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THE POTENTIAL OF COMBINED ETHANOL EXTRACTS OF SAGA AND SEMBUKAN LEAVES IN REDUCING CHOLESTEROL LEVELS IN HYPERCHOLESTEROLEMIC MICE

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ABSTRACT

Background: To date, no research has explored the synergistic potential between saga leaves (*Adenanthera pavonina* L.) and sembukan leaves (*Paederia foetida* L.) in increasing the effectiveness of lowering cholesterol levels. This study aims to evaluate the potential of the combination of saga leaf ethanol extract and sembukan leaf in lowering cholesterol levels in hypercholesterolemia mice.
Methods: The study used 36 mice divided into six treatment groups, including a negative control group, a positive control, a drug control, and a treatment group with different doses of a combination of ethanol extract of Saga leaves and Sembukan leaves. The spectrophotometer measured lipid levels on days 0, 21, and 24. Data analysis was done using the one-way ANOVA test to determine group differences.
Results: The analysis showed that the combination of ethanol extract of saga leaves and sembukan leaves significantly reduced the cholesterol levels of hypercholesterolemia mice (p=0.000). The best dose for cholesterol reduction is 56 mg/20 g body weight (BW) with a 66% reduction.
Conclusion: Combining ethanol extract of saga leaves and sembukan leaves at a dose of 56 mg/20 g body weight (BW) effectively reduced cholesterol levels in hypercholesterolemia mice. This research may contribute to developing alternative natural therapies to lower cholesterol levels and prevent cardiovascular disease.
Keywords: ethanol extract combination, hypercholesterolemia, mice (*Mus musculus*), saga leaves, sembukan leaves

INTRODUCTION

Indonesia is renowned for its rich biodiversity, including medicinal plants used by the community for generations (Rabiatul Adawiyah, Siti Maimunah, & Pienyani Rosawanti, 2019). One form of utilising this natural wealth is through phytotherapy, also known as herbal medicine, which utilises local plants to treat various diseases (Munaeni et al., 2022). Using plants such as saga leaves (*Adenanthera pavonina* L.) and Sembukan leaves (*Paederia foetida* L.) as traditional medicine shows great potential in modern medicine, especially in reducing cholesterol levels. A society that is increasingly aware of the importance of healthy living, including natural remedies, opens up opportunities for further research on the benefits of these medicinal plants in overcoming health problems, such as hypercholesterolemia, which can lead to cardiovascular disease (Nasution, Adi and Santosa, 2015).

Research on the benefits of herbal plants in treating hypercholesterolemia has been conducted. Previously, saga tree bark was effective in controlling blood lipid levels, as explained by a previous study that found that the bark of the saga tree interferes with

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cholesterol biosynthesis and lipid utilisation in individuals with dyslipidemia (Koodalingam *et al.*, 2015). On the other hand, the leaves also show similar potential in lowering cholesterol and triglyceride levels and increasing the levels of *High-Density Lipoprotein* (HDL) cholesterol, as revealed by (Tassa, Sahu, Barman, & Sahu, 2023). Further research also showed that combo leaves can lower cholesterol levels by up to 19.17% at specific doses (Elok Faiqotul Jannah, 2023). Meanwhile, Saga leaves showed a decrease of 25.33% (Tengku Rahmadito Putra Samudra, 2023). These studies demonstrate the significant potential of individual plants; however, no studies have investigated the combined effects of the two to enhance the effectiveness of reducing cholesterol levels.

Although previous studies have shown the potential benefits of sage and sembukan leaves in lowering cholesterol levels, research on combining these two extracts is still limited. No studies have addressed the synergistic potential between these two plants to improve the effectiveness of lowering cholesterol levels, although the combination approach may result in more substantial synergistic effects and reduce side effects or toxicity (Afifah, Apriliana, Setiawan, & Berawi, 2024; Annisa, Sulaiman, Nugroho, & Nugroho, 2021; Nursamtari & Zuhrotun, 2022). This gap presents a significant challenge in the development of more efficient and safe herbal therapies to combat hypercholesterolemia. Research on the combination of extracts from these two plants is needed to determine whether the effects of the combination can provide more benefits than the individual use of each extract.

This study investigates the potential of combining the ethanol extract of saga leaves and sembukan leaves in reducing cholesterol levels in hypercholesterolemia model mice. With this combination approach, it is hoped that a more effective herbal therapy can be developed, with a synergistic effect between the two plants that can better overcome cholesterol problems than using a single extract. The significance of this research lies in its ability to provide safer and more natural treatment alternatives for the community, reduce dependence on chemical drugs that are at risk of causing side effects, and pave the way for the development of herbal medicine based on scientific Specifically, the objectives of this study were: to evaluate the effect of combination extracts on the reduction of cholesterol levels, to determine the optimal dose that provided the most significant reduction, and to assess the potential synergistic effects of the combination of extracts compared to the administration of a single extract.

RESEARCH METHOD

Extraction

The sembukan leaf powder and saga leaf powder were then extracted using the maceration method, which involves soaking each powder in a 96% ethanol solvent at a 1:4 ratio four times, each for 24 hours. After that, filtration is carried out, and the obtained filtrate is then evaporated using a rotary evaporator to produce a viscous extract. The extracts obtained are then weighed using an analytical balance (Riwanti, Izazih, & Amaliyah, 2020).

Classification of Test Animal Groups

The UNUSA Health Research Ethics Committee approved the animal testing protocol with approval number 0026/EC/KEPK/UNUSA/2024. A total of 36 experimental animals were divided into six groups, each consisting of six male mice. The first group served as a control group, receiving no special treatment, and consisted of six healthy mice. The second group served as a positive control of 6 healthy mice given the drug propylthiouracil, which had been measured and suspended in water. The third group acted as a drug control group, in which six healthy mice were given the drug simvastatin. The fourth group received a 14

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mg/20 g body weight (BW) dose, containing six healthy mice with a combination of sembukan leaf and saga leaf extract. The fifth group was given a dose of 28 mg/20 g body weight (BW), which contained six healthy mice with a combination of sembukan leaf and saga leaf extract. The last group was given a dose of 56 mg/20 g body weight (BW), which contained a combination of 6 healthy mice, including those treated with the sembukan leaf and saga leaf extract. During the acclimatisation process, which lasted 1 week, the mice were fed CP155 and given gallons of aqua water to adapt.

Combination Extract Treatment with a ratio of 1:4

The combination of sembukan leaf and saga leaf extract is weighed according to the prescribed dose. For a dose of 14 mg/20 g body weight (BW), as much as 210 mg of a combination of ethanol extracts of sembukan and saga leaves was weighed, then dissolved in aquades to a volume of 10 ml in a measuring flask until it reached the limit mark and homogenised. For a dose of 28 mg/20 g body weight (BW), as much as 420 mg of a combination of ethanol extracts of sembukan and saga leaves was weighed, then dissolved in 10 mL of aqua destillata in a measuring flask until it reached the limit mark and homogenised. For a dose of 56 mg/20 g body weight (BW), as much as 840 mg of a combination of ethanol extracts of sembukan and saga leaves was weighed, then dissolved in aquades to a volume of 10 ml in a measuring flask until it reached the limit mark and was homogenised. The test animals were administered a dose of 1 ml daily, according to the group and the prescribed dose, and this treatment was carried out for 3 days.

Hypercholesterolemia Activity Testing

The test animals were acclimated for 7 days to allow for self-adjustment, and then divided into six groups. In the first stage, mice's initial blood cholesterol levels are measured before adding the extract. Group 1 (negative control) was given standard feed (Centrat CP 511) and drink, group 2 (positive control), group 3 (drug control), group 4 (dose 14 mg/20 g body weight (BW)), group 5 (dose 28 mg/20 g body weight (BW)), and group 6 (dose 56 mg/20 g body weight (BW)). Groups 2 to 6 were administered propylthiouracil at a dose of up to 1.04 grams per day for 14 days to increase cholesterol levels in mice. On day 21, the cholesterol levels of the positive control group were measured to ensure an increase in cholesterol levels after the mice had been fasted for approximately 9 hours. In dose groups 1, 2, and 3, a combination of the ethanol extract of sembukan and saga leaves was administered, while in the control group, simvastatin was used as a comparator for 3 days. On day 24, cholesterol levels in mice were measured after administration of the extract in dose groups 1, 2, and 3, and cholesterol levels in the simvastatin-treated groups were measured.

Test Animal Blood Sampling

The cholesterol level measuring device is prepared by first disinfecting the tail of the mice using a cotton swab moistened with alcohol. Next, the tail is extended and cut about 1 mm from the tail end using sterile scissors. The blood that comes out of the tail of the mice is stored in a tube and then centrifuged at 1500 rpm. After the centrifugation process, the serum is collected to measure the cholesterol levels of the mice.

Measurement of Animal Cholesterol Levels Test

The measurement of cholesterol levels in test animals was carried out using the Cholesterol Oxidase-Peroxidase Aminoantipyrine Phenol (CHOD-PAP) method. The work step begins with the preparation of tools and materials. The reagents are then mapped into tubes according to the respective quantities for blanks, standards, and samples. After that,

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the mixture in the tube is homogenised and incubated for 10 minutes. The measurement results are then read using a spectrophotometer.

Data Analysis

Cholesterol data were analyzed using the Shapiro–Wilk normality test and Levene's homogeneity test. Differences in mean cholesterol levels among groups were analyzed using One-Way ANOVA, followed by *post hoc* testing when significant differences were observed.

RESULTS

Table 1 results showed that saga leaves (*Adenanthera pavonina* L.) and sembukan leaves (*Paederia foetida* L.) produced the same extract yield. From 500 g of simplicia powder of each plant, 41 g of extract was obtained, corresponding to a yield of 8%. This indicates that the maceration method using 96% ethanol provided comparable extraction efficiency for both plant materials.

Table 1. Results of the extraction of saga and sembukan leaves

Simplisia	Powder weight (g)	Extract weight (g)	Rendemen (%)
Saga leaves (*Adenanthera pavonina* L)	500	41	8
Sembukan leaves (*Paederia foetida* L)	500	41	8

Table 2 presents the results of measuring the cholesterol levels of mice after treatment with a combination of ethanol extracts from saga leaves and sembukan leaves. The data recorded were mouse cholesterol levels (in mg/dl) grouped by type of treatment, namely negative control, positive control, drug control, and three combination doses of extracts (14 mg/20g body weight (BW), 28 mg/20g body weight (BW), and 56 mg/20g body weight (BW). The cholesterol levels of mice vary based on the treatment they receive. The negative control had an average cholesterol level of 112.7 mg/dL, while the positive control showed a significant increase to 189.3 mg/dL. The drug control showed a decrease with an average of 117.7 mg/dl, but still higher than the negative control. Treatment with a combination dose of saga and sembukan leaf ethanol extract (dose 1; 14 mg/20g body weight (BW)) resulted in an average cholesterol level of 140.9 mg/dl. Dose 2 (28 mg/20g body weight (BW)) lowered cholesterol levels to 128.7 mg/dl. In comparison, dose 3 (56 mg/20g body weight (BW)) resulted in 124.8 mg/dL cholesterol levels, almost equivalent to those of the negative controls. Before conducting the *One-Way ANOVA Test*, a normality test was carried out using the Shapiro-Wilk test because the sample size was less than 100, showing a significant value greater than 0.05, indicating normally distributed data. The homogeneity test used the Levene test, with a significance level (sig) greater than 0.05, indicating that the data were homogeneous. The One-Way ANOVA test was then performed to determine the average difference between groups, yielding a significant result of 0.000, indicating a statistically significant difference between groups.

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Table 2. Results of measuring cholesterol levels in mice after treatment with ethanol extract of saga and sembukan leaves

Test Animals	Mice Cholesterol Level (mg/dl)					
	Negative Control	Positive Control	Drug Control	Dose 1 (14 mg/20g BW)	Dose 2 (28 mg/20 g BW)	Dose 3 (56 mg/ 20g BW)
1	120.9	191.2	130.6	136.7	122.2	126.8
2	125.5	189.3	128.5	150.7	125.5	121.5
3	102.4	196.5	120.6	139.4	128.9	127.4
4	99.3	194.7	117.5	142.5	116.8	120.9
5	116.8	183.7	96.2	146	132.8	132.6
6	111.5	180.4	112.5	130.2	146.2	119.5
Mean ± SD	112.7	189.3	117.7	140.9	128.7	124.8

DISCUSSION

The reduction in total cholesterol levels in hypercholesterolemic mice represents the main finding of this study and is closely related to the quality of the extracts and the bioactive compounds obtained through the extraction process. Extraction in this study was carried out using the maceration method, which involves immersing the materials to extract active compounds. A total of 500 g of saga leaf and sembukan leaf simplicia were soaked in 2000 mL of 96% ethanol for four days, with a material-to-solvent ratio of 1: (Heckmann et al., 2024; Rafiq et al., 2024) 4. During the immersion process, the solvent penetrates the cell wall and enters the cell cavity containing the active compounds, allowing the dissolved compounds to diffuse out of the cells due to concentration differences between the intracellular and extracellular solutions. After four days, the mixture was filtered to separate the residue from the filtrate, which was then concentrated using a rotary evaporator to obtain a viscous extract. The resulting extract was weighed, yielding 41 g with a yield of 8%. The obtained extract yield indicates that maceration using 96% ethanol was effective and consistent in extracting bioactive compounds from both plant materials. The extraction yield is an important parameter because it reflects not only the amount of extract produced but also the efficiency of the extraction method. Yield is closely related to the concentration of active compounds in the sample; therefore, a higher yield generally indicates a more effective extraction process, ensures sufficient material availability for further analysis, and supports a more accurate evaluation of the pharmacological activity of the extract (Usman & Ibrahim, 2019).

An increase in mouse cholesterol levels was observed in the positive control group induced with propylthiouracil. This is caused by propylthiouracil, a treatment for hyperthyroidism (Suryandari, Yunaini, Sunaryo, Istiadi, & Pratomo, 2024) that can increase cholesterol levels by inhibiting the formation of thyroid hormones. Thyroid hormones play a role in increasing cholesterol through the activation of LDL receptors in the liver and enhancing HDL activity. Meanwhile, in the control group receiving simvastatin suspension, the cholesterol levels of the mice decreased. Simvastatin functions as an antihyperlipidemic and anticholesterol drug (Kassem et al., 2025) by inhibiting the activity of the enzyme 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG CoA-reductase) (Anggi Mutiara Hasanah, 2018). The combination of ethanol extract of the sembukan and saga leaves effectively lowers the blood's total cholesterol levels because it contains flavonoid compounds. Flavonoids can inhibit the development of heart disease through their

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antioxidant properties (Indriyani *et al.*, 2025). The flavonoids work similarly to estrogen, serving as cardioprotective agents (Rislan Faiz Muhammad, 2020). It improves lipid profiles, lowering total cholesterol, LDL, and triglycerides and increasing HDL. The decrease in cholesterol by flavonoids can also be through other mechanisms, namely, increased bile acid secretion and decreased cholesterol metabolism (Anjelin & Amelia, 2023; Chen et al., 2025).

In previous studies on the antihyperlipidemic effects of sembukan and saga leaves, doses of 3.5 mg/g body weight (BW), 7 mg/20 g body weight (BW), and 14 mg/20 g body weight (BW), respectively, were used. Despite the same dose, results showed a different reduction in cholesterol levels at a dose of 14 mg/20 g body weight (BW). In the sembukan leaf test, a decrease in cholesterol levels was recorded at 19.27% (Elok Faiqotul Jannah, 2023), while in saga leaves, it reached 25.33% (Tengku Rahmadito Putra Samudra, 2023). Furthermore, the combination of ethanol extract from the sembukan and the saga leaf was tested at doses of 14 mg/20 g body weight (BW), 28 mg/20 g body weight (BW), and 56 mg/20 g body weight (BW). The most significant reduction in cholesterol levels occurred at a dose of 56 mg/20 g body weight (BW), which resulted in a 66% reduction in cholesterol levels.

CONCLUSION

This study investigates the potential of combining the ethanol extract of saga and sembukan leaves in reducing cholesterol levels in a hypercholesterolemia model mouse. The results showed that the best dose of a combination of ethanol extract of sembukan leaves (*Paederia foetida* L) and saga leaves (*Adenanthera pavonina* L) in lowering cholesterol levels in hypercholesterolemia mice was a dose of 56 mg/20 g body weight (BW). The combination of saga leaf ethanol extract and sembukan leaf was effective in lowering cholesterol levels in hypercholesterolemia rats which could be beneficial for the development of antihyperlipidemic therapy.

RECOMMENDATIONS

Future studies are recommended to evaluate the safety of the combination of saga leaf and sembukan leaf extracts through subacute and chronic toxicity tests to ensure long-term safety. In addition, identification and quantification of the main active compounds, particularly flavonoids and phenolic compounds, are necessary to determine the contribution of each compound to the antihypercholesterolemic activity. Further studies with a wider range of doses and longer treatment durations are also required to determine the optimal dose and the stability of the cholesterol-lowering effect. Moreover, comprehensive lipid profile measurements, including LDL, HDL, and triglycerides, as well as molecular mechanism studies involving lipid metabolism enzymes, are expected to provide a more thorough understanding of the mechanism of action of the extract combination.

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