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In Vitro Test of Fraction N-Hexan, Aquadest and Crude Ethanol Extract of Moringa Leaves (*Moringa oleifera* L.) by Cholesterol Lowering Using a UV-Vis Spectrophotometer

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Abstract: This research is an experimental research study on in vitro test that uses sampling test from herbal medicine. The purpose of this study is to determine the anticholesterol activity and EC50 value of n-hexan farction, aquadest fraction and ethanol extract of moringa leaves. Moringa leaves (*Moringa oleifera* L.) contain secondary metabolite compounds that can lower blood cholesterol levels. The analysis method uses UV-Vis spectrophotometry at a wavelength of 665 nm with a series of test sample concentrations of 100 ppm; 300 ppm; and 500 ppm. Research data shows a decrease in cholesterol levels is directly proportional to the increase in concentration in the sample. The EC50 value of n-hexane partition was 142.57 ppm, aquadest partition was 737.88 ppm, and ethanol extract was 156,7 ppm. This indicates that the lower polarity of the solvent used in the extraction process, better than the anticholesterol activity of the extract. Conclusion, the best anticholesterol activity was found in the n-hexane fraction with an EC50 value of 142.57 ppm.

Keywords: Anticholesterol; Fraxination; Moringa leaves; UV-Vis spectrophotometry

Introduction

Indonesia has abundant natural wealth. Various types of medicinal plants thrive in the nature of Indonesia. This natural wealth provides significant benefits for the health of its population, even for the world's population. Several studies have proven to the world that Indonesia has the potential as a place for the growth and development of medicinal ingredients for the global community. Indonesia possesses abundant organic natural resources and contains millions of chemical compounds (Kristiningrum et al., 2018). The medicinal plants utilized by the community are predominantly climbers, herbs, and trees (Afentina et al., 2025).

Moringa leaves (*Moringa oleifera* L.) are one of the medicinal plants that have been recognized for their rich biochemical profile, including extraordinary nutritional content, such as high protein, vitamins, minerals, and antioxidants. Moringa is known to enhance the immune system and has many health benefits (Chaniago et al. 2025). Furthermore, this plant is relatively easy to obtain, especially in tropical areas like Indonesia, making it a potential ingredient to be developed as a main component for research (Afriza et al., 2023). Moringa leaves are known to contain alkaloids, saponins, phytosterols, tannins, polyphenols, phenolics, and flavonoids, which act as antioxidants (Alverina et al., 2016). Research on the activity of Moringa at a dose of 75 mg/kg has shown a reduction in total blood cholesterol levels in rats by 47.5% (Dwitiyanti et al., 2015).

Cholesterol is a fatty substance that circulates in the blood, with a yellowish color and resembling wax, which is produced by the liver and is necessary for the body. Cholesterol is a group of lipids that are not hydrolyzed and is the main sterol in human body tissue. Cholesterol is significant because it is the most important element in plasma lipoproteins and plasma membranes

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and is a precursor in a large number of steroid compounds. The cholesterol produced by the body consists of 2 (two) types, namely HDL (High-Density Lipoprotein) cholesterol, which is referred to as good cholesterol, and LDL (Low-Density Lipoprotein) cholesterol, which is referred to as bad cholesterol. LDL cholesterol will accumulate in the walls of the coronary arteries, leading to blockages, thus LDL is called bad cholesterol. Excessive cholesterol levels in the blood are referred to as hypercholesterolemia (Berampu et al., 2024).

Hypercholesterolemia is defined as elevated cholesterol levels in the blood that exceed normal values. In addition to hypercholesterolemia, dyslipidemia is a significant primary risk factor for atherosclerotic cardiovascular disease (ASCVD). Epidemiological data also suggest that hypercholesterolemia, and possibly coronary atherosclerosis itself, are risk factors for ischemic cerebrovascular accidents (Jellinger et al., 2017). Abnormal cholesterol levels increase the risk of atherosclerotic plaque formation in blood vessels (Denta et al., 2022). Reducing cholesterol levels in patients at risk for coronary heart disease is crucial to preventing clinical risks and mortality rates in patients (Ferdinand, 2004).

Cardiovascular disease (CVD) is a health problem worldwide and is the leading contributor to global mortality (Varady & Jones, 2005), while high cholesterol levels are a major risk factor for this disease (Jia et al., 2023; Tian et al., 2023). Physical inactivity is a risk factor for cardiovascular disease and various other chronic diseases, such as diabetes, cancer (colon and breast cancer), obesity, high blood pressure, bone and joint diseases (osteoporosis and osteoarthritis), and depression (Widiastuti et al., 2023). The formation of atherosclerotic plaques involves an inflammatory process, which can trigger and accelerate atherosclerosis. Evidence of the role of inflammation in atherosclerotic plaque formation is seen in the elevated levels of inflammatory markers, interleukin-6 and tumor necrosis factor alpha, in individuals with heart disease. Factors causing inflammation can affect endothelial cell dysfunction, particularly in high cholesterol conditions triggered by macrophages or foam cells, due to oxidative stress induced by ox-LDL (Budianto & Akbar, 2022).

The high prevalence of hypercholesterolemia and the associated health risks, such as cardiovascular disease, have prompted significant research into natural alternatives for cholesterol management (Hiswanah, 2025). One such plant that has been recognized for its benefits in lowering cholesterol is moringa leaves. Prevention of atherosclerotic cardiovascular events in high-risk patients can be achieved by managing the reduction of lipoprotein cholesterol particles associated with apolipoprotein B, specifically triglyceride-rich lipoprotein remnant cholesterol and low-density lipoprotein cholesterol (LDL-C), which will be beneficial for the prevention of both primary and secondary attacks (Rosenblit, 2019).

Flavonoid compounds are one of the substances that can lower cholesterol. Flavonoids are compounds that contain C15 atoms, predominantly found in plants, and include flavones, aurones, chalcones, isoflavones, anthocyanins, and leucocyanins. Flavonoids have the potential to reduce cholesterol in the blood by decreasing the absorption of cholesterol and bile acids in the small intestine (Parbuntari et al., 2023), which is consistent with the content of Moringa leaves that contain flavonoids. Muharraran et al. (2023) reported that the extract of Moringa oleifera leaves is more effective as an antibacterial against *Streptococcus mutans* compared to *Enterococcus faecalis*.

In addition, Moringa leaves contain antioxidants that function as inhibitors to prevent oxidation by reacting with reactive free radicals to form relatively stable non-reactive free radicals (Isnaini et al., 2023). The beta-carotene content in Moringa leaves also protects lipid membranes from peroxidation and halts the chain reaction of free radicals (Susanty et al., 2019). Moringa leaves also contain beta-sitosterol, which lowers cholesterol levels by reducing LDL concentrations in plasma and inhibiting the reabsorption of cholesterol from endogenous sources. Flavonoids and polyphenols significantly increase superoxide dismutase (SOD) and catalase levels, while plasma lipids, with the main sources of blood cholesterol derived from the diet (exogenous) and synthesized in the liver (endogenous). Kahirah et al. (2024) also reported that Moringa leaves are effective in addressing anemia in pregnant women. Herawati et al. (2024) reported that consuming Moringa leaves along with papaya leaves, almond milk, green beans, ajwa dates, soy milk, and fennel leaves has a positive impact on lactation.

Another study by Susanti et al. (2020) states that moringa leaf extract lowers blood cholesterol levels in rats because it contains alkaloids, saponins, phytosterols, tannins, phenolics, and flavonoids. The flavonoids in Moringa leaves prevent LDL oxidation and halt the activity of HMG-CoA reductase. Additionally, research conducted by Nurdin et al. (2021) on the antihypercholesterolemic effectiveness of ethanol extract from Moringa leaves proved that the extract has an effect on reducing cholesterol levels in male white rats at a dose of 10.8 mg/200gBB. Kusumawardhani et al. (2024) reported that the n-hexane fraction of the ethanol extract from moringa leaves has the ability to lower LDL levels with an optimal dose of 14.888 mg/kg body weight of rats, comparable to simvastatin 10 mg. Another study in 2018 by Kristiningrum et al. reported that the water fraction of moringa leaves contains

saponins and polyphenols, while the ethyl acetate fraction contains alkaloids, flavonoids, saponins, terpenoids, and polyphenols. The n-hexane fraction contains only alkaloids and terpenoids. In 2016, Olasehinde *et al.* conducted research to determine the antiplasmodial activity of Moringa oleifera leaves in a mouse model (*Mus musculus*) and reported that *Moringa oleifera* showed potential for possible future use as an alternative to some conventional drugs.

Bagheri et al. (2020) reported that both water and methanol extracts showed antibacterial activity against all selected bacteria, while hexane and benzene extracts exhibited antifungal activity against all tested fungi. Additionally, hexane, benzene, and isopropanol extracts showed activity against the Hepatitis B virus. Another study on moringa was reported by Nurafifah et al. (2021), which found that the ethanol extract of Moringa seeds at concentrations of 25%, 50%, and 75% demonstrated strong inhibition against E. coli cultures. Meanwhile, the ethyl acetate fraction of *Moringa oleifera* leaves was reported to lower blood glucose levels, cholesterol, and low-density lipoprotein in a type 2 diabetic rat model. Furthermore, this fraction can improve insulin sensitivity by increasing GLUT4 expression (Hidayati et al., 2018; Khairi et al., 2023).

Based on the description above, this study investigated the cholesterol lowering activity of different extracts of Moringa leaves, specifically n-hexane, aquadest, and ethanol extracts using in vitro methodologies. The novelty of this research lies in its comprehensive evaluation of multiple extraction methods to assess their efficacy in reducing cholesterol levels. While previous studies have primarily focused on single extraction techniques or isolated compounds, this investigation utilizes a comparative approach to enhance our understanding of how different solvent polarities can affect the extraction of bioactive compounds responsible for antihyperlipidemic activity. This dual focus not only identifies the most effective extraction method but also provides insights into the bioactive interactions within the extracts.

Conducting this research is vital for several reasons. First, as public awareness surrounding the side effects of synthetic pharmaceuticals increases, there is a growing demand for natural remedies that can provide similar benefits without adverse effects. Second, the identification of effective natural cholesterol-lowering agents may yield new therapeutic options for managing hypercholesterolemia, thereby contributing to improved cardiovascular health. Lastly, this study could pave the way for further research into the mechanisms through which Moringa extracts exert their effects, ultimately leading to better health outcomes for individuals at risk of cholesterol-related diseases.

Method

Research Type

The type of research used in this study is experimental research. The development model used in this study is the activity test of n-Hexan and Aquadest Fraction and Ethanol Extract of Moringa Leaves (*Moringa oliefera* L.) on In Vitro Cholesterol Lowering.

Tool and Material

The tools used in this research are aluminum foil, analytical balance, beaker glass, clamp, drop pipette, erlenmeyer, filter paper, hairdrayer, hotplate, oven, porcelain cup, rotavapor, separating funnel, magnetic stirrer, stirring rod, measuring glass, measuring flask, mesh sieve no. 40, micro pipette, UV-VIS spectrophotometer, statif and waterbath.

The materials used in this research are aquadest, anhydrous acetic acid, concentrated sulfuric acid (H_2SO_4), moringa leaves, ethanol (C_2H_6O) 96%, chloroform, n-hexane (C_6H_{14}), and pure cholesterol powder.

Research Procedure

Making Simplicia of Moringa Leaves

1000 grams of moringa leaves were collected and weighed. Then, the sample was sorted to select the required parts and separate the unwanted ones. Next, the sample was cleaned using running water to remove any attached foreign materials. The sample was dried using an oven at 50°C for 3 days. The dried sample was then ground into a powder using a blender and sieved with a 40 mesh sieve to obtain the simplicia. The resulting simplicia was weighed, and the percentage of water content was calculated using a formula, as shown in equation 1.

$$Moisture\ content = \frac{initial\ weight - final\ weight}{initial\ weight} \quad (1)$$

Sample Extraction

200 grams of dried simplicia was put into a maceration container. Then, 1000 ml of 96% ethanol solvent was added. The ethanol solvent used must be approximately 1 cm above the surface of the simplicia. The extraction process was carried out by maceration for 3 days and stirred twice a day. After that, the simplicia was separated from the extraction result using a filter cloth. Then, the extraction result was concentrated using a rotavapor until a thick extract was obtained. The extract obtained was calculated for its rendament percentage using the formula, as shown in equation 2.

$$Rendament = \frac{(\ dry\ simplisia\ weight\ )}{(wet\ simplisia\ weight)} x\ 100\ \% \quad (2)$$

Preparation of Extract Fractionation

5 grams of dry ethanol extracts of Moringa leaf was added 50 ml of n-hexane and 50 ml of aquadest in a glass beaker until mixed, then placed in a separating funnel and shaken again. After that, it was left for 24 hours until a clear separation of 2 phases occurred. Then, the fractions were separated, where the aquadest fraction would be located at the bottom layer and n-hexane at the top layer. Next, the results of each fraction were collected in different containers. Furthermore, each fraction was concentrated on a water bath.

Preparation of Cholesterol Standard Solution

10 mg of pure cholesterol powder was dissolved with n-hexane in a 10 ml volumetric flask to obtain a concentration of 1000 ppm. The standard solution was then made with concentrations of 100, 300, and 500 ppm by pipetting 1, 3, and 5 ml of the 1000 ppm standard solution, respectively, and then adding chloroform to each until the volume reached the 10 ml mark.

Determination of Maximum Wavelength

The cholesterol standard solutions with concentrations of 100, 300, and 500 ppm, and 1000 ppm, were used to scan the highest wavelength in UV-Vis spectrophotometry. 2 ml of each concentration of the solution was taken and mixed with 2 ml of acetic anhydride and 0.1 ml of concentrated H2SO4. The mixture was left for 10 minutes, then the maximum wavelength was measured on a UV-Vis Spectrophotometer at a wavelength of 400-800 nm.

Preparation of Test Solution

The test solution was made by making a series of concentrations of 100, 300, 500 ppm, and 1000 ppm of n-hexane and aquadest fractions and Moringa leaf ethanol extract. Each concentration was put into a 10 ml volumetric flask and dissolved with the corresponding solvent up to the mark.

Anticholesterol Activity Test

The test solutions, namely n-hexan and aquadest fractions, and ethanol extract of Moringa leaf, were taken as 2 ml each and put into a test tube. Then, 2 ml of cholesterol solution with a concentration of 400 ppm was added to each test solution. Each solution was then homogenized on a hotplate stirrer at a speed of 1500 rpm for 10 minutes. 3 ml of the supernatant was taken and added with 2 ml of acetic acid and 0.1 ml of concentrated H2SO4. Next, the absorbance of each mixture was measured using a UV spectrophotometer at a wavelength of 665 nm. The percentage decrease in cholesterol levels was calculated using the following formula, as shown in equation 3.

$$A = \frac{C-B}{C} X100\% \quad (3)$$

Description:
A : Percentage of lowering cholesterol
B : Absorbancy of final cholesterol value
C : Absorbancy of initial cholesterol value

Measurement of Effective Concentration Value (EC 50)

To calculate the EC 50 value, we can use the linear regression formula, as shown in equation 4.

$$y = bx + a \quad (4)$$

Description:
y = percentage of cholesterol reduction
x = concentration of test solution
a = intercept of the regression line with the y-axis
b = slope of the regression line

The EC 50 value can be calculated by substituting y = 50 into the linear regression formula, as shown in equation 5.

$$50 = ax + b$$
$$X = (50 - b) / a \quad (5)$$

Description: The x value obtained is the EC 50 value.

Data Analysis

The data obtained in this study is quantitative. Quantitative data were obtained from the EC_{50} values of n-hexan and aquadest fractions and ethanol extract of Moringa leaves (*Moringa oleifera* L.). EC50 is the concentration of the test solution that can reduce cholesterol levels by 50% which it is related to the ability of the test solution as an anti-cholesterol. If EC 50 value is lower, it means that a subtance have the stronger the anticholesterol activity (Lutfiyati et al., 2021).

Result and Discussion

Results of Characteristics Data of Simplisia and Ekstrak of Moringa Leaves (Moringa oleifera L.)

Moringa leaves (*Moringa oleifera* L.) leaves were used as the sample in this study because they empirically contain active compounds, namely alkaloids and flavonoids, which have potential cholesterol lowering effects. The research began with the preparation of dry simplicia by drying 1000 grams of wet moringa leaf simplicia until 240 grams of dry simplicia were obtained. Drying was carried out using an oven at 50^{O}C for 3 days. Drying at a low temperature was intended to maintain the active chemical content in the simplicia from being damaged. Based on the results of the processing of the moringa leaf simplicia, the water

content was 0.76. This is in accordance with the theory in the Indonesian Medicinal Material (MMI) which states that a good water content in simplicia is usually less than 10%.

Then, the extraction was continued using the maceration method with 1000 ml of 96% ethanol solvent to make an extract from 200 grams of dry simplicia. According to Artati et al. (2025) ethanolis selected as the solvent for the extraction of moringa leaves due to its polar nature, which facilitates the dissolution of various bioactive compounds such as flavonoids, tannins, and phenolic compounds that are prevalent in plant materials. The choice of 96% ethanol specifically enhances the extraction efficiency of these polar phytochemicals, as higher concentrations of ethanol can effectively disrupt cell walls and release intracellular compounds. Additionally, ethanol's ability to extract a wide range of metabolites, including both hydrophilic and lipophilic compounds, makes it an ideal solvent for comprehensive phytochemical analysis.

The extract yield was 35.2 grams. The extract rendament percentage obtained was 17.6%. This aligns with the theory in Materia Medika Indonesia (MMI), which states that a good rendament of extract is greater than 10%. The results related to the characteristics of the medicinal herbs and extracts are shown in Table 1.

Table 1. Results of Characteristics Data of Simplisia and Extract of Moringa Leaf (*Moringa oleifera* L.)

Characteristics Data	Standard (MMI)	Result (%)
Moisture Content	<10%	0.76
Rendament	>10%	17.6

The Results of Characteristic Data of the Fraxination of Moringa leaveextract (Moringa oleifera L.)

The fraxination of the concentrated extract was carried out by taking 5 grams of the extract and adding 50 ml of aquadest solvent and 50 ml of n-hexane. The mixture was then placed in a separating funnel and allowed to stand for 1 day to separate into two phases. The n-hexane phase was at the top layer, while the water phase was at the bottom layer, due to n-hexane's specific gravity of 0.6174 g/ml, which is lower than water's 1 g/ml. The result of characteristic data of the fraxination of moringa leaves extract (Moringa oleifera L.) show n-hexan rendement at 27.6% and aquadest at 27%. Based on the rendement results of both fractions, it still meets the good rendement according to MMI. This result as shown in table 2.

Table 2. Results of Characteristics Data of the Fraxination

Fraxion	Dry Weight Fraxion		Rendament (%)
N-heksan	5 g	1.38	27.6
Aquadest	5 g	1.35	27.0

The Results of Absorbance Data of Test Samples and Comparison Standards at 400-800 nm Wavelength

Preparation of the standard solution was done by mixing 10 mg of pure cholesterol powder into 10 ml of chloroform to create a concentration of 1000 ppm. Then, 1, 3, and 5 ml were transferred to obtain concentrations of 100, 300, and 500 ppm. Next, 2 ml of acetic anhydride and 0.1 ml of H_2SO_4 were added, mixed until homogeneous, and covered with aluminum foil. The maximum wavelength obtained was around 665 nm. This wavelength is the most suitable for seeing the extent of light absorption. Knowing this wavelength is crucial for more accurate measurements.

The standard curve, the coefficient value (R) in the cholesterol standard curve in this study is R2 = 0.9652, the value of a = 1.4965 and the value of b = 0.6593. This correlation coefficient is used to see how linear an analysis is, where the value of a shows the intercept of the curve on the y-axis, and the value of b shows how steep the curve is. The result of absorbance data of test samples and comparison standar can see at table 3.

Table 3. Absorbance Data of Test Samples and Comparison Standards at 665 nm Wavelength

C (ppm)	Average Absorbance of Sample (Value + Deviation Standard)			
	N-Heksana Fraxion	Ethanol Extract	Aquadest Fraxion	Pure Cholesterol
100	0.571+0.004	0.533+0.001	0.166+0.001	0.673+ 0.003
300	2.040+0.019	1.199+0.004	0.394+0.001	2.662+ 0.029
500	2.817+0.043	1.368+0.006	0.453+0.002	3.666+ 0.033

Description: C is Concentration and ppm is part per million

Based on the table above, it can be seen that as the sample concentration increases, so the absorbance measured by the spectrophotometer also increases. N-hexan fraxion is the best absorbance better than ethanol extract and aquadest fraxion. This data are used to making the linear regression formula. The curves of standard can be seen in Figure 1.

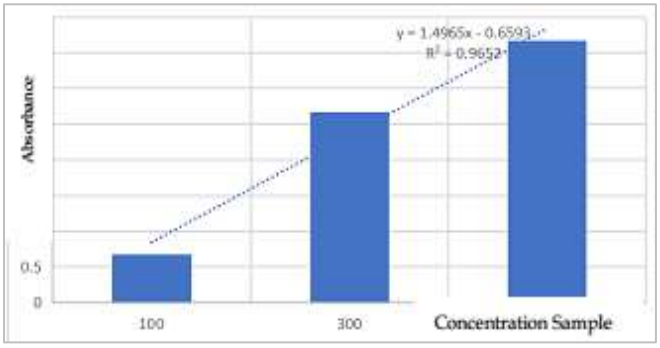

Figure 1. Standard curve of cholesterol

The Result Data on Lowering of Cholesterol Levels in Samples

Activity test for anticholesterol was done with start to make a test solution with a concentration of 1000 ppm. The concentrations of the solution to be used are 100, 300, and 500 ppm. As the concentration increases, the solution becomes thicker. To make the test solution, we weighed 1 mg of aquadest, 1 mg of n-hexane, and 1 mg of ethanol extract from Moringa (Moringa oleifera L.) leaves, then dissolved them in 10 ml of solvent each. For each concentration, we made 10 ml of solution, and for testing, we took 2 ml from each solution and put it into an erlenmeyer flask, which was then covered with aluminum foil. The Erlenmeyer flask was covered with aluminum foil to prevent the test solution from exposed to light. Then, we added 2 milliliters of cholesterol solution with a concentration of 400 ppm. Next, we stirred the solution for 10 minutes using a hotplate stirrer at a speed of 1500 rpm to mix the sample solution with cholesterol evenly. After that, we took 3 ml of supernatant and added 2 milliliters of acetic anhydride and 0.1 milliliter of concentrated H2SO4. According to Ilyas et al. (2020), acetic anhydride was added to extract cholesterol and produce an acetyl derivative of steroids, while H2SO4 was added to produce a color reaction that produces a green-colored solution. The green color comes from the steroid content, which consists of cholesterol. The formed component will be measured using a UV-VIS spectrophotometer at a wavelength of 665 nm. Data on lowering of cholesterol levels in sample can show at table 4.

Table 4. Data on Lowering of Cholesterol Levels in Samples

C (ppm)	Lowering Chlolesterol (%)		
	N- Heksan Fraxion	Ethanol Extract	Aquadest Fraxion
100	14.53277	20.80238	75.33432
300	14.42953	49.70638	83.47315
500	15.15602	58.49515	86.25607

Description : C = Concentration

Result of EC50 Values of the N-hexan and Aquadest Fraction and Ethanol Extract of Moringa Leaves (Moringa oleifera L.)

The EC50 (Effective Concentration) value is a measure that indicates how much of a substance is needed to reduce cholesterol levels by half from the initial amount. To find this EC50 value, a linear regression formula y = bx + a is used. This explains the relationship between the concentration of the sample tested (x value) and the average percentage decrease in cholesterol (y value). Based on what is shown in Figure 2, the EC50 value for the N-Hexane partition sample is 142.57 ppm. For the sample extracted with ethanol, the EC50 value is 156.71 ppm. Meanwhile, the EC50 value for the sample from the aquadest partition is 737.88 ppm. The best EC50 value is the N-hexan partition with the value of 142.57 ppm. According to Anggraini et al. (2018), the EC50 value is negatively related to the ability of a material to function as an anti-cholesterol. Therefore, the lower the EC50 value, the stronger the anti-cholesterol ability. The result shown on table 5.

Table 5. EC50 Values of The N-hexan and Aquadest Fraction and Ethanol Extract of Moringa Leaves (Moringa oleifera L.)

Sample	Result (ppm)	Standard*
N-Heksan Fraction	142.57	If EC 50 value is lower, it means that a subtance have the stronger the anticholesterol activity
Ekstrak Etanol	156.71	
Aquadest Fraction	737.88	

Direction: *The aquadest fraction has the best EC 50 value, thus having the best anticholesterol activity

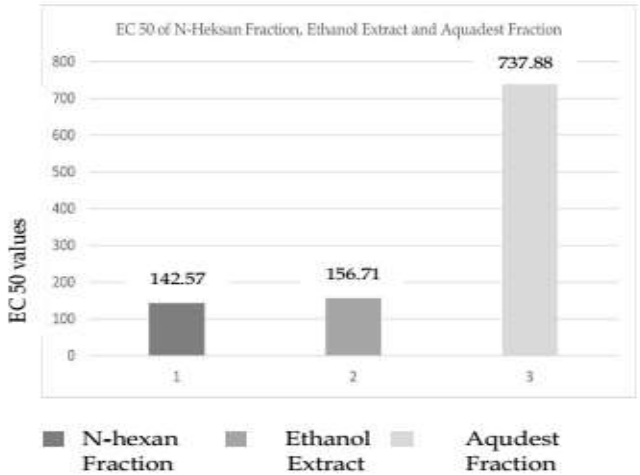

Figure 2. EC 50 values for n-hexan fraction, ethanol extract and aquadest fraction of moringa leaves (*Moringa oleifera* L.)

Conclusion

Based on the research results, it can be concluded that the n-hexane partition, ethanol extract, and aquadest partition from *Moringa oleifera* L. leaves have cholesterol-lowering activity, demonstrating EC_{50} values of 142.57 ppm for the n-hexane partition; 156.71 ppm for the ethanol extract; and 737.88 ppm/mL for the aquadest partition. The n-hexane fraction exhibited the highest (most potent) anticholesterol activity with an EC50 value of 142.57 ppm.

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Author Contributions

Conceptualization, Y.U. and M.B; methodology, Y.U. and M.B; formal analysis, Y.U.; investigation, Y.U. and M.B; resources, Y.U. and M.B; writing—preparation of original draft, Y.U.; writing—reviewing and editing, Y.U.; visualization, Y.U.; supervision, Y.U and M.B..; project administration, M.B.; obtaining funding, Y.U. and M.B. All authors have read and approved the published version of the manuscript.

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Conflicts of Interest

The author declares that there is no conflict of interest in this research.

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