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PROFILING GC-MS ETHANOL EXTRACT OF PUTRI MALU LEAVES (*Mimosa pudica L.*) AND IT'S POTENTIAL AS ANTI-CHOLESTEROL IN VITRO

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ABSTRACT

Cholesterol plays a crucial role in cell building and hormone production in the body, but excessive levels can lead to various diseases. Elevated cholesterol levels increase the risk of cardiovascular diseases such as atherosclerosis. Natural remedies for high cholesterol treatment are commonly sourced from medicinal plants containing many phytochemicals. One such plant is the Putri Malu (Mimosa pudica L.) leaves . This study aims to evaluate the chemical composition of Putri Malu (Mimosa pudica L.) leaves extract and its potential anti-cholesterol effects in vitro. The extraction process used in this study was maceration, followed by GC-MS profiling analysis and the Liebermann Burchad method with UV-Vis spectrophotometry. The findings of this study revealed the presence of five chemical constituents in the extract. Among them, the second and fourth peaks had the highest abundance of acacetin at 14.08% and diosmetin at 73.79%, respectively. The ethanol extract of Putri Malu leaves demonstrated strong anti-cholesterol activity in vitro with an IC50 value of 24.8993, indicating its potential as an agent for treating hypercholesterolemia. Further research is required to evaluate this extract's efficacy in vivo and investigate its underlying mechanism of action.

Keywords: *Anti-cholesterol, extract of putri malu leaves, GC-MS profiling analysis, Liebermann Burchard, maceration*

INTRODUCTION

Elevated levels of cholesterol in the human body can lead to cardiovascular disease. Regular consumption of foods that contain high amounts of cholesterol can increase blood cholesterol levels. Studies have revealed that hyperlipidemia, including increased total cholesterol levels in plasma, is a risk factor for coronary heart disease [1]. However, conventional drugs used to treat hypercholesteremia are known to cause side effects such as rhabdomyolysis, myoglobinuria, and myopathy. Therefore, there is a need to develop cholesterol-lowering products that utilize natural ingredients to make them more affordable and safer for consumers [2].

Cholesterol is an essential substance that the body needs to perform vital functions, but excessive amounts can cause atherosclerosis and increase the risk of

coronary heart disease [3]. Two types of cholesterol are produced in the body: high-density lipoprotein (HDL) cholesterol, which is good for the body, and low-density lipoprotein (LDL) cholesterol, which is bad for the body. When the levels of LDL cholesterol are too high, a condition known as hypercholesterolemia occurs, which increases the risk of premature atherosclerotic cardiovascular disease [4].

Hypercholesterolemia is defined as a condition where the total cholesterol level is 190 mg/dL or higher. Based on a study conducted by the Ministry of Health in 2016, 42% of 25,234 subjects from 34 provinces in Indonesia had hypercholesterolemia [5]. High cholesterol levels in the body can lead to the onset of various diseases, with cardiovascular disease being one of the most prevalent. In addition, Genetics and diet contribute to increased cholesterol levels in the blood [6].

Currently, medicinal plants are being developed as a treatment for cholesterol. Many of these plants contain phytochemicals with antioxidant properties that can prevent and treat various complications related to oxidative stress [6,7]. Flavonoids are an active compound found in these plants, which have many benefits for the body. One of these benefits is that flavonoids can reduce cholesterol levels by eroding cholesterol deposits on the walls of coronary blood vessels. This can help prevent other diseases caused by cholesterol, such as hypertension, stroke, and heart disease [8].

Flavonoids also work to reduce triglycerides (TGA) and increase high-density lipoprotein (HDL) [9]. They do this by inhibiting the action of the 3-hydroxy 3-methylglutaryl coenzyme A reductase (HMG Co-A reductase) enzyme, which is responsible for producing cholesterol in the body [10]. *Mimosa pudica L*. is a plant reported to have antidiabetic, antihyperlipidemia, and anti-hepatitis effects, as well as various potentials such as antimicrobial, anti-inflammatory, hepatoprotective, and antioxidant. The ethanol extract of Mimosa pudica L. leaves contains compounds such as terpenoids, flavonoids, glycosides, alkaloids, phenols, tannins, quinines, saponins, and coumarins. Meanwhile, the ethanol extract of Mimosa pudica L. root contains flavonoids, tannins, and steroids [11].

One way to determine the potential of Putri Malu (Mimosa pudica L.) leaves as an anti-cholesterol agent is by conducting Gas Chromatography-Mass Spectrometry (GCMS) profiling to analyze the chemical compounds in the leaves. The GCMS profiling shows each compound component's fragmentation pattern and total abundance [12]. Based on this, this study aims to identify the chemical compounds present in Putri Malu (Mimosa pudica L.) leaves extract and evaluate its anti-cholesterol effect in vitro [13].

METHOD

Material and Instrument

The ingredients used in this study were the extract of Putri malu, Raw Cholesterol (Sigma Aldrich), Chloroform p.a, Anhydrous Acetic Acid, Concentrated H_2SO_4 p.a. The tools used in this study were analytical balance (Ohaus Pioneer with a sensitivity of 0.0001 g), aluminium foil, vortex, double beam UV-VIS spectrophotometer (Shimadzu UV VIS 1700), Hellma Analytic cuvette, glassware such as a measuring flask (Pyrex), beaker glass, test tube, volume pipette, rotary evaporator and Mass

Spectrometry-GCMS (Hewlett-Packard 6890), with an Agilent 19091S-433 HP-5MS column.

Determination of the Putri Malu (Mimosa pudica L.) Plant

The determination of the Putri embarrassed plant was carried out in the Biology lab of STIFAR YAPHAR SEMARANG.

Sampling and Processing

Putri Malu (Mimosa pudica L.) leaves as much as 5 kg was collected, washed and cleaned of dirt, then dried in the sun with a black cloth. They were then mashed using a blender to obtain a powder. The powder obtained was sieved using mesh sieve no 30/40.

Putri Malu (Mimosa pudica L.) Leaves Plant Extraction

200 grams of Putri Malu (Mimosa pudica L.) leaves were extracted by maceration method using 96% ethanol solvent as much as 2 L (1 : 10) for 3 days and then continued with the maceration process. The extract was concentrated with a rotary evaporator until it became a thick extract which was then carried out for phytochemical screening.

Analysis of Putri Malu (Mimosa pudica L.) Leaves Extract with GC-MS

The chemical profile of Putri Malu (Mimosa pudica L.) leaves extract was screened using Gas Chromatography-Mass Spectrometry-GCMS (Hewlett-Packard 6890) with an Agilent 19091S-433 HP-5MS column measuring 30 meters in length and 250 meters in diameter. One litre of Putri Malu (Mimosa pudica L.) leaves extract (dissolved in methanol) was injected into the GC-MS. Helium gas was used as the carrier gas at a 1 mL/minute flow rate, and the oven temperature was set at 325°C. The initial temperature of the oven was 150°C with a temperature rate of 2°C/minute. This process ran for 10°C/minute and then increased to 240°C with a holding time of 11 minutes. The total running time was 22 minutes with a scanning range of 50-550 sma. The screening of the chemical structure profile was based on the analysis of mass spectrum fragmentation patterns. It was compared with the mass spectrum in the National Institute of Standards and Technology (NIST) and the Wiley compound profile database [13].

Anti-cholesterol Test (*Lieberman Bourchad)*
Cholesterol (control group)

The cholesterol stock solution was prepared by dissolving 50.8 mg of standard cholesterol powder in 50.0 ml of analytical-grade chloroform, resulting in a stock concentration of 1016 ppm.

Standard Curve

To determine the linearity, 5 series of standard curves were prepared with concentrations of 60, 80, 100, 120, and 200 ppm, which were directly made in test tubes. 2.0 ml of anhydrous acetic acid and 0.1 ml of concentrated H_2SO_4 were added to each solution, homogenized using a vortex, and covered with aluminium foil on the tube's outer layer. The absorbance was measured at the maximum wavelength of 662.9 nm after 7 minutes of incubation. A correlation curve was then constructed between the concentration and the absorbance.

RESULTS AND DISCUSSION

Phytochemical Screening of The Ethanol Extract of Putri Malu (Mimosa pudica L.) Leaves

Cholesterol is a type of fat that circulates in the bloodstream and appears as yellowish-like wax produced by the human body, especially in the liver. It is naturally formed and has several functions, including

producing sex hormones, adrenal cortex hormones, vitamin D, and forming bile salts that help the intestines absorb fat. As a result, high cholesterol levels can cause cardiovascular disease, making it necessary to have alternative herbal remedies that can lower cholesterol levels in the blood.

In this research, a maceration method was used to extract the leaves of the Mimosa pudica plant. The solvent was 96% ethanol, and maceration was carried out for three days. From 220.404 grams of Mimosa pudica plant powder, a concentrated extract of 41.746 grams was obtained with a yield of 18.94%. In addition, further screening of phytochemicals was performed to identify the secondary metabolite content in the extract.

The results of the phytochemical screening of the ethanol extract of Putri Malu (Mimosa pudica L.) leaves can be seen in Table 1.

Table 1. Phytochemical Screening E. Putri Shame Leaves

No	Phytochemical Screening	Results
1.	Flavonoid	+
2.	Alkaloid	+
3.	Tanin	+
4.	Saponin	+
5.	Steroid/ Triterpenoid	-
6.		-

Profiling GC-MS Ethanol Extract of Putri Malu (Mimosa pudica L.) Leaves

The extract from the leaves of Mimosa pudica showed positive results for the presence of alkaloids, flavonoids, tannins, and steroids. Furthermore, a qualitative analysis was conducted using Gas Chromatography-Mass Spectroscopy (GC-MS) to compare the results. The screening of phytochemicals was followed by quantitative analysis using Gas Chromatography-Mass Spectroscopy (GC-MS), and the results were analyzed using GC-MS [14]. Based on the chemical profiling using GC-MS, it was observed that the chromatogram results showed 5 compound peaks with apparent abundance (Figure 1). Among these peaks, the second and fourth peaks showed the highest abundance. The structure of each compound was determined based on its unique fragmentation pattern and distinctive base peak [11].

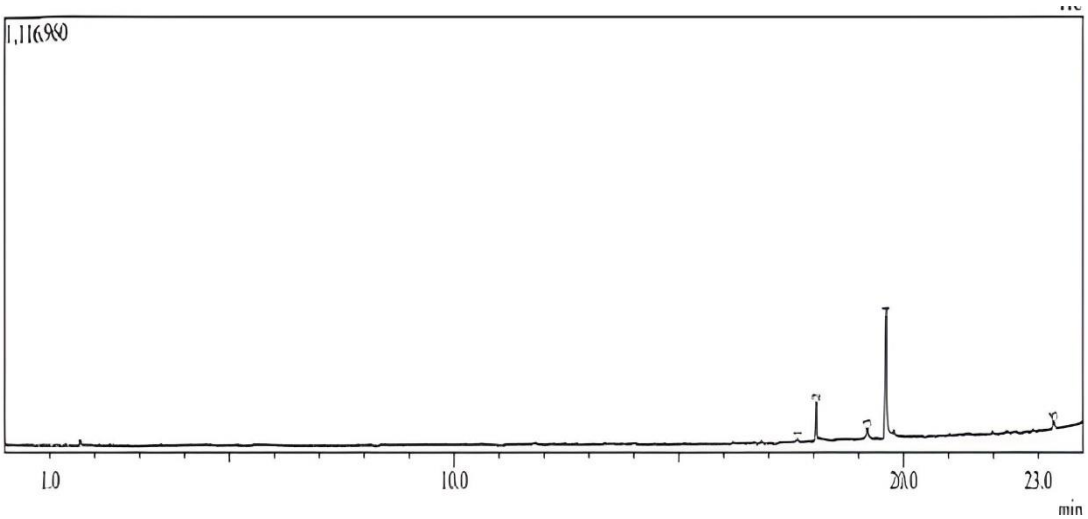

Figure 1. GC Results from Putri Malu (Mimosa pudica L.) Leave Extract.

Table 2. Phytochemical components identified in Putri Malu (Mimosa pudica L.) leaves extract by GC-MS analysis

No	Compound Name	% Area	Retention Time	MR	SIM
1	Dihydroflavonol	1.54	17.637	239	83
2	Acacetin/2,4,5 dihydroxyl 7-methoxyflavone	14.08	18.059	269	84
3	$C_{13}H_{16}N_2O_2S$/1,2 Benzisothiazole,3-(Hexahydro-1H-azepin-1-y)1,1-dioxide	5.78	19.194	264	92
4	Diosmetin	73.79	19.616	299	88
5	$C_{15}H_{26}O$/ 1H-Cyclopenta[a]pentalen-7-ol,decahydro-3,3,4,7a-tetramethyl-(CAS)	4.82	23.340	207	83

From Table 2, it can be shown that the compounds that have the greatest abundance in the extract of Putri Malu (Mimosa pudica L.) leaves are the compounds at peaks no. 2 and 4, namely acacetin and diosmetin. The following is the fragmentation pattern of the acacetin and diosmetin compounds:

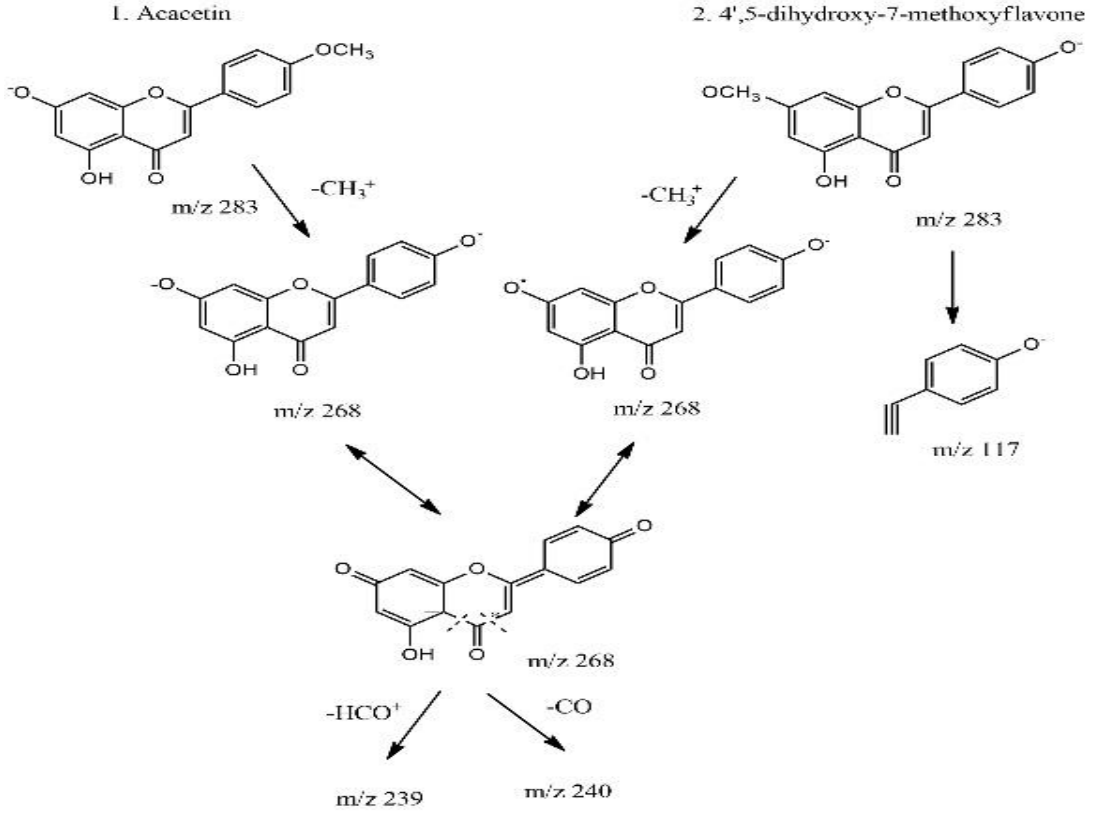

Figure 2. Acacetin Fragmentation Pattern

The ion-product spectrum of the two isomeric compounds Acacetin and 2,4,5-hydroxy-7-methoxyflavone, Mr 283, showed similarities; the m/z 268 ion was the dominant fragment for both compounds due to the formation of a very stable anion radical structure. Both compounds also showed a loss of CH_3. Together and CO or HCO,

forming minor ions m/z 240 and 239, Loss of HCO (29) from the deprotonated phenolic compound, respectively [15]. The peak corresponding to the m/z 117 B-ring fragment production of 40.5-dihydroxy-7-methoxy flavone is the only difference observed in the ion-product spectra of the two isomeric compounds. It thus allows the differentiation of the two compounds [16].

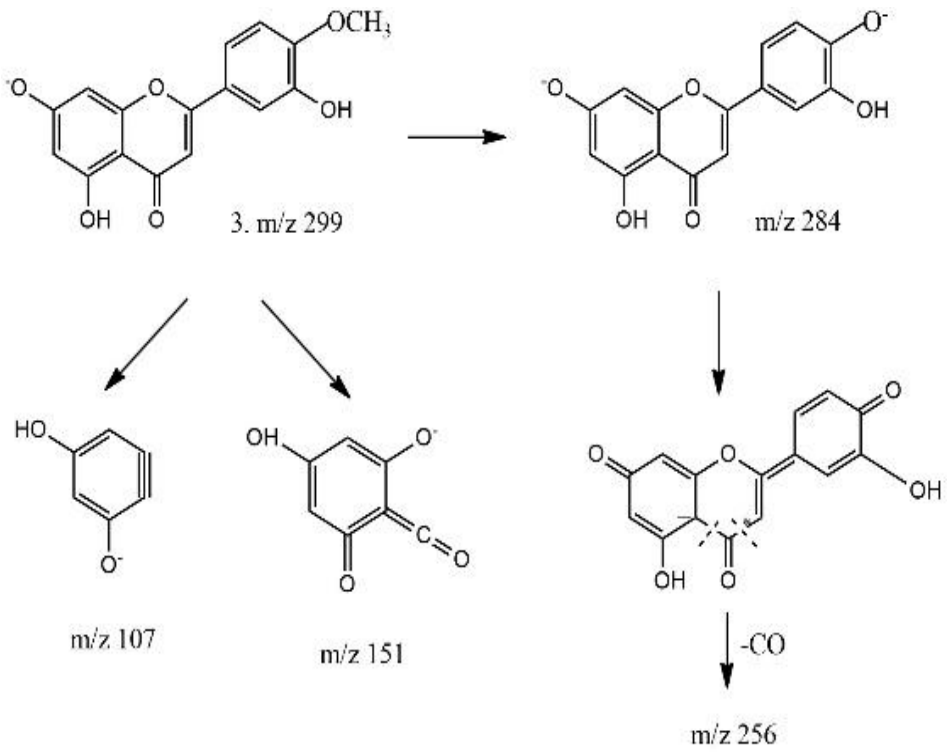

Figure 3. Diosmetin Fragmentation Pattern

Diosmetin/3,4,7-trihydroxy 3 methoxyflavone, and Mr 299/300, both exhibited loss of methyl as the most prominent fragmentation, yielding m/z 284. Other fragments arise from concurrent loss of CH_3 and CO and B-ring fragmentation. Diosmetin provides [M-H15-28] at m/z 256 and A-ring fragments m/z 107 and 151, whereas 3,4,7-trihydroxy-3-methoxy flavone provides m/z 135 A-ring fragments and ring fragments -B of m/z 148 COO m/z 117 m/z 239 m/z 240 The fragmentation pattern suggested is more a feature of the position of the 3- and 5-hydroxyl groups than the position of the B-ring methoxyl substituent [16].

The extract of Mimosa pudica leaves was found to contain alkaloids, flavonoids, tannins, and steroids based on the results of the chemical extraction. Subsequently, Gas Chromatography-Mass Spectroscopy (GC-MS) was used to conduct qualitative analysis and compare the results. The phytochemical screening was carried out using Gas Chromatography-Mass Spectroscopy (GC-MS), and the results of the quantitative analysis were obtained. According to the results of the chemical profiling using GC-MS, five compound peaks were identified

with apparent abundance, as shown in Figure 1. The peak with the highest abundance was found on the second and fourth peaks. The structure of each compound was determined based on its fragmentation pattern and distinctive base peaks.

Cholesterol Test

After the specific flavonoids, acacetin and diosmetin, were identified, their anti-cholesterol activity was tested on the extract of Mimosa pudica leaves. Acacetin is a natural flavonoid with antioxidant, anti-inflammatory, and antidepressant properties. It can also regulate protein and lipid metabolism, thus preventing free radicals from oxidizing cholesterol and causing arteriosclerosis [17,18].

The effect of acacetin on the atherosclerotic process, plasma inflammatory factors, and lipid metabolism were investigated. Acacetin significantly increased the viability of EA.hy926 cells by reducing the ratio of apoptotic and necrotic cells to 3 mol/L. Additionally, 3 mol/L acacetins decreased ROS levels and increased the reductase protein's expression through the MsrA and Nrf2 pathways via Nrf2 phosphorylation and Keap1 degradation. In vivo, acacetin treatment greatly attenuated atherosclerosis by increasing the aortic root's circulation and reductase levels, reducing plasma inflammatory factors' levels, and accelerating lipid metabolism. This indicates acacetin's anti-oxidative and anti-atherosclerotic effects, which show potential therapeutic value for atherosclerotic-related cardiovascular diseases [19,20]. Diosmetin is a flavonoid with antioxidant and anti-inflammatory effects and can regulate blood pressure and vascular changes [21,22]. Diosmetin is an aglycone of diosmin glycoside flavonoid, and its various therapeutic effects indicate excellent antioxidant activity both in vitro and in vivo [23]. Vascular changes are associated with dyslipidemic diseases that lead to heart disease. The anti-cholesterol test was conducted using the Liebermann-Burchard method [20] on the ethanol extract of Mimosa pudica leaves.

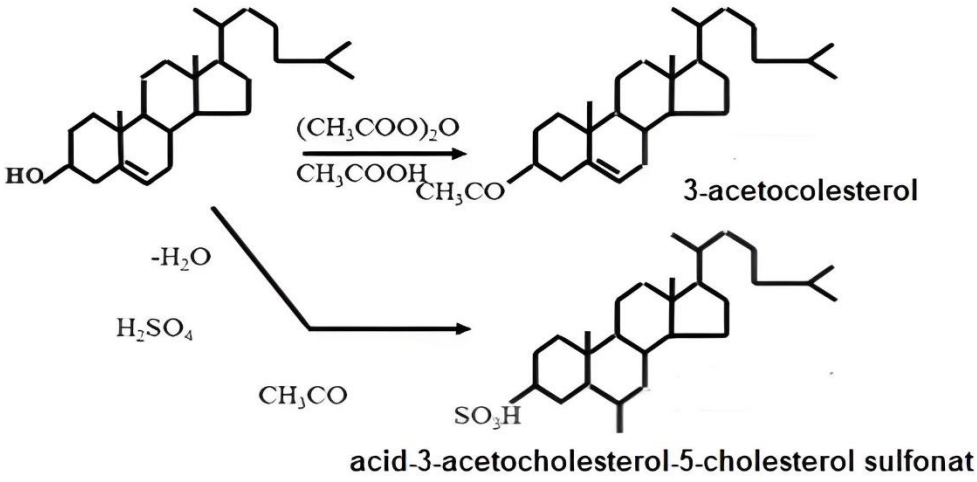

Figure 4. Cholesterol test reaction

Table 3. Data on Decrease in Cholesterol Levels in Putri Malu (Mimosa pudica L.) Leaves Extract

concentration	absorbance		% inhibition	IC 50 ppm
	blank	sample		
20,56	0,793	0,397	49,93695	**24,8993**
61,68	0,793	0,384	51,57629	
102,8	0,793	0,353	55,4855	
143,92	0,793	0,341	56,99874	
185,04	0,793	0,353	55,4855	

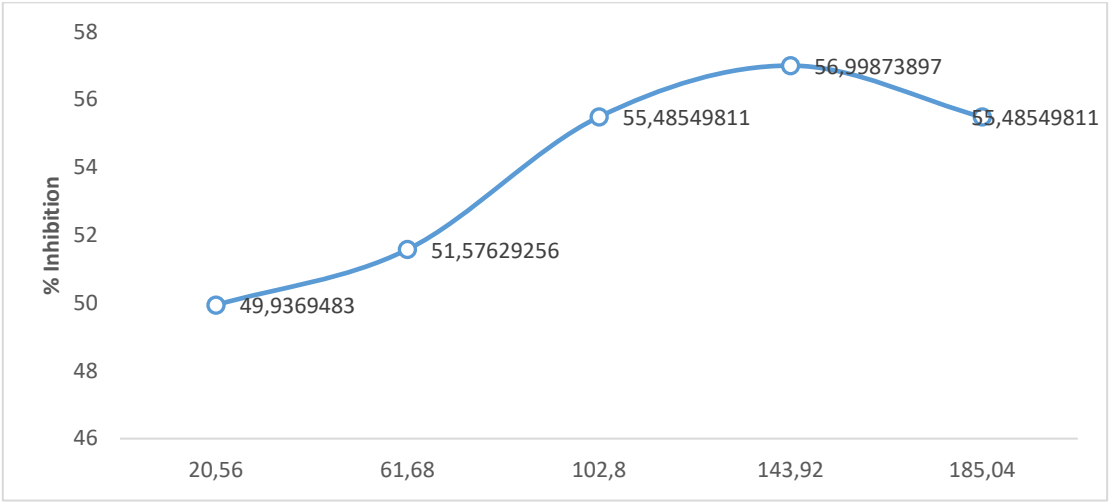

Figure 5. Cholesterol Levels Reduction Curve

After analyzing the table and diagram provided, it is evident that the absorbance decreases as the concentration of the extract increases while the percentage of cholesterol reduction increases. The extract binds to more cholesterol at higher concentrations, leaving less unbound cholesterol in the sample. As a result, when the Lieberman-Burchard reagent is added, the color of the test solution fades, resulting in a low absorbance value [24-26]. The color change indicates the presence of free cholesterol bound to the reagent. The extract from the leaves of Putri Malu (Mimosa pudica L.) with a concentration of 185.04 ppm exhibited a 55.4855% reduction in cholesterol levels, indicating its potential as an in vitro cholesterol-lowering agent. These findings align with the results of the UV Vis spectrophotometric method used to test the anti-cholesterol activity of the compounds. The IC50 value of 24.8993 ppm indicates an anti-cholesterol solid ability. It is worth noting that this study's limitations include the fact that the active compounds are still in the form of extracts, and it would be better to obtain fractions or isolates of the active compounds. The next step is to test the Putri Leave Extract in vivo.

CONCLUSION

The objective of this study was to identify the chemical compounds present in the leaves extract of Putri Malu (Mimosa

pudica L.) and to evaluate its anti-cholesterol effect in vitro. The findings showed that five chemical compounds were present in the extract, with the second and fourth peaks containing the most abundant compounds, acacetin, with an abundance of 14.08% and Diosmetin, with an abundance of 73.79%. Furthermore, the study results revealed that the ethanol extract of Putri Malu (Mimosa pudica L.) had anti-cholesterol activity in vitro, with an IC50 value of 24.8993, indicating a strong anti-cholesterol ability in vitro. Therefore, it can potentially be a therapeutic agent in treating hypercholesterolemia. However, it should be noted that this study only evaluated the extract's in vitro effects; further studies are needed to evaluate its effectiveness in vivo.

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