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In Vitro Cholesterol-Reducing Activity of Ethanolic Extract of Namnam Leaves (*Cynometra cauliflora* L)

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ABSTRACT

Hypercholesterolemia remains an important risk factor for cardiovascular disease and often requires long-term therapy, which may be accompanied by unwanted effects, including myopathy and hepatotoxicity. This has driven interest in safer plant-derived alternatives. Namnam leaves (Cynometra cauliflora L) contain flavonoids, saponins, and tannins, and an in vivo study reported lipid-lowering effects of their ethanol extract; however, no study has directly evaluated their in vitro anticholesterol activity. This study aimed to evaluate the in vitro anticholesterol activity of ethanolic extract of namnam leaves (Cynometra cauliflora L) and determine its EC50 value. The leaves were macerated with 96% ethanol, and extract was tested using the liebermann-burchard reaction measured by UV-Vis spectrophotometry. Anticholesterol activity was assessed at 100, 200, 300, 400, and 500 ppm, with simvastatin as the positive control. Data were analyzed using two-way ANOVA followed by Tukey's HSD test ($\alpha = 0.05$). The extract showed increasing cholesterol reduction with increasing concentration, with mean (± SD) inhibition values of 7.99 ± 0.19%, 21.44 ± 0.12%, 35.27 ± 0.19%, 51.20 ± 0.19%, and 67.45 ± 0.19%, respectively. Linear regression yielded y=0.1487x-7.9805 (R=0.998), and the EC50 value was 389.916 ppm, compared with 148.94 ppm for simvastatin, indicating that simvastatin was approximately 2.6-fold more potent. At 500 ppm, the extract's inhibition did not differ significantly from that of simvastatin at 400 ppm ($p = 1.000$). Phytochemical screening confirmed the presence of flavonoids, saponins, tannins, and steroids in the extract. These findings indicate concentration-dependent in vitro cholesterol-reducing activity of the ethanolic extract of namnam leaves in this chemical assay, which warrants further mechanistic and biological confirmation before any therapeutic or pharmacological equivalence with simvastatin can be inferred.

Keyword: *Anticholesterol; namnam leaves; EC_{50}; in vitro*

Introduction

Hypercholesterolemia, defined as a total blood cholesterol concentration exceeding 240 mg/dL, has become increasingly prevalent due to lifestyle changes such as habitual high-fat diet consumption(Lestari et al., 2020). Globally, it affects 37% of adult men and 40% of adult women, with prevalence highest in Western Europe (~54%) (Lestari et al., 2020); in Indonesia, the 2023 National Health Survey reported that 27.8% of the population aged ≥15 years had borderline cholesterol levels and 11.7% were in the high-risk category(Badan Kebijakan Pembangunan

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Kesehatan, 2023), with aging, sex, body mass index, and blood pressure among the key contributing factors (Bijani et al., 2020). Elevated cholesterol drives atherosclerosis, in which lipid plaques accumulate in arterial walls and raise blood pressure (Suci & Adnan, 2020), frequently coexisting with hypertension (Ivanovic & Tadic, 2015) and contributing to coronary heart disease, stroke, and metabolic syndrome, making it one of the principal modifiable risk factors for cardiovascular morbidity and mortality worldwide.

Management of hypercholesterolemia generally combines non-pharmacological and pharmacological strategies. Dietary modification remains the first-line approach, with recommendations limiting daily cholesterol intake to below 300 mg, total fat to less than 25% of daily energy requirements, and saturated fat to under 10% of total energy intake (Yugharyanti et al., 2024). Reduction of low-density lipoprotein (LDL) cholesterol has become the principal target of lipid-lowering therapy as a preventive measure against cardiovascular disease (Sianto et al., 2022). Pharmacologically, synthetic agents such as atorvastatin, nicotinic acid, and fenofibrate are widely prescribed; however, their long-term use has been associated with adverse effects, including myopathy, renal impairment, and hepatotoxicity (Pradana & Suryanto, 2017). These limitations have driven growing scientific interest in safer, plant-derived alternatives capable of lowering cholesterol with a more favorable safety profile.

Indonesia, as a tropical country with abundant biodiversity, harbors numerous medicinal plants containing secondary metabolites with potential lipid-lowering properties. Flavonoids represent one such class of compounds, exerting cholesterol-lowering effects through a mechanism analogous to that of statins, namely by downregulating the expression of the enzyme HMG-CoA reductase while upregulating LDL receptor (LDLR) gene expression (Abbas et al., 2024). It should be noted that these enzymatic and receptor-level mechanisms have been established in prior biological studies and were not directly measured by the chemical assay used in the present study; they are presented here as literature-based rationale rather than as mechanisms demonstrated by the present findings. Empirical support for this potential has been demonstrated in several local plants; for instance, the ethanol extract of gedi leaves (*Abelmoschus manihot* (L.) Medik) exhibited in-vitro cholesterol inhibition of up to 50.33% at a concentration of 5,007.8 ppm (Ilyas et al., 2020), underscoring the feasibility of screening Indonesian flora for novel anticholesterol agents.

Namnam (*Cynometra cauliflora* L.) is a native Southeast Asian species traditionally consumed as food and used as an herbal remedy. Phytochemical screening of its leaves has revealed pharmacologically relevant concentrations of flavonoids, saponins, phenolics, tannins, and steroids (Anliza & Rachmawati, 2024), while methanolic leaf extracts have further been shown to contain

proanthocyanidins, catechins, and flavonoid glycosides such as vitexin, isovitexin, and quercetin-3-O-glucoside (Ado et al., 2015). These constituents have been linked to a range of pharmacological activities, including potent antioxidant, antihyperglycemic, and anticholinesterase effects (Ado et al., 2015) and an in-vivo study further reported that ethanol extract of *C. cauliflora* leaves reduced body weight, blood triglycerides, and LDL levels in obese mice, with vitexin proposed as the principal compound responsible for this antihyperlipidemic effect (Seyedan et al., 2019). Given that flavonoids are central to the cholesterol-lowering mechanism described above, and that namnam leaves are rich in flavonoid compounds, this plant represents a promising yet largely unexplored candidate for anticholesterol agent development.

Despite this favorable phytochemical profile and preliminary evidence of lipid-modulating activity in an in-vivo model, no study to date has specifically evaluated the in-vitro anticholesterol activity of namnam leaf extract, and existing pharmacological reports on this species remain confined to its antioxidant, antidiabetic, and antihyperlipidemic effects rather than direct cholesterol-inhibitory capacity. This represents a clear research gap, as an in-vitro assay is essential to establish a direct, mechanism-level indication of cholesterol-binding or cholesterol-inhibitory potential prior to more resource-intensive in-vivo confirmation. To address this gap, the present study evaluated the in-vitro anticholesterol activity of the ethanol extract of namnam leaves using the Liebermann–Burchard colorimetric method coupled with UV-Vis spectrophotometry, with simvastatin employed as the positive control. The extract's inhibitory activity was quantified across a range of concentrations to determine its half-maximal effective concentration (EC_{50}), thereby providing the first empirical evidence on the anticholesterol potential of *Cynometra cauliflora* L. leaves and contributing novel baseline data to support its future development as a plant-based lipid-lowering agent. It is important to clarify that the Liebermann–Burchard assay employed here is a chemical, non-cellular reaction that measures the reduction of free cholesterol available to react with the reagent, and does not, by itself, demonstrate an enzymatic or receptor-mediated mechanism of action. Simvastatin was included in this chemical assay as a benchmark positive control for comparative potency rather than as evidence that the extract and simvastatin share the same mechanism of action, since simvastatin exerts its clinical cholesterol-lowering effect through HMG-CoA reductase inhibition in a biological system, whereas its behavior in the present chemical system reflects direct chemical interaction with cholesterol in the reaction mixture.

Methods

Data Collection Technique

The instruments were an analytical balance (OHAUS), moisture analyzer (OHAUS), rotary evaporator (BUCHI R-300), centrifuge (Hettich EBA 200), oven (Memmert), UV-Vis spectrophotometers (Thermo Scientific Genesys 150 and Thermo Spectronic Genesys 2), hot plate stirrer, volumetric flasks, test tubes, separating funnel, measuring cylinders, beakers, glass jars, micropipette, and muffle furnace. The materials were namnam leaves (Cynometra cauliflora L.; 3 kg), cholesterol standard ($C_{27}H_{46}O$), simvastatin ($C_{25}H_{38}O_5$), 96% ethanol, concentrated sulfuric acid, acetic anhydride, distilled water, aluminum chloride, Dragendorff's, Mayer's, and Wagner's reagents, hydrochloric acid, ferric chloride, chloroform, ammonia, and magnesium metal.

Procedure

Preparation of Simplicia

Fresh namnam leaves were sorted, washed under running water, air-dried in the shade, pulverized, and passed through an 80-mesh sieve (Zahara et al., 2023).

Preparation of Crude Extract

A total of 500 g of simplicia powder was macerated with 2,000 mL of 96% ethanol for 24 hours. The residue was re-macerated twice with 1,000 mL of 96% ethanol. All filtrates were combined and concentrated using a rotary vacuum evaporator at 50–60°C (Ilyas et al., 2020). The crude extract was then weighed.

Moisture Content

One gram of extract was weighed and placed on the moisture analyzer pan. The sample was analyzed at 105°C, and the moisture content displayed by the instrument was recorded (Rodhiyah et al., 2024).

Ash Content

Total ash content was determined by weighing 1.5 g of extract into a pre-weighed empty crucible, incinerating the sample in an electric muffle furnace at 550°C until ashing was complete, cooling the crucible in a desiccator, and reweighing it (Kementerian Kesehatan Republik Indonesia, 2008).

Phytochemical Screening

Tannins

One milliliter of extract was treated with three drops of 10% $FeCl_3$ solution. A blackish-green color change indicated a positive result for tannins (Anliza & Rachmawati, 2024).

Flavonoids

One milliliter of extract was treated with a small amount of magnesium powder and several drops of concentrated HCl. Formation of a reddish-brown color indicated a positive result for flavonoids (Qomaliyah et al., 2023).

Alkaloids

One milliliter of extract was mixed with 5 mL chloroform and several drops of ammonia, then concentrated. The residue was treated with 2 N HCl and shaken until homogeneous, and the resulting acid layer was divided into three portions for testing with Dragendorff's, Mayer's, and Wagner's reagents. A white precipitate with Mayer's reagent, an orange precipitate with Dragendorff's reagent, or a brown precipitate with Wagner's reagent indicated a positive result for alkaloids (Anliza & Rachmawati, 2024).

Saponins

Three milliliters of extract were mixed with 5 mL distilled water and shaken vigorously for 10 seconds. Formation of a stable foam persisting for at least 10 minutes indicated a positive result for saponins (Singh & Kumar, 2017).

Terpenoids/Steroids

One milliliter of extract was evaporated to dryness and treated with acetic anhydride, chloroform, and concentrated sulfuric acid added carefully along the tube wall (Liebermann–Burchard reaction). A red-orange or purple color indicated the presence of triterpenoids, whereas a green or blue color indicated the presence of steroids (Widya et al., 2023).

Anticholesterol Activity Test

Preparation of Cholesterol Standard Solution

A cholesterol stock solution (1,000 ppm) was prepared by dissolving 100 mg of cholesterol in chloroform in a 100 mL volumetric flask, with gentle heating at approximately 45°C until completely dissolved (Lindawati & Ningsih, 2020).

Preparation of Sample Solution

Test solutions of the namnam leaf ethanol extract were prepared at 100, 200, 300, 400, and 500 ppm by pipetting 1, 2, 3, 4, and 5 mL, respectively, of the extract stock solution into 10 mL volumetric flasks and diluting to volume with chloroform (Lindawati & Ningsih, 2020).

Preparation of Simvastatin Standard Solution

Simvastatin working solutions of 100, 200, 300, 400, and 500 ppm were prepared in chloroform using the dilution procedure described for the sample solutions (Lindawati & Ningsih, 2020).

Determination of Maximum Wavelength (λmax)

One milliliter of the 1,000 ppm cholesterol standard solution was diluted with chloroform to 10 mL to obtain a 100 ppm solution, then reacted with 2.0 mL acetic anhydride and 0.1 mL concentrated H_2SO_4, covered with aluminum foil, and incubated for 15 minutes. The mixture was scanned with a UV-Vis spectrophotometer over 600–750 nm to identify the wavelength of maximum absorbance (Lindawati & Ningsih, 2020).

Determination of Operating Time

A 1 mL aliquot of cholesterol standard solution was diluted with chloroform to 10 mL and reacted with 2.0 mL acetic anhydride and 0.1 mL concentrated H_2SO_4 in a foil-covered flask. Absorbance was recorded every minute from 0 to 30 minutes at λmax; the operating time was taken as the point at which absorbance became stable (Lindawati & Ningsih, 2020).

Cholesterol Standard Curve Measurement

A calibration curve was constructed using eight cholesterol concentrations (30, 40, 50, 60, 70, 80, 90, and 100 ppm), obtained by diluting 0.3–1.0 mL of the 1,000 ppm stock solution to 10 mL with chloroform. Each solution was reacted with 2.0 mL acetic anhydride and 0.1 mL concentrated H_2SO_4, incubated for the established operating time, and measured at λmax to generate the calibration curve (Widya et al., 2023).

LOD and LOQ

The limit of detection (LOD) and limit of quantitation (LOQ) were calculated from the standard deviation of the response and the slope of the calibration curve (Wijayanti & Rochmah, 2024).

Preparation of Test Solution

A 5.0 mL aliquot of each sample or simvastatin solution at concentrations of 100, 200, 300, 400, and 500 ppm was pipetted and mixed with 5.0 mL of the 200 ppm cholesterol solution. The mixture was homogenized, and 5.0 mL of the resulting solution was transferred into a centrifuge tube. The mixture was centrifuged for 2 minutes, and the supernatant was collected for absorbance measurement (Lindawati & Ningsih, 2020).

Measurement of Cholesterol Absorbance

The supernatant was reacted with 2.0 mL acetic anhydride and 0.1 mL concentrated H2SO4, covered with aluminum foil, incubated for the established operating time, and measured at λmax. The blank consisted of 5.0 mL chloroform, and the negative control consisted of 5.0 mL of 200 ppm cholesterol solution; both were treated with the same reagents. All measurements were performed in triplicate (Lindawati & Ningsih, 2020). The percentage of cholesterol reduction and the EC_{50} value were determined from the calibration and treatment data (Kusumaningtyas et al., 2025).

Statistical Analysis

The data were expressed as the mean ± standard deviation of three independent replicates. The percentage of cholesterol reduction was analyzed using two-way analysis of variance (ANOVA), with treatment and concentration as fixed factors and their interaction included in the model. Homogeneity of variance was tested using Levene's test, while the normality of the model residuals was assessed using the Shapiro–Wilk and Kolmogorov–Smirnov tests (Handoyo et al., 2025). Tukey's Honestly Significant Difference (HSD) test was performed when the

ANOVA results were significant. A significance level of α = 0.05 was used. All statistical analyses were performed using IBM SPSS Statistics.

Results and Discussion

1.1 Plant Authentication and Extraction Yield

Botanical determination performed at the Directorate of Scientific Collection Management, National Research and Innovation Agency (BRIN), Cibinong, confirmed that the leaf material used in this study was *Cynometra cauliflora* L. (family Fabaceae), verifying the identity of the plant prior to extraction.

Maceration of 500 g of namnam leaf simplicia in 96% ethanol yielded 66 g of crude extract, corresponding to an extraction yield of 13.144% (Table 1), which exceeds the minimum requirement of 10.0% for a satisfactory plant extract (Departemen Kesehatan Republik Indonesia, 2000) and is broadly comparable to the 10.1% yield reported for a methanolic extract of the same leaves (Zulkifli et al., 2025). The extract had a moisture content of 3.43% and a total ash content of 9.405% (Table 1), both within the accepted limits of below 10% and 16.6%, respectively (Kementerian Kesehatan Republik Indonesia, 2017). A moisture content within this range indicates that the extract was sufficiently dry to limit microbial contamination (Firawati et al., 2024) and to avoid an underestimation of the true concentration of active compounds, which could otherwise distort the calculated inhibition percentages and EC_{50} value (Amalia & Anggraini, 2023), while the ash content reflects the residual mineral and inorganic matter remaining after complete combustion of the organic material (Azis et al., 2026; Maryam et al., 2020).

Table 1. Non-Specific Quality Parameters of the Ethanol Extract of Namnam Leaves

Parameter	Result (%)
Extraction yield	13.144
Moisture content	3.43
Total ash content	9.405

1.2 Phytochemical Screening

Phytochemical screening (Table 2) showed that the ethanol extract of namnam leaves contained tannins, flavonoids, saponins, and steroids, while alkaloids and terpenoids were not detected. This qualitative profile agrees closely with that reported for a methanolic extract of the same species, which likewise tested positive for flavonoids, phenolics, saponins, tannins, and steroids and negative for alkaloids (Zulkifli et al., 2025). The blackish-green color obtained in the tannin test reflects the formation of a coordinate covalent complex between the ferric ion and phenolic hydroxyl groups (Handayani et al., 2024), whereas the reddish-brown color in the flavonoid test arises from magnesium-mediated

reduction of the flavonoid benzopyrone nucleus under acidic conditions (Tri Hardiningsih et al., 2025). The stable foam obtained in the saponin test is consistent with the surfactant-like behavior of saponins, which lower the surface tension of water (Simbolon et al., 2025), and the greenish-blue coloration in the steroid test corresponds to the conjugated chromophore generated when concentrated sulfuric acid acts as an oxidizing and dehydrating agent on the steroid nucleus (Fransiska et al., 2021; Mierza et al., 2023). The absence of a precipitate with Mayer's, Dragendorff's, and Wagner's reagents indicated that alkaloids were not present at detectable levels (Iqbal & Jariah, 2025).

Among the metabolite classes identified, flavonoids are of particular relevance to the anticholesterol activity evaluated below, as they have been reported to lower LDL cholesterol by upregulating hepatic LDL-receptor expression, binding apolipoprotein B, and inhibiting HMG-CoA reductase, the rate-limiting enzyme of cholesterol biosynthesis (Azhari et al., 2017). The presence of flavonoids, together with saponins and tannins, therefore provides a plausible phytochemical basis for the cholesterol-lowering activity observed in the following section.

Table 2. Phytochemical Screening of the Ethanol Extract of Namnam Leaves

Compound	Reagent	Result	Positive Indication
Tannins	10% FeCl3	+	Blackish-green solution
Alkaloids	Mayer's	-	White precipitate
	Dragendorff's	-	Orange precipitate
	Wagner's	-	Brown precipitate
Flavonoids	Mg powder + conc. HCl	+	Brown to reddish solution
Saponins	Distilled water	+	Stable foam for 10 minutes
Steroids	Chloroform + acetic anhydride + conc. H2SO4	+	Green/blue solution
Terpenoids	Chloroform + acetic anhydride + conc. H2SO4	-	Red/orange/purple solution

1.3 Anticholesterol Activity of Namnam Leaf Extract and Simvastatin

The anticholesterol activity of the ethanol extract of namnam leaves and of simvastatin, used as the positive control, was evaluated in vitro using the Liebermann–Burchard colorimetric reaction coupled with UV-Vis spectrophotometry, an approach widely favored for cholesterol assays because of its simplicity, sensitivity, and specificity toward the steroid nucleus (Da Ressurreição et al., 2022). Acetic anhydride acetylates the steroid nucleus and

removes residual moisture from the reaction medium, while concentrated sulfuric acid catalyzes a sequence of sulfonation, dehydration, and conjugation reactions that convert cholesterol into a polyene cation chromophore responsible for the blue-green color that develops during the assay (Xiong et al., 2007). Because the intensity of this color, and therefore the absorbance recorded at λmax, is directly proportional to the concentration of unreacted free cholesterol remaining in solution, a lower absorbance in a sample-treated tube relative to the negative control reflects a greater capacity of the extract or simvastatin to reduce the amount of free cholesterol available to react with the reagent.

Prior to quantitative analysis, the operating time of the cholesterol–Liebermann–Burchard complex was established by monitoring absorbance over a 30-minute period at an optimum wavelength of 608 nm (Table 3). Absorbance increased progressively during the first 14 minutes, plateaued between minutes 14 and 16, and thereafter declined gradually, indicating that the colored complex had reached maximum, stable formation before beginning to fade. A reaction time of 15 minutes was therefore adopted for all subsequent measurements. This stabilization window closely parallels the 15–16-minute optimum reported for the same reagent system by Da Ressurreição et al. (2022), supporting the reliability of the present measurement conditions. The subsequent decline in absorbance beyond minute 16 is consistent with the inherent instability of the Liebermann–Burchard chromophore, which is prone to further oxidative and photolytic degradation on prolonged exposure to the strongly acidic reaction medium and to ambient light (Xiong et al., 2007); wrapping the reaction tubes in aluminum foil during incubation therefore remained essential to preserving measurement accuracy.

Table 3. Operating Time of the Cholesterol

Time (minutes)	Absorbance
1	0.374
6	0.429
14	0.483
16	0.483
18	0.477
22	0.465
30	0.442

A calibration curve was subsequently constructed from eight cholesterol standard concentrations (30–100 ppm) to enable quantitative estimation of free cholesterol in the test solutions. Linear regression of the mean absorbance values produced the equation $y = 0.0039x + 0.1032$, with a correlation coefficient (r) of 0.9993 ($r^2 = 0.9986$; Figure 1), indicating a strong linear relationship between

cholesterol concentration and absorbance across the concentration range tested and confirming that the assay conditions were suitable for quantitative. The sensitivity of the method was further supported by a limit of detection of 3.296 ppm and a limit of quantitation of 10.987 ppm, calculated from the standard deviation of the response and the slope of the calibration curve, confirming that the assay could reliably detect and quantify cholesterol well below the working concentration range used in the subsequent inhibition assay.

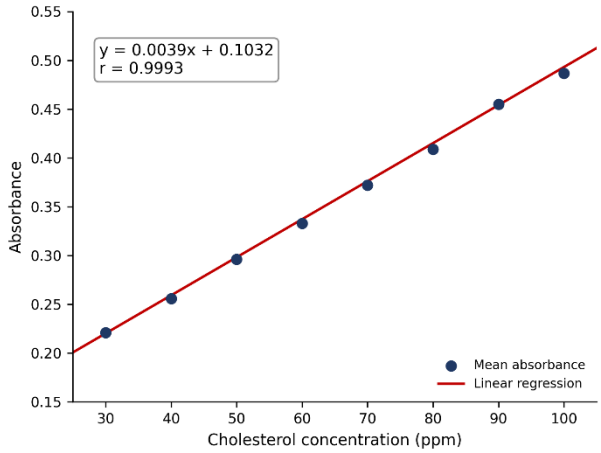


Figure 1. Regression graph of the cholesterol standard calibration curve (y = 0.0039x + 0.1032; r = 0.9993)

The anticholesterol activity of the namnam leaf extract and of simvastatin, assessed at concentrations of 100–500 ppm, is summarized in Table 5. For both substances, absorbance declined and the percentage of cholesterol reduction increased consistently with increasing concentration, reflecting a typical concentration-dependent inhibitory response. Linear regression of the extract data yielded the equation y = 0.1487x − 7.9805 (R = 0.998), from which an EC_{50} of 389.916 ppm was derived, whereas simvastatin produced a steeper dose–response relationship with an EC_{50} of 148.94 ppm. This roughly 2.6-fold difference indicates that simvastatin was substantially more potent than the crude ethanol extract on a weight basis, which is unsurprising given that simvastatin acts as a direct, high-affinity competitive inhibitor of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, the rate-limiting enzyme of the mevalonate pathway, whereas the activity of the crude extract derives from a mixture of comparatively weaker, lower-affinity phytochemicals rather than a single optimized active compound (Sultan et al., 2019).

Table 5. Anticholesterol Activity of Namnam Leaf Ethanol Extract and Simvastatin

Concentration (ppm)	**Extract)**		**(Simvastatin)**	
	Absorbance	**% Inhibition**	**Absorbance**	**% Inhibition (Mean ± SD)**

		(Mean ± SD)		
100	0.757	7.99 ± 0.19	0.442	46.21 ± 0.25
200	0.646	21.44 ± 0.12	0.378	54.03 ± 0.12
300	0.532	35.27 ± 0.19	0.323	60.68 ± 0.19
400	0.401	51.20 ± 0.19	0.267	67.53 ± 0.12
500	0.268	67.45 ± 0.19	0.210	74.42 ± 0.25
Regression equation	y = 0.1487x − 7.9805 (R = 0.998)		y = 0.07x + 39.5743 (R = 0.9996)	
EC_{50} (ppm)	389.916		148.94	

Values are expressed as mean ± SD (n = 3 independent replicates per concentration). A two-way ANOVA (Treatment × Concentration) confirmed significant main effects of Treatment ($F(1,20) = 124237.157$; $p < 0.001$) and Concentration ($F(4,20) = 51970.321$; $p < 0.001$), as well as a significant Treatment × Concentration interaction ($F(4,20) = 6811.907$; $p < 0.001$), indicating that the two substances did not follow parallel dose–response patterns. Levene's test confirmed homogeneity of variance ($F(9,20) = 0.670$; $p = 0.726$), and model residuals were approximately normally distributed (Shapiro–Wilk, $p = 0.057$), satisfying the assumptions required for ANOVA. Tukey's HSD post-hoc test indicated that all pairwise comparisons among the ten treatment–concentration groups were statistically significant ($p < 0.001$), except for extract at 500 ppm versus simvastatin at 400 ppm (mean difference = 0.08 percentage points; $p = 1.000$), indicating that at its highest tested concentration the extract achieved activity statistically comparable to simvastatin at a lower concentration.

Despite being less potent than the synthetic standard, the in-vitro potency of the namnam extract compares favorably with several other Indonesian plant extracts evaluated using the same colorimetric method. Ethanol extract of gedi leaves (*Abelmoschus manihot* (L.) Medik), for instance, exhibited a considerably higher EC_{50} of 5,007.8 ppm (Ilyas et al., 2020), more than tenfold weaker than the namnam extract, whereas ethanol extract of cocoa (*Theobroma cacao*) peel and extract of kale (*Brassica oleracea* var. acephala) achieved lower EC_{50} values of 67.785 ppm and 32.350 ppm, respectively (Amalia & Anggraini, 2023; Kusumaningtyas et al., 2025). This considerable spread in potency across botanically unrelated species indicates that anticholesterol activity measured by the Liebermann–Burchard assay depends strongly on the specific phytochemical composition and concentration of bioactive metabolites present in a given extract, rather than reflecting a uniform property of plant-derived material in general. These cross-study EC_{50} comparisons should nonetheless be interpreted with caution, as the cited studies differed in

reaction conditions such as cholesterol concentration, incubation/operating time, solvent system, and concentration ranges tested, all of which can influence the absolute EC_{50} value obtained.

The significant Treatment × Concentration interaction identified by two-way ANOVA ($F(4,20) = 6811.907$; $p < 0.001$) indicates that the extract and simvastatin did not follow parallel dose–response trajectories: the difference in cholesterol-reduction activity between the two substances progressively narrowed with increasing concentration, from 38.22 percentage points at 100 ppm to 6.97 percentage points at 500 ppm (Tukey HSD, $p < 0.001$ at every matched concentration). Notably, at the highest concentration tested, the extract (500 ppm, 67.45 ± 0.19%) achieved a level of cholesterol reduction that did not differ significantly from simvastatin at 400 ppm (67.53 ± 0.12%; mean difference = 0.08 percentage points; Tukey HSD, $p = 1.000$). This convergence suggests that, although the extract requires substantially higher concentrations than simvastatin to achieve comparable chemical activity in this assay, sufficiently high concentrations of the extract can approach the magnitude of activity produced by the positive control, which is consistent with the extract's mechanism relying on the cumulative, lower-affinity contribution of multiple phytochemicals rather than the single, high-affinity enzymatic target exploited by simvastatin.

The cholesterol-lowering capacity of the namnam extract is consistent with the flavonoid, saponin, and tannin content confirmed by phytochemical screening (Table 2). Flavonoids have been reported in the literature to reduce cholesterol availability through several possible converging mechanisms, including direct inhibition of HMG-CoA reductase, upregulation of hepatic LDL-receptor expression, and enhancement of cholesterol excretion (Ayunda & Malita, 2024); their antioxidant activity may also help limit the oxidative modification of lipids that would otherwise exacerbate cholesterol accumulation (Azhari et al., 2017), although these specific mechanisms were not directly measured in the present chemical assay. Saponins may likewise form insoluble complexes with cholesterol and bile acids, potentially lowering the pool of free cholesterol available for micellar solubilization, while tannins may precipitate cholesterol-containing complexes through hydrogen bonding and hydrophobic interaction with the steroid nucleus; these remain plausible explanations for the observed activity rather than mechanisms directly confirmed by this study. Because the crude extract used in this study contains all of these compound classes simultaneously, the activity observed likely reflects a combined, additive effect rather than the action of a single isolated constituent. These in-vitro findings are further corroborated by an in-vivo report in which ethanol extract of namnam leaves lowered total cholesterol, LDL, and triglyceride levels in high-fat-diet-induced obese mice, an effect attributed in part to a substantial saponin and flavonoid content of 629.1 mg diosgenin equivalents/g

extract and 55.0 mg rutin equivalents/g extract, respectively (Seyedan et al., 2019). Taken together, the present in-vitro results provide direct evidence of a cholesterol-inhibitory mechanism that is consistent with, and helps explain, the previously reported in-vivo lipid-lowering activity of this species.

Overall, these results establish that the ethanol extract of namnam leaves exhibited genuine, concentration-dependent cholesterol-reducing activity in this in-vitro chemical assay, albeit at a substantially higher effective concentration than simvastatin. These findings warrant further investigation, including mechanistic, cellular, animal, and eventually clinical studies, before the extract's therapeutic potential as a cholesterol-lowering agent can be established; the present data alone do not support a claim of a natural alternative to existing cholesterol-lowering therapy, and identification of its principal active constituents remains an important next step.

Conclusion

The ethanol extract of namnam (*Cynometra cauliflora* L.) leaves, obtained by maceration with 96% ethanol, met the standard quality requirements for a plant extract and was confirmed to contain tannins, flavonoids, saponins, and steroids. Using the Liebermann–Burchard colorimetric method, the extract exhibited concentration-dependent anticholesterol activity in vitro, with mean inhibition rising from 7.99% to 67.45% across the 100–500 ppm concentration range and an EC_{50} of 389.916 ppm, compared with 148.94 ppm for simvastatin as the positive control. These findings indicate that the ethanol extract of namnam leaves exhibited concentration-dependent cholesterol-reducing activity in this in-vitro chemical assay and warrants further investigation.

Suggestion

Additional mechanistic, cellular, animal, toxicity, and eventually clinical studies are necessary before any therapeutic cholesterol-lowering claim can be made, and the comparison with simvastatin should be interpreted cautiously given the differing mechanisms underlying the two substances' activity. Future work should also report the standard deviation of replicate measurements together with appropriate significance testing, and pursue isolation and identification of the principal active constituents responsible for the observed cholesterol-reducing activity.

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