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Anticholesterol activity of arumanis mango (*Mangifera indica* L.) and white turmeric (*Curcuma mangga* Val.) extract in dietary rats

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Abstract

Altered plasma cholesterol levels, a common indicator of susceptibility to cardiovascular disease, characterize hypercholesterolemia. Currently, health issues have shifted from infectious diseases to degenerative diseases. Chemical treatments often present numerous side effects for patients, prompting a growing interest in traditional medicines. Natural medicinal plants are now widely available and offer fewer side effects as an alternative form of treatment. Since they have been used traditionally for treating ailments, particularly dyslipidemia, research has been done on the possible therapeutic effects of arumanis mango (*Mangifera indica* L.) leaves and white turmeric (*Curcuma mangga* Val.) This study aimed to evaluate the cholesterol-lowering effects of a combination of arumanis mango leaf extract and white turmeric extract in hypercholesterolemic rats. To simulate cholesterol elevation, rats fed a yeast-enriched diet are used in this study to test the cholesterol-lowering effectiveness of a mixture of extracts from the leaves of arumanis mango and white turmeric. This research adopted an experimental laboratory method with a pre-post test design involving four groups: Group I as the normal control, Group II as the negative control, Group III as the positive control treated with 100 mg/kg body weight fenofibrate, and Group IV as the treatment group induced with yeast-enriched feed for 28 days and subsequently treated with a combination of arumanis mango leaf extract and white turmeric extract at a dose of 150 mg/kg body weight. The results showed a substantial difference between the negative control and treatment groups, with a 55.66% reduction in cholesterol in the test animals. This suggests that combining arumanis mango leaf extract and white turmeric extract reduces cholesterol levels in the test animals.

1. Introduction

Alterations in plasma cholesterol levels characterize hypercholesterolemia, a common indicator associated with an increased risk of cardiovascular disease. Currently, health concerns have shifted from infectious diseases to degenerative diseases, which are believed to be influenced by lifestyle changes in the millennial generation. Contributing factors include smoking from an early age, poor dietary habits, environmental factors, lack of physical activity, excessive consumption of fat and cholesterol-rich foods, insufficient fiber intake, and genetic factors, all of which can trigger degenerative diseases (Yani *et al.*, 2020).

Hypercholesterolemia is a key factor that leads to various other health complications due to the lack of attention given to its management. According to the Indonesian Endocrinology Association (PARKENI), the management of hypercholesterolemia includes non-pharmacological interventions known as Therapeutic Lifestyle Changes (TLC) and the use of cholesterol-lowering drugs (Mahwal *et al.*, 2022).

One potential natural remedy for hypercholesterolemia is arumanis mango and white turmeric. Arumanis mango is a widely cultivated variety in Indonesia known for its sweet taste and aromatic fragrance. Beyond its fruit, the leaves of arumanis mango contain bioactive compounds such as flavonoids, tannins, and triterpenoids, which have been studied for their pharmacological properties, including anti-diabetic, anti-cancer, anti-diarrheal, antibacterial, anti-hyperlipidemic, renoprotective, and analgesic effects (Mahdiyah and Husni, 2019). Meanwhile, white turmeric is a lesser-known member of the *Curcuma* genus that has been traditionally used in herbal medicine. It contains curcuminoids and essential oils that exhibit anti-inflammatory, antioxidant, and cholesterol-lowering

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properties.

Based on research findings, it can be concluded that the extract of arumanis mango leaves and white turmeric has potential as an anti-dyslipidemia agent. The flavonoid, triterpenoid, and tannin content in these plants is thought to reduce lipid levels in the blood. Additionally, tannins act as antioxidants, which may prevent endothelial dysfunction that could lead to elevated total cholesterol, triglycerides, and LDL levels (Nugraha and Hasanah, 2018).

Given these research results, further studies are necessary to examine the combined effects of these two plants, as their potential anti-cholesterol activity makes them promising candidates for use in combination therapy. Therefore, this study aimed to explore the therapeutic effects of a combination of arumanis mango leaf extract and white turmeric extract.

2. Materials and methods

2.1 Tools and materials

The tools used in this study include injection syringes, pots, stoves, beakers, measuring cylinders, stirring rods, test tubes, drop pipettes, analytical scales, evaporators, and cholesterol measurement devices. The materials used consist of NaCMC, a fenofibrate suspension (100 mg/kg BW), a combination of arumanis mango leaf extract and white turmeric extract, yeast-enriched feed, and 24 male rats as test animals.

2.2 Preparation of simplisia

The preparation of simplisia and extraction was carried out using the maceration method with 70% ethanol as a solvent to obtain a concentrated extract of arumanis mango and white turmeric. The process involved weighing 100 g of each dried powdered simplisia and submerging it in 1 L of 70% ethanol in a maceration vessel. The mixture was stirred for 20 min daily over five days to ensure complete extraction. Afterwards, the extracts were concentrated using a rotary evaporator until a thick extract was obtained (Djoko *et al.*, 2020).

2.3 Extraction process

The extraction was performed using the maceration method. Every 100 g of dried powdered simplisia was soaked in 1 L of 70% ethanol in a maceration vessel for five days, with daily stirring for 20 min to ensure complete extraction (Rawung *et al.*, 2020).

2.4 Extract standardization

After extraction, standardization and phytochemical screening were performed to identify secondary metabolites using the tube test method. This allowed for the detection of the secondary metabolites present in the arumanis mango leaf extract and white turmeric extract.

2.5 Phytochemical screening of extract

Phytochemical screening tests were conducted for flavonoids, tannins, saponins, and terpenoids. The flavonoid test involved adding 10 mL of water to the arumanis mango leaf extract. The mixture was heated for 5 min, filtered, and the filtrate was added to 0.1 g of Mg, 1 ml of concentrated HCl, and 1 ml of amyl alcohol. The solution was shaken vigorously, and the presence of flavonoids was indicated by a red, yellow, or orange color in the amyl alcohol layer (Ramadhan *et al.*, 2020).

The tannin test involved dissolving arumanis mango and white turmeric extracts in 100 mL of hot water, cooling the solution, and filtering it. Approximately 5 mL of filtrate were added to a test tube with 3 drops of 1% $FeCl_3$ solution. A positive result was indicated by a violet-green or green-black color upon reaction with $FeCl_3$ (Widiastuti *et al.*, 2023).

For the saponin test, 0.5 g of extract was dissolved in 10 mL of hot distilled water, cooled, and shaken vigorously for 10 s. A positive reaction was confirmed by the formation of foam for 10 min with a height of 1-10 cm, and the foam remained after the addition of 1 drop of 2 N hydrochloric acid (Ramadhan *et al.*, 2020).

The test for triterpenoids and steroids was conducted using the Lieberman-Burchard reaction. In this test, 0.5 g of extract was dissolved in 0.5 mL of chloroform, followed by the addition of 0.5 mL of acetic anhydride. Approximately 2 mL of concentrated sulfuric acid was then added along the wall of the test tube. A brown or violet ring at the boundary of the solution indicated the presence of triterpenoids, while a greenish-blue ring indicated the presence of steroids (Hasim *et al.*, 2019).

2.6 Anti-cholesterol activity assay

Cholesterol levels were measured in male Wistar rats (*Rattus norvegicus*) induced with yeast-enriched feed. Blood samples were taken from the caudal veins on day 0 after induction, on day 3 after induction with yeast-enriched feed and administration of the arumanis mango leaf and white turmeric extract combination, and subsequently on days 7, 14, 21, and 28. Blood sampling was conducted at the Toxicology Pharmacology Laboratory of Universitas Muhammadiyah Gombong. The treatment groups were categorized based on the duration of extract administration as follows: P1 (day 1 of treatment), P2 (day 7), P3 (day 14), P4 (day 21), and P5 (day 28).

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2.7 Data analysis

The percentage reduction in cholesterol levels was calculated using the equation. The calculated percentage reductions were analyzed statistically using SPSS software (version 16), with a comparative analysis conducted through a One-Way ANOVA test at a 95% confidence level. A Post Hoc test was then performed to determine significant differences between treatment groups. A p-value of less than 0.05 indicated a statistically significant difference between the groups.

$$\% \text{ Reduction} = \frac{\text{Initial cholesterol level (H0)} - \text{Final cholesterol level (H28)}}{\text{Initial cholesterol level (H0)}} \times 100\%$$

3. Results

3.1 Extraction

The determination of arumanis mango leaves and white turmeric was conducted at the Faculty of Biology, Ahmad Dahlan University, Yogyakarta, Indonesia. Before the collection of materials, an ethical approval was submitted and granted with the approval number: 022402020 from the Research Ethics Committee of Ahmad Dahlan University, Yogyakarta. The preparation of simplisia was followed by extraction using the maceration method. The yield obtained from arumanis mango leaves was 35.19 g, with a yield percentage of 35.19%, while the yield from white turmeric was 30.19%. The extraction results are summarized in Table 1.

3.2 Phytochemical screening

Phytochemical screening was conducted to identify the secondary metabolites present in arumanis mango leaf extract and white turmeric extract using the tube test method. The results, summarized in Table 2, confirm previous findings that the use of 70% ethanol as an extraction solvent is more effective in isolating secondary metabolites from both arumanis mango leaves and white turmeric extract (Nugraha *et al.*, 2022).

3.3 Anti-cholesterol activity

The cholesterol levels were measured in male Wistar rats that had been induced with cholesterol through a yeast-enriched feed. Blood samples were collected from the caudal veins on day 0 (baseline), day 3 after induction with yeast-enriched feed, and following the administration of a combination of arumanis mango leaf extract and white turmeric extract on days 7, 14, 21, and 28. The findings suggest that the administration of yeast-enriched feed affects blood cholesterol levels, as seen in Table 3.

4. Discussion

The preparation of simplisia followed by extraction using the maceration method with 70% ethanol as the solvent resulted in a concentrated extract of arumanis mango and white turmeric. The extraction process involved weighing 100 g of each dried powdered simplisia, which was then submerged in 1 L of 70% ethanol in a maceration vessel. The mixture was stirred daily for 20 min over five days to ensure complete extraction (Hradaya and Husni, 2021). The extract was concentrated using a rotary evaporator to obtain a thick extract, yielding 35.19 g from arumanis mango leaves and 30.19 g from white turmeric extract. Studies suggest that using a higher ethanol concentration during maceration increases quercetin content in the extract (Ramadhan *et al.*, 2020). These findings indicate that solvent concentration plays a crucial role in maximizing the extraction of bioactive compounds, such as quercetin, supporting ethanol as an effective medium for obtaining secondary metabolites from plant materials.

Phytochemical analysis confirmed the presence of

Table 1. Extraction results of arumanis mango and white turmeric.

	Weight (g)	Solvent (L)	Thick Extract (g)	Yield (%)	Requirements
Arumanis mango leaves	100	1	35.19	35.19	<7.2%
White turmeric	100	1	30.19	30.19	<7.2%

Source: Yani *et al.* (2020).

Table 2. Phytochemical screening results of arumanis mango and white turmeric.

Extract	Parameter	Reagent	Color reaction	Result
Arumanis mango leaves	Flavonoid	NaOH 10%	Dark red	+
	Tannin	$FeCl_3$	Blackish green	+
	Saponin	-	Foamy	+
	Terpenoid	Concentrated H_2SO_4	Blue ring	+
White turmeric	Flavonoid	NaOH 10%	Dark red	+
	Tannin	$FeCl_3$	Blackish green	+
	Saponin	-	Foamy	+
	Terpenoid	Concentrated H_2SO_4	Greenish blue ring	+

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Table 3. Blood cholesterol levels (mg/dL) for each group.

Group	P0	P1	P2	P3	P4	P5	%Reduction
Normal control	73.68±3.04	74.85±3.65	74.98±3.66	83.17±8.97	81.15±6.76	132.02±62.34	-
Negative control	74.58±2.58	203.5±5.99	199.83±11.29	203.5±5.99	109.33±11.70	246±66.89	71.28
Positive control (100 mg/kgBW fenofibrate)	75.57±2.15	74.97±2.36	79.00±9.06	80.40±7.74	83.17±8.97	75.67±4.19	74.57
Combination of arumanis mango and white turmeric extract	75.92±2.27	175.83±13.67	175.83±13.67	165.83±16.44	127.33±6.10	96.67±24.09	74.65

Values are presented as mean±SD. P0: baseline; P1: treatment day 1; P2: treatment day 7; P3: treatment day 14; P4: treatment day 21; and P5: treatment day 28.

key bioactive compounds in the extracts. The flavonoid test was positive, indicated by the appearance of red, yellow, or orange coloration in the amyl alcohol layer, while the tannin test showed positive results with a violet -green or green-black color upon reacting with $FeCl_3$ (Nugraha *et al.*, 2022). The presence of saponins was confirmed by foam formation persisting for 10 min, and the triterpenoid and steroid tests, using the Lieberman-Burchard reaction, indicated triterpenoids with a brown or violet ring and steroids with a blue-green ring (Rawung *et al.*, 2020). These results highlight the potential of arumanis mango and white turmeric as sources of bioactive compounds with therapeutic properties, particularly in cholesterol reduction and antioxidant activity.

Blood sampling conducted at the Pharmacology and Toxicology Laboratory, Muhammadiyah Gombong University, revealed significant differences in cholesterol levels in rats induced with yeast-enriched feed and treated with the combination extract compared to the positive control group. Figure 1 illustrates the cholesterol levels (mg/dL) in test animals divided into four groups: K1 (negative control), K2 (positive control), K3, and K4 (treatment groups administered a combination of arumanis mango extract and white turmeric extract at a dosage of 150 mg/kg BW). The positive control group (K2) demonstrates the highest cholesterol levels among all groups, whereas the treatment groups (K3 and K4) show a marked reduction in cholesterol levels compared to the positive control. Statistical analysis confirmed that the group treated with 150 mg/kg BW of arumanis mango leaves and white turmeric extracts exhibited significantly stronger anti-cholesterol activity than the negative control group (p<0.05). The reduction in blood cholesterol levels observed in rats induced with yeast-enriched feed demonstrated a relatively higher percentage decrease in the combination extract group. ANOVA results suggested that yeast-enriched feed influences blood cholesterol levels, though limitations such as variations in feed intake and initial glucose levels introduced variability in cholesterol data.

Despite these limitations, the combination of

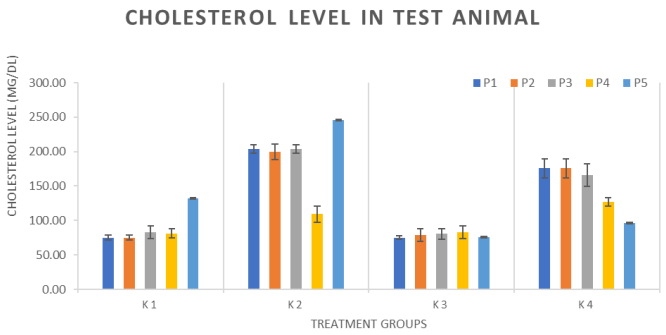

Figure 1. Results of average total cholesterol levels measured using the Easy Touch strip device. K1: normal control; K2: negative control; K3: positive control treated with 100 mg/kg body weight fenofibrate; K4: Combination of arumanis mango extract and white turmeric extract dosage 150 mg/kg BW; P1: treatment day 1; P2: treatment day 7; P3: treatment day 14; P4: treatment day 21; and P5: treatment day 28.

arumanis mango leaves and white turmeric extracts demonstrated promising potential as a natural anti-cholesterol agent. The flavonoid content in the extracts is believed to suppress blood lipid levels, while tannins act as antioxidants that may inhibit endothelial dysfunction, preventing increases in total cholesterol, triglycerides, and LDL levels (Nugraha *et al.*, 2022). Additionally, arumanis mango leaf extract has been shown to reduce lipid levels, decrease intracellular lipid content, and enhance adiponectin production, making it a potential pharmacological agent for obesity or metabolic syndrome (Sferrazzo *et al.*, 2019). Studies also indicate that arumanis mango leaves possess strong antioxidant and anti-inflammatory properties, which contribute to lowering cholesterol and lipid levels while reducing inflammation associated with dyslipidemia (Kutawa *et al.*, 2021).

These findings suggest that a combination of arumanis mango leaf extract at 150 mg/kg BW and white turmeric extract at 150 mg/kg BW holds strong potential as an anti-cholesterol agent through its antioxidant and anti-inflammatory mechanisms. The lipid-lowering effects and safety of long-term use make this combination a promising candidate as an alternative or adjunctive therapy for cholesterol management, particularly in yeast-enriched diet-induced models, as

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illustrated in Figure 2.

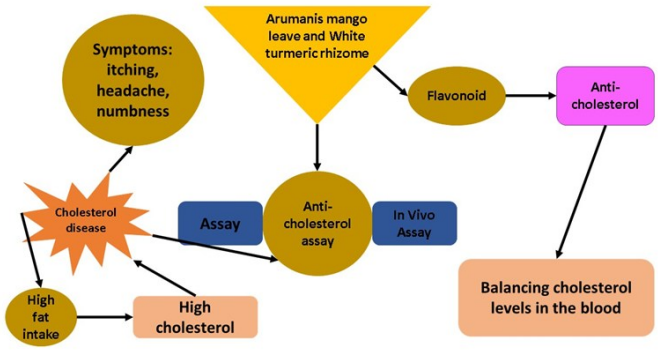

Figure 2. Flowchart of anti-cholesterol agent assay.

4. Conclusion

The study demonstrated that the combination of arumanis mango leaf extract and white turmeric extract exhibits anti-cholesterol activity in rats fed with yeast-enriched feed. Administration of the extract combination at 150 mg/kg BW reduced cholesterol levels compared to the negative control and showed a reduction comparable to the positive control (fenofibrate). These findings suggest that this combination has potential as a natural cholesterol-lowering agent, although further studies, including statistical analysis, are required to confirm its significance.

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Conflict of interest

The authors declare no conflict of interest.

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