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Antihypercholesterolemic Activity of the Ethanol Extract Combination of Leaves *Calotropis gigantea* L. and *Adenanthera pavonina* L.

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ABSTRACT

Hypercholesterolemia is a significant risk factor for cardiovascular disease, and its prevalence continues to increase. Pharmacological therapy using statins is effective but may cause side effects when used long-term. Therefore, exploring natural ingredients as an alternative antihypercholesterolemic therapy is essential. This study aimed to evaluate the activity of a combination of ethanol extracts of Calotropis gigantea L. leaves and Adenanthera pavonina L. on the cholesterol levels of male mice induced with propylthiouracil (PTU). The study was conducted experimentally using a post-test-only control-group design with 36 male mice divided into six groups: negative control, positive control, drug control (simvastatin), and three treatment groups receiving extract combinations at doses of 14, 28, and 56 mg/g BW. The research stages included seven days of animal adaptation, induction of hypercholesterolemia with PTU for 14 days, administration of the extract combination at doses of 14, 28, and 56 mg/g BW for four days, and blood sampling for total cholesterol measurement using an automated chemical spectrophotometer. The results showed a significant increase in cholesterol levels in the positive control group, while simvastatin produced the most significant reduction. The extract combination gradually decreased cholesterol levels with increasing dose, with the highest dose approaching the effect of simvastatin. The paired t-test showed a p-value < 0.05, indicating significant differences between treatments. The combination of Calotropis gigantea L. and Adenanthera pavonina L. leaf extracts has potential as a herbal agent to support antihypercholesterolemic therapy.

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A. INTRODUCTION

Hypercholesterolemia is a specific type of hyperlipidemia characterized by metabolic disorders due to increased blood cholesterol levels (Wang et al., 2025). Greece was recently reclassified from a low-risk to moderate country in terms of cardiovascular disease risk, with 27% of cardiovascular deaths caused by hypercholesterolemia (Stergiou et al., 2023). The development of atherosclerosis and the risk of subsequent cardiovascular disease can be reduced by treatment with lipid-lowering drugs, with statins being the first drug of choice. In addition to lipid-lowering drugs, Lifestyle management, including adherence to a heart-healthy diet, is a cornerstone in medicine (Toft-Nielsen et al., 2025). Although synthetic drugs such as statins are effective, their long-term use can cause side effects and require high costs, so safer and more affordable treatment alternatives are needed, including those from natural ingredients.

Several studies have evaluated *Calotropis gigantea*, both ethanol and methanol extracts from leaves and other parts, and have exhibited antioxidant activity (Sawong et

al., 2022), anti-inflammatory (Sivapalan et al., 2023), and potential lipid reduction in test animal models (Ngibad et al., 2025). Ethanol extract of *C. gigantea leaves* is reported to be rich in phenolics and flavonoids that act as powerful antioxidants (Alafnan et al., 2021). In addition, the plant contains various important phytochemical groups such as triterpenoids, phenolics, cardiac glycosides, and flavonoids (Kaewpaeng et al., 2025). Flavonoids from the natural product itself have been widely reported to be beneficial in the treatment of hypercholesterolemia with minimal side effects (Hanis et al., 2025). Meanwhile, *Adenanthera pavonina* is known to contain alkaloids, saponins, flavonoids, carbohydrates, anthocyanins and betayanins, quinones, cardiac glycosides, terpenoids, triterpenoids, coumarins, steroids, and acids (Owoeye et al., 2023). Pharmacological studies show that this plant has antinociceptive, cytoprotective, anti-inflammatory, antihyperglycemic, and hypolipidemic effects (Krishnan et al., 2022). These bioactive compounds make *A. pavonina* a potential candidate in the development of herbal antihypercholesterolemia agents.

Although previous studies have shown the potential of Calotropis gigantea and Adenanthera pavonina as antihypercholesterolemia agents, Most studies still focus on using a single extract from each plant. Until now, no reports have evaluated the combined effect of ethanol extracts from C. gigantea and A. pavonina leaves on lowering blood cholesterol levels. Research results Ngibad et al. (2025) shows that Calotropis gigantea leaf extract can significantly lower cholesterol levels, although the effects are not as strong as those of simvastatin or some of the other extracts tested. This activity is associated with bioactive compounds, such as flavonoids and phenolics, which help reduce LDL oxidation. Meanwhile, Edi (2022) reports that polycosanol (lignofiber ester) from A. pavonina seed oil may inhibit the increase in total cholesterol in quail yolks with a concentration of at least 0.25% in feed. However, no studies have tested the combination of these two leaf extracts. Therefore, further studies are needed to explore its potential synergistic effects on cholesterol-lowering.

Based on this discussion, this study aims to evaluate the antihypercholesterolemic activity of a combination of ethanol extracts from Calotropis gigantea and Adenanthera pavonina leaves in animal models. The novelty of this research lies in the combination approach to two plant extracts that have previously been studied separately. By combining the two extracts, it is hoped that a more substantial synergistic effect can be obtained in lowering cholesterol levels, while providing an alternative to herbal therapies that are safer than synthetic drugs. This research is expected to enrich the scientific literature on the use of local medicinal plants as candidates for antihypercholesterolemia phytopharmaceuticals and support the development of effective and affordable complementary therapies.

B. RESEARCH METHOD

This study uses an experimental laboratory design with *a post-test only design with a* control *group design* (Fathunikmah et al., 2025). This design is a randomized controlled trial that aims to compare the effects and efficacy between treatment groups by using experimental animals as research objects (Nugroho et al., 2025). The following is a diagram of the research flow:

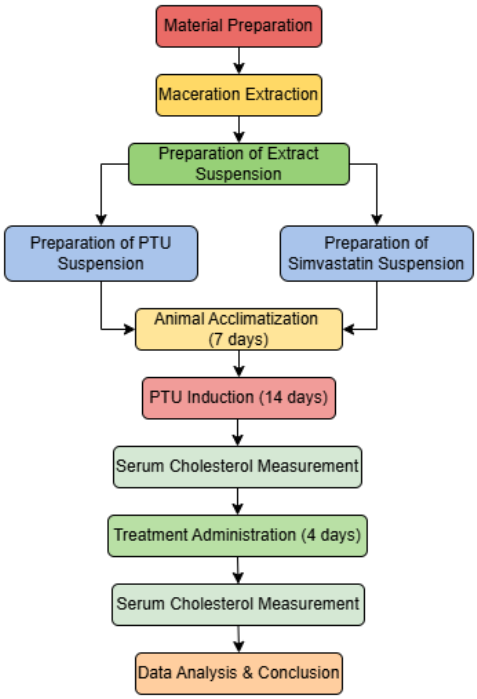

Figure 1. Research flow diagram

1. Extract Manufacturing and Suspension

Calotropis gigantea L. *and Adenanthera pavonina* L. *leaf simplicia powder* that had undergone sieving was extracted by maceration with 96% ethanol as the solvent at a ratio of 1:4 for 3 days (3×24 hours). The maceration results are filtered, then the filtrate is evaporated with a rotary evaporator until a concentrated extract is obtained (Kirana et al., 2025), which is further weighted using the analytical balance.

The concentrated extracts of the two simplicia were then weighed according to the test dose, namely 14 mg/20 g BW, 28 mg/20 g BW, and 56 mg/20 g BW. Each dose is placed in a measuring flask, and aqueducts are added to bring the volume to 10 mL to obtain the appropriate concentration. The solution is homogenized by stirring to get a stable suspension of the extract.

In addition, comparative suspensions were prepared: simvastatin and propylthiouracil (PTU). For the manufacture of simvastatin suspension, one simvastatin tablet is weighed, then ground to a smooth powder. The obtained powder is suspended in an aqueous solution to a final volume of 10 ml and homogenized to produce a simvastatin suspension. Meanwhile, the PTU suspension containing 1.04 g of propylthiouracil was weighed and crushed into a fine powder. The powder is then added to aqueous solutions up to a volume of 50 ml, so that a propyltiourasil suspension with a concentration of 100 mg is obtained (Arifah et al., 2022).

2. Animal Treatment Stage and Effectiveness Testing

The next stage is the treatment of test animals, using up to 36 male mice weighing 20–30 g. All animals were randomly divided into six groups, each consisting of six. The treatment group consisted of: negative control (standard feed without treatment), positive control (propyltiouracil-induced without therapy), drug control (propyltiouracil-induced and simvastatin given), and three groups of combination treatment of *Calotropis gigantea* L. and *Adenanthera pavonina* L. leaf extracts with doses of 14, 28, and 56 mg/20 g BW, respectively, also induced by propyltiouracil.

Before treatment, all animals were acclimatized for seven days under controlled laboratory conditions to adapt to the experimental environment.

After the acclimatization period, the initial blood cholesterol levels of mice were measured. The entire group, except negative controls, was induced with propylthiouracil for 14 consecutive days to improve blood cholesterol levels. On the 21st day, a remeasurement was performed to confirm an increase in cholesterol. Furthermore, the treatment group was given a combination of Calotropis gigantea L. and Adenanthera pavonina L. leaf extracts according to the dosage (14, 28, and 56 mg/20 g BW), and the drug control group was given simvastatin. In contrast, the negative control group received standard feed without induction. The treatment was given for 4 days, and the mice's blood cholesterol levels were measured again to assess its effectiveness.

Blood sampling was carried out by disinfecting the tail of mice using an alcoholic cotton swab, then cutting off the tail end ±1 mm with sterile scissors to obtain blood (Syafira et al., 2022). A total of 1 mL of blood was placed in a vacuum tube containing anticoagulants and centrifuged at 3000 rpm for 10 minutes. The serum formed is separated from the blood cells, and subsequently the cholesterol level (Asrori et al., 2022) Analyzed using the SINOWA BS-3000P Chemistry Analyzer spectrophotometer.

C. RESULTS AND DISCUSSION

1. Extraction

Based on the results in **Table 1**, the extraction of *Calotropis gigantea* leaves yielded 3% with a concentrated extract of 15 g, while Adenanthera pavonina leaves yielded 6.1% with a concentrated extract of 81 g.

Table. 1 Results of leaf extraction of *Calotropis gigantea* and *Adenanthera pavonina*

Simplisia	Powder Weight (g)	Weight of Concentrated Extract(g)	Yield (%)
Calotropis gigantea	500 gram	15 gram	3 %
Adenanthera pavonina	500 gram	81 gram	6.1 %

The results of the comparison of the yield of the leaf extracts *of Calotropis gigantea* and *Adenanthera pavonina* showed that there was a variation in the amount of concentrated extracts produced. The content of secondary metabolites in each plant, where A likely influences this difference in yield value*. Pavonina* is known to contain more bioactive compounds, such as flavonoids, alkaloids, saponins, and polyphenols, allowing for greater extraction of these compounds (Krishnan et al., 2022). In contrast, low yields in *C. gigantea* do not necessarily indicate low biological activity, because even though the extract yield is small, this plant is still rich in important active compounds such as triterpenoids, Phenolics, flavonoids, and cardiac glycosides that play a role in pharmacological effects (Yadav et al., 2025).

In addition, the extraction method with ethanol solvents also affects the amount of yield, because the properties of the solvent determine the number of polar and semipolar compounds that can be soluble (Warnis & Artika, 2021). These results are in line with previous research Wijaya et al. (2022) and Ningsih et al. (2018) which states that temperature factors and extraction time can influence the difference in yield, Although the basic principle remains the same, which is to draw the active compound from the sample. Extended extraction time can increase yield by prolonging the interaction between solvents and materials, allowing more compounds to diffuse out of

the cell. Thus, despite the higher yields *of A. pavonina*, extracts from both plants still have the potential to be further tested as antihypercholesterolemia agents.

2. Effectiveness of Combination Extracts Against Antihypercholesterolemia

Based on the measurement results (**Table 2**), the negative control group was set as a reference (100%). The positive control group showed a relatively high increase in cholesterol, averaging 169.3%. In contrast, the drug control group showed a significant decrease with an average of 62.1%. The combination treatment with the extract showed a trend of decreasing cholesterol levels with increasing dosage. Dose 1 (14 mg/g BW) lowered the average cholesterol level to 96.1%; dose 2 (28 mg/g BW) to 82.3%; and dose 3 (56 mg/g BW) to 76.2%, which is close to the drug's control effect.

Table. 2 Results of measurement of cholesterol levels in mice

Cholesterol Up Mencit (mg/dL)						
Test animals	**Negative control**	**Positive control**	**Drug control**	**Dose 1 (14 mg/g BW)**	**Dose 2 (28 mg/g BW)**	**Dose 3 (56 mg/g BW)**
1	120.9 (100%)	191.2 (158.1%)	130.6 (68.3%)	176.4 (71%)	136.5 (71.4%)	157.3 (82.3%)
2	125.5 (100%)	189.3 (150.8%)	128.5 (67.9%)	162.1 (85.6%)	147.2 (77.8%)	191.1 (101.0%)
3	102.4 (100%)	196.5 (191.9%)	120.6 (61.4%)	166.4 (172.4%)	130.5 (135.2%)	131.6 (136.4%)
4	99.3 (100%)	194.7 (196.1%)	117.5 (60.3%)	150.1 (77.1%)	121.1 (62.2%)	135.2 (69.4%)
5	116.8 (100%)	183.7 (157.3%)	96.2 (52.4%)	119.5 (65.1%)	133.6 (72.7%)	110.7 (60.3%)
6	111.5 (100%)	180.4 (161.8%)	112.5 (62.4%)	152.3 (84.4%)	140.2 (77.7%)	144.5 (80.1%)
Average	**100%**	**169.3 %**	**62.1 %**	**96.1 %**	**82.3 %**	**76.2 %**

The results in Table 2 show that the positive control group experienced an average increase in cholesterol levels of 169.3% compared to the negative control (100%), indicating that a hypercholesterolemia induction model has been successfully established. In contrast, the control group showed a significant reduction in cholesterol levels, averaging 62.1%, suggesting it can be used as a comparison for the effectiveness of the combination of extracts tested.

A combination of Calotropis gigantea and Adenanthera pavonina leaf extracts showed a trend of decreasing cholesterol levels with increasing dosage. At dose 1 (14 mg/g BW), the average cholesterol level decreased to 96.1%, indicating that the decrease remained relatively small and close to the negative control value. The effectiveness was more clearly seen at dose 2 (28 mg/g BW), with an average of 82.3%, and was even stronger at dose 3 (56 mg/g BW), with an average of 76.2%, approaching the drug's control effect. These results indicate a dose-dependent response, with higher doses providing more substantial antihypercholesterolemic effects.

The cholesterol-lowering effect in this study increased with increasing dose of the combination of extracts, indicating the antihypercholesterolemia activity of both plants. This finding is thought to be related to the bioactive compounds in Calotropis gigantea and Adenanthera pavonina, which are known to have cholesterol-lowering properties. C. gigantea contains flavonoids and phenolics (Devanesan & AlSalhi, 2024). Flavonoids have been reported to lower LDL cholesterol through antioxidant mechanisms, inhibition of HMG-CoA reductase, and increased bile acid metabolism, thus supporting

the findings of cholesterol reduction in this study. (Arifah et al., 2022). In addition, the presence of phenolic compounds also contributes to the reduction of cholesterol through increased excretion of bile acids (Suryani, 2025), which aligns with the trend toward lower cholesterol levels in the treatment group. Meanwhile, A. pavonina is known to be rich in phenolics, flavonoids, alkaloids, steroids, saponins, triterpenoids, and glycosides (Begum et al., 2023). Saponins have been reported to inhibit cholesterol reabsorption through binding with cholesterol and bile acids in the intestinal lumen (Al Amin et al., 2023), so this mechanism may explain the cholesterol reduction observed at higher doses of extracts in this study.

Based on the results of the normality and homogeneity tests, the cholesterol level data met the requirements for normality and homogeneity of variance, with p-values> 0.05. Furthermore, to assess the activity of the extract combination as an antihypercholesterolemia agent, the analysis was performed using a paired t-test. The study showed a p-value of 0.000 ($p < 0.05$), indicating a significant difference in cholesterol levels after combining C. gigantea and A. pavonina leaf extracts.

D. CONCLUSIONS AND SUGGESTIONS

This study demonstrates that the combination of ethanol extracts of *Calotropis gigantea* L. and *Adenanthera pavonina* L. leaves exhibits significant antihypercholesterolemia activity in mice. The positive control group showed a significant increase in cholesterol, while the drug control showed the optimal decrease. Combination administration of extracts showed a pattern of lowering cholesterol levels with increasing dosage, with the highest dose approaching the effectiveness of drug control. Statistical analysis using a paired T-test yielded a p-value of <0.05, indicating a significant difference in cholesterol levels before and after the administration of the extract, namely a substantial reduction in total cholesterol in each treatment group. This antihypercholesterolemia effect is thought to be related to the content of flavonoids, phenolics, and saponins, which act through antioxidant mechanisms, inhibition of HMG-CoA reductase, and increased cholesterol excretion through bile acid binding. Further research is suggested to examine long-term toxicity, blood biochemical parameters, and organ histopathology to ensure safety and expand understanding of the combined mechanism of action of *leaf extracts of Calotropis gigantea* L. and *Adenanthera pavonina* L., as an antihypercholesterolemia agent.

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