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Optimization of the Combination Ratio of Biduri and Bidara Leaf Extracts in Reducing Cholesterol in Mice

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ABSTRACT

This study aimed to evaluate the effectiveness of a combination of ethanol extracts of Biduri (Calotropis gigantea L.) and Bidara (Ziziphus spina-christi L.) leaves in reducing cholesterol levels in mice induced with hypercholesterolemia, as well as to determine the optimal dose and combination ratio. This experimental laboratory study involved several stages, including extract preparation, preparation of extract suspensions, preparation of simvastatin and propylthiouracil (PTU) suspensions, selection and maintenance of experimental animals, administration of treatments, and measurement of blood cholesterol levels. A total of 72 male mice were divided into 12 treatment groups: negative control, positive control, drug control, and nine groups receiving combinations of extracts with ratios of 1:1, 1:2, and 2:1 at three doses (14, 28, and 56 mg/20 g BW). Hypercholesterolemia was induced using PTU for 14 days, followed by oral administration of the extract combinations for 3 days. Cholesterol levels were measured using a spectrophotometer and analyzed using One Way ANOVA. The results showed that the combination of Biduri and Bidara extracts significantly reduced cholesterol levels ($p < 0.05$), with the highest reduction observed in the 2:1 ratio at a dose of 56 mg/20 g BW, showing a 59.18% decrease (135.47 mg/dL) compared to the positive control. These findings indicate a synergistic effect between the two extracts and suggest their potential as a natural hypocholesterolemic agent.

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A. INTRODUCTION

Hypercholesterolemia is a health disorder caused by the accumulation of cholesterol levels in the blood. Excess cholesterol in the blood can easily adhere to the inner walls of blood vessels, increasing the risk of narrowing the vascular pathways (Yudha & Suidah, 2023). Hypercholesterolemia is considered a major health problem in the modern era, playing a significant role in increasing morbidity and mortality due to cardiovascular diseases (Amin et al., 2025). The World Health Organization reports that approximately 39% of the global population experiences elevated cholesterol levels, and each year, 17 million people die due to heart disease and stroke associated with dyslipidemia. (World Health Organization (WHO), 2025). In Indonesia, the prevalence of hypercholesterolemia has increased due to lifestyle changes, including a higher consumption of high-fat foods, lack of physical activity, and genetic factors (Riyadina, 2019). The high incidence and associated complications indicate that hypercholesterolemia is not only a global health threat but also an urgent health issue that requires safe, effective, and widely accessible therapeutic approaches.

Hypercholesterolemia is characterized by elevated total cholesterol levels in the blood, which contributes to the formation of atherosclerotic plaques in the blood vessels. These plaques narrow the arterial lumen, disrupting blood flow and triggering atherosclerosis, coronary heart disease, and stroke. This condition is one of the major risk factors contributing to premature mortality in the productive age group (Yudha et al., 2021). The management of hypercholesterolemia is usually carried out through pharmacological therapy using lipid-lowering drugs, one of which is statins (Kallapur & Sallam, 2023).

Statins are considered first-line therapy because they inhibit the activity of HMG-CoA reductase, an enzyme involved in cholesterol synthesis. Although effective, long-term use carries the risk of side effects such as hepatotoxicity, myalgia, and impaired kidney function (Rossini et al., 2022). Furthermore, limited access to synthetic drugs among lower- and middle-income populations also poses a barrier to long-term therapy (Kaundal & Kumar, 2025). Therefore, there is a need for alternative therapies based on natural products that are safer, more affordable, and utilize the potential of local biodiversity.

Indonesia is recognized as a megadiverse country with a wealth of medicinal plants that have great potential to be developed as phytopharmaceutical agents (Maulidya et al., 2025). One plant species that has long been used in traditional medicine is Biduri (Calotropis gigantea L.). Biduri leaves are rich in bioactive compounds such as flavonoids, alkaloids, and saponins, which have been reported to possess antihyperlipidemic activity (Munandar et al., 2025). Flavonoids act as antioxidants by inhibiting LDL oxidation, while saponins can bind cholesterol in the intestines, thereby reducing lipid absorption (Laka et al., 2022). The study by Ngibad et al. (2025) demonstrated that Calotropis gigantea L. was able to reduce total cholesterol in mice by up to 28 mg/dL, reinforcing evidence that Biduri possesses relevant hypocholesterolemic activity.

In addition, Bidara leaves also have remarkable potential, containing compounds such as flavonoids, triterpenoids, and polyphenols with strong antioxidant activity (Safitri et al., 2022). The study by Lailatusholihah et al. (2023) demonstrated that Bidara leaf extract is rich in flavonoids, which play a role in supporting the reduction of serum cholesterol levels. A recent study by Shivanandappa et al. (2023) reported that Bidara leaf extract was able to reduce total cholesterol levels, enhance insulin sensitivity, and improve ovarian structure in PCOS rats. These effects are likely attributed to bioactive compounds such as quercetin, which provide significant therapeutic activity. The findings indicate that Bidara has broad potential as a natural hypolipidemic agent.

Nevertheless, existing studies have primarily focused on the use of single extracts, either Biduri or Bidara. To date, no research has evaluated the pharmacological interaction potential of these two plants when used in combination, whether synergistic, additive, or antagonistic. Therefore, this study provides a foundation for the development and testing of a combined antihyperlipidemic plant formula, which previously has only been studied separately, while also determining the optimal composition. Accordingly, this research plays a crucial role in evaluating the effectiveness of a combination of ethanol extracts of Biduri and Bidara leaves in reducing cholesterol levels, as well as identifying the most optimal combination ratio.

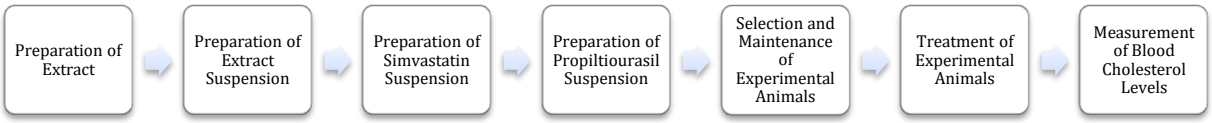
B. RESEARCH METHODS

This study was an experimental laboratory investigation using mice as research subjects. Experimental studies are a type of research that involves directly administering treatments to subjects and subsequently observing their effects on the variables of interest.

1. Materials and Equipment

The materials used in this study included Bidara (Ziziphus spina-christi L.) and Biduri (Calotropis gigantea L.) leaves, 96% ethanol, experimental animals (mice), cotton, tissue, simvastatin tablets, alcohol for disinfection, propylthiouracil (PTU), and distilled water. The equipment used consisted of a spectrophotometer, 1 mL/1 cc syringes, measuring flasks, stirring rods, analytical balance, mouse cages, gloves, beakers, sonde, and scissors.

2. Research Procedure



a. Preparation of Biduri Leaf and Bidara Leaf Extracts

A total of 2000 g of powdered biduri leaves and 2500 g of powdered bidara leaves, which had been finely ground, were extracted using the maceration technique. Each type of powdered material was soaked in 96% ethanol at a ratio of 1:4 (w/v) for three consecutive 24-hour periods (Ayalew & Bekele, 2021; Lohvina et al., 2021; Sanz et al, 2021). After completion of the maceration process, the solution was filtered, and the resulting filtrate was concentrated using a rotary evaporator until a viscous extract was obtained. The viscous extract was then weighed using an analytical balance to determine the extraction yield from each simplicia material.

b. Preparation of Combined Biduri Leaf and Bidara Leaf Extract Suspension

The biduri leaf extract and bidara leaf extract were weighed according to the required doses and then suspended in distilled water to a final volume of 10 mL in a volumetric flask, followed by homogenization. The suspension was prepared in three ratio variations, namely 1:1, 1:2, and 2:1, with doses of 14 mg/20 g body weight (BW), 28 mg/20 g BW, and 56 mg/20 g BW, respectively.

c. Preparation of Simvastatin Suspension

The simvastatin suspension was prepared by weighing one simvastatin tablet, which was then crushed and suspended in distilled water to a final volume of 10 mL, followed by thorough mixing until a homogeneous suspension was obtained.

d. Preparation of Propylthiouracil (PTU) Suspension

The propylthiouracil (PTU) suspension was prepared by weighing 1.04 g of PTU powder, which was then finely ground and suspended in distilled water to a final volume of 60 mL, yielding a concentration of 100 mg/60 mL.

e. Selection and Maintenance of Experimental Animals

A total of 72 healthy male mice *(Mus musculus)* weighing 20–30 gram were used as experimental subjects and divided into 12 groups, each consisting of six mice. The mice were housed in plastic cages with rice husk bedding, provided with standard feed and ad libitum access to drinking water, and acclimatized for 7 days prior to treatment (Zhang et al., 2020). Group K1 served as the negative control without treatment, Group K2 as the positive control receiving PTU at a dose of 1 cc/day, and Group K3 received PTU plus simvastatin at a dose of 1 cc/day. Groups K4–K6 were administered a combination of biduri and bidara extracts (1:1 ratio) at doses of 14, 28, and 56 mg/20 g body weight (BW), respectively; Groups K7–K9 received the combination at a 1:2 ratio at the same dose levels; while Groups K10–K12 were administered the combination at a 2:1 ratio at doses of 14, 28, and 56 mg/20 g BW, respectively.

f. Treatment of Experimental Animals

All mice underwent a 7-day acclimatization period prior to treatment. Baseline blood cholesterol levels were measured after acclimatization. Group K1 (negative control) received standard feed only without any additional treatment. Groups K2–K12 were administered propylthiouracil (PTU) at a dose of 1 cc/day for 14 consecutive days to induce hypercholesterolemia. On day 21, blood cholesterol levels were measured to confirm the increase in cholesterol levels. In addition to PTU administration, Group K3 received simvastatin at a dose of 1 cc/day as a reference drug (drug control). The treatment groups (K4–K12), after hypercholesterolemia induction, were orally administered a combination of biduri and bidara leaf extracts at ratios of 1:1, 1:2, and 2:1, with three dose variations (14 mg/20 g BW, 28 mg/20 g BW, and 56 mg/20 g BW), at a volume of 1 cc/day for three consecutive days. At the end of the treatment period, blood cholesterol levels were measured to evaluate the cholesterol-lowering effects of each extract combination and dose.

g. Measurement of Blood Cholesterol Levels

After ensuring that the cuvette was cleaned with distilled water, 500 µL of cholesterol reagent was prepared and mixed with 5 µL of mouse serum. The mixture was homogenized, incubated for 20 minutes, and the absorbance was measured using a spectrophotometer.

3. Waste Management

a. Experimental Animals

Mice used in Groups 1–12 were anesthetized with chloroform in a closed chamber. After loss of consciousness, the animals were euthanized by cremation (incineration).

b. Equipment

All used equipment was thoroughly washed and dried. Disposable items such as yellow tips, gloves, cotton, and masks were placed in plastic bags and disposed of in yellow biohazard (infectious waste) containers.

4. Data Analysis

This study was categorized as a laboratory experimental study aimed at evaluating the effects of specific treatments on test subjects under controlled conditions. All data collected during the study were analyzed using the One-Way Analysis of Variance (One-Way ANOVA) to determine the presence of statistically significant differences among treatment groups.

C. RESULTS AND DISCUSSION

1. Extraction Yield of Biduri and Bidara Leaf Extracts

Table 3. Extraction Yield of Biduri and Bidara Leaf Extracts

No.	Material	Extraction Yield		
		Initial Weight	**Extract Weight**	**Yield**
1.	Biduri Leaf Powder	2000 g	136.9 g	6.8%
2.	Bidara Leaf Powder	2500 g	76.6 g	3.1%

The results showed that the biduri leaf extract exhibited a higher extraction yield (6.8%) compared to the bidara leaf extract, which yielded only 3.1%. These findings indicate that biduri leaves contain secondary metabolites that are more readily soluble and extractable in 96% ethanol than those present in bidara leaves. Extraction yield is influenced not only by the quantity of bioactive compounds present but also by the physical characteristics of plant tissues, extraction parameters (such as time,

temperature, and solvent-to-material ratio), and the polarity of the solvent used (Mitar & Kardum, 2020). According to Maynita et al. (2023) extraction yield is strongly affected by plant species, extraction method, solvent type, and the condition of the simplicia used.

2. Reduction in Cholesterol Levels

Table 4. Reduction in Cholesterol Levels of Mice

Description		P1	P2	P3	P4	P5	P6	Mean
Negative Control		112,49	101,21	98,81	86,71	116,32	124,91	**106,74**
Positive Control		184,71	196,21	189,4	208,23	192,49	196,87	**194,65**
Drug Control		77,68	99,29	115,41	96,88	122,72	109,91	**103,65**
Ratio 1:1	**Dose 1**	153,1	171,8	158	168,7	170,9	171,5	**165,67**
	Dose 2	145,5	162,1	160,2	155,6	165	159,4	**157,97**
	Dose 3	138	149,3	152,9	158,1	159	145,7	**150,50**
Ratio 1:2	**Dose 1**	157,43	162,81	148,2	151,34	163,78	154,62	**156,36**
	Dose 2	167,25	139,82	154,9	162,72	148,22	149,5	**153,74**
	Dose 3	119,72	132,81	158,9	143,34	138,9	131,8	**137,58**
Ratio 2:1	**Dose 1**	169,2	143,81	148,28	157,6	161,3	140,9	**153,52**
	Dose2	161,82	132,2	141,62	157,4	144,8	136,1	**145,66**
	Dose 3	113,11	139,82	152,49	145,6	133,2	128,6	**135,47**

In the biological activity assay, the total cholesterol level in the negative control group reached an average of 194.65 mg/dL, whereas in the positive control group treated with simvastatin, it drastically decreased to 103.65 mg/dL. Treatment with the combined biduri and bidara leaf extracts demonstrated potential as a cholesterol-lowering agent. The 1:1 combination at Dose 2 resulted in an average cholesterol level of 157.97 mg/dL, while the 2:1 combination at Dose 3 was able to reduce cholesterol levels to 135.47 mg/dL. These results indicate that although biduri extract has a higher extraction yield, its combination with bidara exhibits a more pronounced pharmacological effect. This finding is consistent with the study by Lailatusholihah et al. (2023). which reported that n-hexane extract of bidara leaves was able to reduce cholesterol levels by 70.40%. Therefore, the use of extract combinations has the potential to provide a stronger synergistic effect compared to the use of a single extract.

Data analysis was conducted using SPSS to support the results of this study. The analysis indicated that the data met the assumptions of normal distribution and homogeneity ($p > 0.05$), thereby satisfying the criteria for parametric testing. The One-Way ANOVA test revealed significant differences among the treatment groups ($p < 0.05$), and post hoc analysis confirmed that Dose 2 in the 1:1 combination exhibited a significant

difference compared to the other doses. These findings suggest that this dose can be considered the minimum effective dose.

D. CONCLUSIONS AND RECOMMENDATIONS

The administration of an ethanol extract combination of biduri and bidara leaves was scientifically effective in reducing cholesterol levels in mice induced with hypercholesterolemia. Statistical analysis using the One-Way ANOVA method demonstrated significant differences among the treatment groups ($p < 0.05$). The most optimal dose for lowering cholesterol was Dose 3 (56 mg/20 g BW), achieving a reduction of up to 59.18%. These findings confirm that the combination of biduri and bidara extracts has promising potential as a natural hypocholesterolemic agent.

For future research, it is recommended to conduct further phytochemical analyses to identify the main bioactive compounds responsible for the cholesterol-lowering effect. Additionally, toxicity testing and broader preclinical studies should be performed to evaluate the safety and efficacy of this extract combination before clinical application.

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