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Effectiveness Ethanol Extract of *Tinospora crispa* L, *Cymbopogon citratus*, *Alpinia purpurata K Schum*, and *Alpinia galanga* L in Lowering Mouse Cholesterol Levels

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ABSTRACT

Hypercholesterolemia is a lipid metabolism disorder that increases the risk of cardiovascular disease. Although statins are effective, long-term use may cause side effects, prompting the exploration of natural alternatives that contain bioactive flavonoids, phenolics, and terpenoids with hypocholesterolemic activity. This study aimed to evaluate the cholesterol-lowering effects of ethanol extracts of Brotowali (*Tinospora crispa* L.), Lemongrass Stem (*Cymbopogon citratus*), Red Galangal (*Alpinia purpurata K. Schum.*), and White Galangal (*Alpinia galanga* L.) in hypercholesterolemia-induced male mice. A true experimental study was conducted using 36 male mice divided into six groups: Negative Control (I), Positive Control (II), Drug Control (Simvastatin) (III), and three extract groups (IV–VI) receiving doses of 14, 28, and 56 mg/20 g BW. After a seven-day adaptation period, the animals were administered propylthiouracil for 14 days to induce hypercholesterolemia, which was confirmed by blood cholesterol measurement on Day 15. Subsequently, simvastatin or the extracts were administered for three days, and cholesterol levels were measured on Day 18 using a spectrophotometric method. The results showed that all extracts reduced cholesterol levels compared to the Positive Control (194.65 ± 8.26 mg/dL), but were less effective than simvastatin (103.15 ± 15.54 mg/dL). Brotowali reduced levels to 140.90 ± 10.10 mg/dL; Lemongrass Stem to 143.47 ± 21.65 mg/dL. Red Galangal reached 138.79 ± 10.69 mg/dL and White Galangal 139.03 ± 16.53 mg/dL at the highest dose (56 mg/20 g BW). In conclusion, the four ethanol extracts showed potential as natural antihypercholesterolemic agents, with Red and White Galangal demonstrating the greatest cholesterol-lowering effects at a dose of 56 mg/20 g BW, although their efficacy remained lower than simvastatin.

A. INTRODUCTION

Hypercholesterolemia is a specific type of hyperlipidemia characterised by metabolic disorders due to increased blood cholesterol levels (Wang et al., 2025). Individuals with hypercholesterolemia are at high risk of developing premature cardiovascular disease (Toft-Nielsen et al., 2025). Research by Analysed data collected from 2.144 patients with hypercholesterolemia (LDL cholesterol ≥180 mg/dL) who underwent clinical assessment and genetic diagnosis for familial hypercholesterolemia (FH) between 2000 and 2022 (Tada et al., 2025). Diet and lifestyle are significant predictors of hypercholesterolemia (Svendsen et al., 2025). Treatment of hypercholesterolemia is generally done by administering synthetic drugs such as statins. Still, long-term use can cause side effects, including muscle symptoms and liver dysfunction (Song et al., 2024). Therefore, safer and more effective treatment alternatives are needed, one of which is through the use of natural ingredients that contain bioactive compounds.

Several traditional medicinal plants have been studied for their potential to lower blood cholesterol levels. Brotowali (*Tinospora crispa* L.) is known to contain alkaloid compounds, glycosides, diterpenoids, triterpenoids, lactones, steroids, and flavonoids (Zuhri et al., 2024). *Tinospora crispa* is beneficial in treating conditions such as hyperglycemia, fatty liver disease, fever, jaundice, hyperlipidemia, and other related conditions (Sar et al., 2025). Lemongrass stems (*Cymbopogon citratus*) have phenolic and flavonoid content (Jaouad et al., 2025). Red galangal (*Alpinia purpurata K. Schum*.) contains eugenol, flavonoids, hydroxybenzoic acid, hydroxycinnamic acid, hydroxyphenyl propane, saponins, and tannins (Aisy et al., 2023). White galangal (*Alpinia galanga* L.) is rich in flavonoid compounds, essential oils, terpenes, and saponins that are reported to have antioxidant activity (Liu et al., 2025). According to Flavonoids from natural products are beneficial for treating hypercholesterolemia and are believed to have minimal side effects (Hanis et al., 2025).

Several previous studies have demonstrated that each of these plant extracts exhibits hypolipidemic activity (Abu et al., 2015). Report that *Tinospora crispa* L. extract lowers glucose levels in rats on a high-fat diet through improved insulin resistance. Research by demonstrates that *Cymbopogon citratus* extract is effective in reducing total cholesterol, triglycerides, LDL, and blood glucose levels, with minimal side effects (Aly et al., 2024). Meanwhile, It was found that *Alpinia galanga* L. extract was able to lower total cholesterol, triglycerides, and phospholipids, and increase HDL in Wistar mice (Achuthan & Padikkala, 1997). However, scientific studies that comprehensively evaluate the effectiveness of Brotowali ethanol extracts, lemongrass stems, red galangal, and white galangal on cholesterol levels in mice are still limited.

This study aimed to determine the effectiveness of ethanol extracts from Brotowali (*Tinospora crispa* L.), lemongrass stems (*Cymbopogon citratus*), red galangal (*Alpinia purpurata K. Schum*.), and white galangal (*Alpinia galanga* L.) in lowering cholesterol levels in hypercholesterolemic mice. Previous studies have mainly investigated the antihyperlipidemic effects of these plants individually. The novelty of the present study lies in the comparative evaluation of four ethanol plant extracts under the same experimental conditions using a hypercholesterolemic mouse model. This approach provides new evidence regarding the relative effectiveness of each extract in reducing blood cholesterol levels and identifies the most promising plant source for further development as a natural antihypercholesterolemic agent.

B. METHODS

This study employed an experimental design using a pretest–posttest control group model. In this design, subjects are randomly assigned to either the experimental group or the control group, and measurements are taken before and after the intervention (Yilmaz & Karaoglan Yilmaz, 2023). The following is a diagram of the research flow:

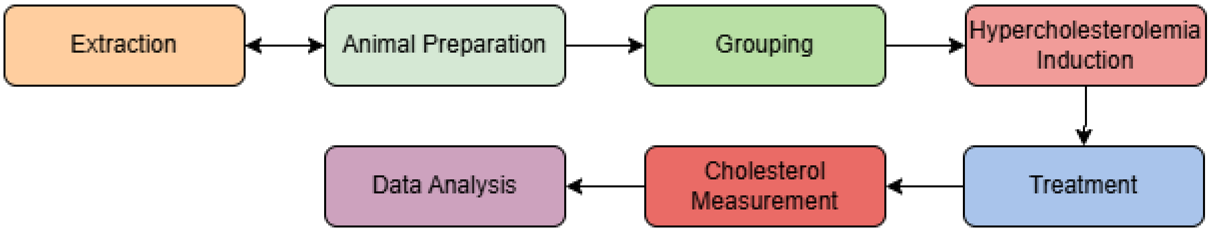

Figure 2. Research flow diagram

1. Extraction Process

Each leaf of Brotowali (*Tinospora crispa* L.), stems of Lemongrass (*Cymbopogon citratus*), Red Galangal (*Alpinia purpurata K. Schum*.), and White Galangal (*Alpinia galanga* L.) material was washed thoroughly under running water, air-dried without direct sunlight, and subsequently oven-dried (Sahu et al., 2025) for 2 hours until a constant dry weight was obtained. The dried simplicia were then ground into fine powder using a blender. Each powdered plant material was extracted separately using the maceration method. A total of 1 part simplicia powder was immersed in 4 parts of 96% ethanol (1:4 w/v) for 4 days (4 × 24 hours). Filtration was performed every 24 hours, and fresh solvent was added to remacerate the residue. All collected filtrates were pooled and concentrated using a rotary evaporator at 40 °C to obtain thick ethanol extracts. Extracts were stored in airtight containers at 4 °C until use.

2. Animal Preparation

The test animals used in this study were male mice (*Mus musculus*) with an average body weight of 20–30 grams and aged 2–3 months. Maintenance was carried out at the Animal Laboratory of Maarif Hasyim Latif University, Sidoarjo. Before treatment, mice underwent a 7-day acclimation period to adapt to their new environment (Li et al., 2025). The animals were fasted for 8 hours before induction. During the acclimatisation period, animals were housed under controlled temperature and humidity conditions, with a 12 h light/12 h dark cycle (Xiao et al., 2025). During rearing, mice were fed standard feed and drinking water ad libitum to meet their nutritional needs.

A propylthiouracil (PTU) suspension was prepared by weighing 1.04 g of PTU (Huang et al., 2024), triturating it in a mortar, and dissolving it in distilled water to a final volume of 50 mL; the homogenized suspension was then administered orally at a dose of 1 mL/animal/day for 14 consecutive days to induce hypercholesterolemia. Additionally, a simvastatin suspension was prepared by weighing 10 mg of simvastatin tablet powder (Hashim et al., 2025) and dissolving it in distilled water to a final volume of 10 mL. After homogenization, the suspension was administered orally at 1 mL/animal/day to the drug-control group. Hypercholesterolemia induction was confirmed on Day 15 by measuring serum cholesterol levels. The doses of PTU and simvastatin were selected based on previously published studies demonstrating their effectiveness in inducing hypercholesterolemia and reducing cholesterol levels in experimental animals (Huang et al., 2024; Hashim et al., 2025). The extract doses of

14, 28, and 56 mg/20 g BW were chosen to represent low, medium, and high dose levels in order to evaluate dose-dependent antihypercholesterolemic activity.

This study used six groups of mice, each consisting of six. The sample size was determined using the Federer formula [(t−1)(n−1) ≥ 15], where t represents the number of treatment groups and n represents the number of animals per group. Based on six experimental groups, a minimum of four mice per group was required. To improve the reliability of the results and to anticipate possible animal loss during the study, six mice were included in each group. Group I served as the negative control, Group II as the positive control, and Group III as the drug control. In contrast, groups IV, V, and VI received a combination treatment of Brotowali ethanol extract (*Tinospora crispa* L.), lemongrass stalks (*Cymbopogon citratus*), red galangal (*Alpinia purpurata K. Schum.*), and white galangal (*Alpinia galanga* L.) at doses of 14 mg/20 g BW, 28 mg/20 g BW, and 56 mg/20 g BW. Before treatment, all test animals were adapted to CP511 feed for seven days, then fasted for eight hours. Afterwards, the blood of the negative control group was taken for an initial cholesterol level check. The next day, groups II to VI were fed CP511 and propylthiouracil for 14 days to induce an increase in cholesterol levels. Then, on the 15th day, blood was taken to confirm the increase in cholesterol levels. Furthermore, group II was given only propylthiouracil, and group III received a combination of propylthiouracil and simvastatin. In contrast, groups IV, V, and VI received propylthiouracil and a combination of brotowali ethanol extract, lemongrass stalks, red galangal, and white galangal according to the predetermined dosage. The administration of simvastatin and a combination of extracts was carried out simultaneously for three days. On the 18th day, blood was taken to evaluate the decrease in cholesterol levels in mice.

Cholesterol levels were measured by disinfecting the tails of mice using a cotton swab soaked in alcohol. The tail was then extended and cut approximately 1 mm from the tip using sterile scissors to obtain a blood sample. The blood sample was collected in a 1 mL test tube and centrifuged at 3000 rpm for 10 minutes. The serum was separated from the blood cells after centrifugation (Motallebi et al., 2025). Total cholesterol levels were subsequently measured using the SINOWA BS-3000P Chemistry Analyzer according to the manufacturer's instructions. Prior to sample analysis, the SINOWA BS-3000P Chemistry Analyzer was calibrated according to the manufacturer's instructions to ensure the accuracy and reliability of cholesterol measurements. Commercial cholesterol reagent kits were used following standardized laboratory procedures, and all measurements were performed under identical analytical conditions to ensure the accuracy, validity, and reliability of the obtained results.

C. RESULT AND DISCUSSION

1. Extraction

The extraction results in **Table 1**, using an ethanol solvent on 500 g of simplicia powder, showed a variation in yield between samples. Brotowali (*Tinospora crispa* L.) produces an extract of 15 g with a yield of 3%, while lemongrass stalks (*Cymbopogon citratus*) produce 19.1 g with a yield of 3.8%. A higher yield value was obtained on red galangal (*Alpinia purpurata K. Schum.*) of 55.574 g with a yield of 11%, and the highest yield was achieved by white galangal (*Alpinia galanga* L.), which is 84 g with a yield of 16%.

Table 1. Ethanol Extract Preparation of Four Medicinal Plants

Simplisia	Powder Weight (g)	Extract Weight (g)	Rendemen (%)
Brotowali (*Tinospora crispa* L)	500 g	15 g	3 %
Lemongrass Stem (*Cymbopogon citratus*)	500 g	19.1 g	3.8 %
Red Galangal (*Alpinia purpurata K schum.*)	500 g	55.574 g	11 %
White Galangal (*Alpinia galanga* L)	500 g	84 g	16 %

In **Table 1**, the relatively low yield in brotowali and lemongrass stems can be caused by the low content of polar-semi-polar compounds that can be soluble in ethanol, so that only a small part of the active metabolites are successfully attracted by the solvent. Research Sukiman et al. (2022) reports indicate that the extraction method has a significant impact on the yield of *Tinospora crispa*, where optimisation of conditions can substantially increase yields. In *Cymbopogon citratus*, research Ashaq et al. (2024) shows that the yield of essential oils and extracts is influenced by the conditions of simplicia (fresh or dried), the extraction method, and the length of the process. This explains the low results in this study because the extraction was performed using ethanol, whereas most lemongrass studies employed more distillation methods for essential oils.

On the other hand, white galangal yields the highest results because it is known to contain considerable amounts of essential oil, flavonoids, and phenolics, which are generally polar to semi-polar, making them easier to extract with ethanol. Research by Na Nongkhai et al. (2024) indicates that the ethanol extract of *Alpinia galanga* yields a high quantity of diverse active compounds. Red galangal yields a considerable amount, although it is still lower than that of white galangal. Research related to *Alpinia purpurata* shows the presence of hydrosols that are strongly influenced by heating temperature and distillation time. Therefore, it is essential to determine the optimal heating temperature and distillation time to obtain a high-quality hydrosol (Sivem et al., 2022).

The use of ethanol as a solvent in this study is particularly relevant because ethanol is a universal solvent that can dissolve a wide range of active compounds, from polar to semi-polar. Other factors that also affect the yield are the moisture content of the simplicia, the maceration technique used, and the condition of the simplicia powder, such as particle size and dryness level. This is in line with a review by Ozdemir et al. (2024) this emphasises that solvent selection, temperature, and material-to-solvent ratio are essential factors in determining yield.

2. Effectiveness of Reducing Cholesterol Levels In Hypercholesterolemia Rats

Based on the results of **Table 2**, which measured the total cholesterol levels of rats after treatment, it was found that the negative control group had a cholesterol level of 106.41 ± 12.81 mg/dL, the positive control group had 194.65 ± 8.26 mg/dL, and the drug control group had 103.15 ± 15.54 mg/dL. Brotowali extract showed cholesterol levels of 160.51 ± 10.39 mg/dL (dose of 14 mg/20 g BW), 147.99 ± 5.69 mg/dL (28 mg/20 g BW), and 140.90 ± 10.10 mg/dL (56 mg/20 g BW). Lemongrass Stem Extract yielded values of 153.82 ± 29.17 mg/dL, 148.18 ± 17.07 mg/dL, and 143.47 ± 21.65 mg/dL. Red Galangal Extract of 151.04 ± 11.28 mg/dL, 151.15 ± 10.19 mg/dL, and 138.79 ± 10.69 mg/dL. Meanwhile, White Galangal extract showed values of 155.55 ± 8.32 mg/dL, 152.44 ± 12.94 mg/dL, and 139.03 ± 16.53 mg/dL.

Table 2. Cholesterol Levels in Rats After Extract Treatment

No.	Negative Control	Positive Control	Drug Control	Dose 1 14mg/20g BW	Dose 2 28mg/20g BW	Dose 3 56mg/20g BW
Brotowali Extract (*Tinospora crispa* L)						
1	112.49	184.71	77.68	174.53	150.43	146.45
2	101.21	196.21	99.29	165.18	146.66	130.67
3	98.81	189.40	115.41	169.34	151.23	153.4
4	86.71	208.23	96.88	149.46	155.36	128.74
5	116.32	192.49	122.72	155.62	137.76	135.61
6	124.91	196.87	109.91	146.91	144.52	148.54
Mean ± SD	106.41 ± 12.81	194.65 ± 8.26	103.15 ± 15.54	160.51 ± 10.39	147.99 ± 5.69	140.90 ± 10.10
Lemongrass Stem Extract (*Cymbopogon citratus*)						
1	112.49	184.71	77.68	102.15	147.81	115.80
2	101.21	196.21	99.29	168.22	121.96	130.63
3	98.81	189.40	115.41	138.81	135.34	127.91
4	86.71	208.23	96.88	165.82	168.82	168.82
5	116.32	192.49	122.72	170.61	153.64	152.22
6	124.91	196.87	109.91	177.32	163.52	167.46
Mean ± SD	106.41 ± 12.81	194.65 ± 8.26	103.15 ± 15.54	153.82 ± 29.17	148.18 ± 17.07	143.47 ± 21.65
Red Galangal Extract (*Alpinia purpurata K Schum.*)						
1	112.49	184.71	77.68	157.43	135.21	131.24
2	101.21	196.21	99.29	132.91	155.81	143.63
3	98.81	189.40	115.41	164.21	159.40	139.81
4	86.71	208.23	96.88	153.70	137.25	156.61
5	116.32	192.49	122.72	149.11	161.89	130.29
6	124.91	196.87	109.91	148.89	157.32	129.15
Mean ± SD	106.41 ± 12.81	194.65 ± 8.26	103.15 ± 15.54	151.04 ± 11.28	151.15 ± 10.19	138.79 ± 10.69
White Galangal Extract (*Alpinia galanga* L)						
1	112.49	184.71	77.68	165.05	130.34	112.71
2	101.21	196.21	99.29	149.21	158.21	139.20
3	98.81	189.40	115.41	152.81	153.31	134.21
4	86.71	208.23	96.88	163.73	166.34	160.11
5	116.32	192.49	122.72	159.32	144.67	157.62
6	124.91	196.87	109.91	145.19	161.86	132.33
Mean ± SD	106.41 ± 12.81	194.65 ± 8.26	103.15 ± 15.54	155.55 ± 8.32	152.44 ± 12.94	139.03 ± 16.53

The results of **Table 2** shows that there was a difference in total cholesterol levels between mice given Brotowali extract, Lemongrass Stems, Red Galangal, and White Galangal compared to the Control Group. The negative control group had cholesterol levels of 106.41 ± 12.81 mg/dL, while the positive control achieved the highest values, which were 194.65 ± 8.26 mg/dL. This suggests that the induction of hypercholesterolemia successfully increases cholesterol levels (Tada & Harada-Shiba, 2025). The control group of the drug (simvastatin) showed levels of 103.15 ± 15.54 mg/dL, close to negative control values. This difference confirms that the administration of the extract has a cholesterol-lowering effect compared to the positive control. However, the effect has not been matched by the control of the drug.

Statistical analysis showed that cholesterol level data were normally distributed and homogeneous ($p > 0.05$). One-way ANOVA revealed a significant difference among groups ($p < 0.05$), indicating that the treatments affected cholesterol levels. Post hoc analysis confirmed significant differences between the negative and positive control groups, the positive control and drug control, the drug control and all extract dose groups, as well as between the negative control and all extract dose groups. However, no significant differences were observed among the extract dose groups (doses 1, 2, and 3).

Brotowali extract exhibits a stable cholesterol-lowering pattern as the dose increases. At 14 mg/20 g BB, the level reached 160.51 ± 10.39 mg/dL, decreased to 147.99 ± 5.69 mg/dL at a dose of 28 mg/20 g BB, and reached 140.90 ± 10.10 mg/dL at 56 mg/20 g BB. This pattern confirms the dose-dependent effect, as reported by Batra et al. (2023) this indicates that increasing the dosage of *Tinospora crispa* extract enhances its pharmacological activity. Brotowali contains flavonoids, alkaloids, and diterpenoids that are antioxidants (Sanpinit et al., 2023) and can inhibit the synthesis of cholesterol in the liver (AMOM et al., 2011). Although the reduction achieved was consistent, the value was still higher than that of drug control.

Lemongrass Stem Extract also showed a downward trend, with values of 153.82 ± 29.17 mg/dL at 14 mg/20 g BB, 148.18 ± 17.07 mg/dL at 28 mg/20 g BB, and 143.47 ± 21.65 mg/dL at 56 mg/20 g BB. Considerable SD values, especially at low doses, showed variation in response between test animals. The main components of lemongrass stems are citral, flavonoids, and phenolics (Jaouad et al., 2025). It is known to have hypocholesterolemia activity through antioxidant mechanisms and inhibition of HMG-CoA reductase (Somparn et al., 2018).

Red Galangal Extract shows a different pattern. At a dose of 14 mg/20 g BB, cholesterol levels were 151.04 ± 11.28 mg/dL, which is almost the same as the dose of 28 mg/20 g BB, at 151.15 ± 10.19 mg/dL. A more pronounced decrease occurred at the dose of 56 mg/20 g BB, which was 138.79 ± 10.69 mg/dL. This suggests that the hypocholesterolemia effects of red galangal appear significant at high doses. Flavonoid compounds, essential oils, and phenolics in red galangal (Aisy et al., 2023) plays a role in lowering cholesterol by increasing bile acid excretion and inhibiting lipogenesis.

White Galangal Extract produces cholesterol levels of 155.55 ± 8.32 mg/dL at a dose of 14 mg/20 g BB, decreased to 152.44 ± 12.94 mg/dL at a dose of 28 mg/20 g BB, and reached 139.03 ± 16.53 mg/dL at 56 mg/20 g BB. Similar to red galangal, a more pronounced decrease occurs at the highest dose. This is consistent with research who evaluated *Alpinia galanga* as an antidyslipidemia (Manasa & Tumaney, 2022).

When comparing extracts at high doses (56 mg/20 g BB), the cholesterol levels of mice decreased to 140.90 mg/dL (Brotowali), 143.47 mg/dL (Lemongrass Stem), 138.79 mg/dL (Red Galangal), and 139.03 mg/dL (White Galangal). This value indicates that Red Galangal and White Galangal have a more substantial cholesterol-lowering effect than Brotowali and Lemongrass Stems. However, they have not been able to match the effectiveness of simvastatin (103.15 mg/dL). Thus, the entire extract has potential as a natural antihypercholesterolemic agent, but its potency is still below that of standard therapy.

D. CONCLUSION AND SUGGESTIONS

Based on the results of this study, ethanol extraction of four medicinal plants produced different yield values, with white galangal (*Alpinia galanga L.*) showing the highest yield (16%), followed by red galangal (*Alpinia purpurata K. Schum.*) (11%), lemongrass stem (*Cymbopogon citratus*) (3.8%), and brotowali (*Tinospora crispa L.*) (3%). The administration of these extracts reduced total cholesterol levels in hypercholesterolemic rats compared with the positive control group. Statistical analysis showed that the data were normally distributed and homogeneous ($p > 0.05$), and one-way ANOVA indicated a significant difference among treatment groups ($p < 0.05$), confirming that the treatments influenced cholesterol levels. Post hoc analysis revealed significant differences between the negative and positive control groups, the positive control and drug control, and between the positive control and all extract-treated groups, while no significant differences were observed among the extract dose groups. At the highest dose (56 mg/20 g BW), red galangal and white galangal showed slightly greater reductions in cholesterol levels (138.79 mg/dL and 139.03 mg/dL, respectively) compared with brotowali (140.90 mg/dL) and lemongrass stem (143.47 mg/dL), although all extracts were less effective than the drug control (simvastatin). These findings indicate that the tested plant extracts may have potential as natural antihypercholesterolemic agents. Further studies are recommended to optimize extraction techniques, identify and characterize the active compounds responsible for the observed effects, and evaluate additional lipid profile parameters such as LDL, HDL, and triglycerides with broader dose variations and longer treatment periods.

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