beling leaves contain antioxidant compounds, including polyphenols, flavonoids, catechins, alkaloids, caffeine, saponins, and tannins (Ramadhani *et al*., 2021). Flavonoids can interact with free radicals by directly scavenging oxygen radicals and inhibiting enzymes that stimulate free radical formation, such as cyclooxygenase and lipoxygenase. In lowering cholesterol levels, these antioxidant compounds are believed to function by inhibiting the HMG-CoA reductase enzyme, which acts as a catalyst in cholesterol synthesis (Lestari *et al*., 2025). The higher the antioxidant activity, the greater the inhibition of HMG-CoA reductase and lipase (Yunarto *et al*., 2019).

In addition to antioxidant activity, other bioactive compounds such as saponins also contribute to cholesterol reduction through different pathways. Saponins have been shown to lower cholesterol levels and act as anticancer agents. Saponins are antioxidant compounds that can be used as anti-inflammatory, antimicrobial, antiviral, and antiparasitic agents. Saponins can suppress the expression of lipogenic transcription factors, such as SREBP-1c, and increase the expression of genes involved in fatty acid oxidation, including PPARα and ACOX1, thereby reducing triglyceride accumulation in the liver (Lin *et al*., 2022). Other compounds that can lower cholesterol levels are alkaloids and tannins. Alkaloids are known to inhibit the action of the pancreatic lipase enzyme, resulting in increased fat excretion in the feces. Meanwhile, tannins contribute to lowering cholesterol and LDL levels by stimulating the conversion of cholesterol into bile acids and increasing their excretion in the feces (Tanggu *et al*., 2021).

According to the literature, various groups of compounds such as saponins, alkaloids, and tannins have been reported to contribute to cholesterol-lowering effects through different mechanisms. Flavonoids are the most dominant bioactive compounds and play a crucial role in cholesterol reduction. Binahong leaf extract contains a high level of flavonoids (71.8 mg/g), along with phenols, tannins, and saponins that also contribute to its biological activity (Yolanda *et al*., 2024). Similarly, the ethanolic extract of Keji Beling leaves contains 1.333% flavonoids, together with phenols (0.773%), tannins (1.319%), and alkaloids (0.643%) *(Rivai et al*., 2019). The abundance of flavonoids in both extracts supports their potential as hypolipidemic agents by mechanisms such as inhibiting LDL oxidation, enhancing lipid metabolism, and modulating enzymes involved in cholesterol biosynthesis.

Table 2. Results of Kruskal–Wallis and Post Hoc Tests on Total Cholesterol Levels (Primary Data, 2025)

Comparison Group	Significance Value (p)	Description
Overall (Kruskal–Wallis test)	0.001	Significant difference between groups
Negative control vs. Positive control	0.000	Significant difference
Negative control vs. Combination 1:1	0.000	Significant difference
Negative control vs. Combination 1:3	0.021	Significant difference
Negative control vs.	0.005	Significant

Combination 3:1	difference

Table 2 shows that the statistical analysis indicates a statistically significant difference among the groups. All combination treatments of Binahong and Keji Beling leaf extracts (ratios 1:1, 1:3, and 3:1) significantly reduced total cholesterol levels ($p < 0.05$) compared to the negative control. The 1:1 ratio showed the greatest cholesterol-lowering effect among all treatment groups.

Conclusion

The research results concluded that a combination of ethanol extracts from binahong leaves and keji beling leaves can reduce total cholesterol levels in mice. The most effective ratio was 1:1, which achieved a 45.3% reduction (113.4 mg/dl), followed by a 3:1 ratio with a 27.80% reduction (70.8 mg/dl), and a 1:3 ratio with a 14.17% reduction (40.4 mg/dl).

Declaration of Competing Interest

The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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