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Anticholesterol Assay of Ethanol Extract of Ginseng Leaves *(Talinum paniculatum)*

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Abstract: Instant eating patterns such as fast food have become a habit in society. Fast food has a high fat content and minimal fiber, so it is often associated with an increase in total cholesterol levels in the blood plasma. Ginseng leaves *(Talinum Paniculatum)* have anticholesterol properties due to the chemical compounds such as flavonoids, saponins, and tannins. Flavonoid compounds are able to release cholesterol found in the walls of blood vessels. The purpose of this study was to determine whether there was anticholesterol activity and EC_{50} value in the ethanol extract of ginseng leaves with a concentration range of 1500; 2500; 3500; 4500; and 5500 ppm. The samples extracted by maceration method with 96% ethanol obtained % an extract yield of 12.22%. The ethanol extract of ginseng leaves was tested for anticholesterol activity using the Lieberman-Burchard method and then measured using UV-Vis spectrophotometry at a wavelength of 669 nm for 5 minutes. The results reported that the ethanol extract of ginseng leaves possessed an anticholesterol activity with an EC_{50} value of 4040.7 ppm.

Keywords: *Anticholesterol; ethanol extracts; ginseng leaves; spectrophotometer*.

INTRODUCTION

As society adjusts to a more modern way of life, fast food and other instant foods are growing in popularity. Because fast food contains a lot of fat and little fiber, it is known to raise blood cholesterol levels (Bachmid, 2015).

Cholesterol is a waxy fatty compound secreted by the liver and present in the blood, which is yellowish and is very important for bodily functions (Mulyani, 2019). The body requires cholesterol as a source of energy, as a building block in the synthesis of steroid hormones, and to aid in the formation of cell walls. The liver is responsible for producing up to 80% of the cholesterol found in the body, the remaining 20% comes from the food and drink we normally consume (Anggraini & Ali, 2017).

South Sulawesi is home to a large number of medicinal plants. (Emelda, A. *et al*., 2023). One of them is flower fame leaves, widely used in South Sulawesi, locally known as "Ginseng Bugis".

Numerous studies have demonstrated that the flavonoids, saponins, tannins, alkaloids, and other chemicals present in ginseng leaves have an impact on boosting blood circulation to the peripheral and central nervous systems. Additionally, ginseng leaves have immunomodulatory and antibacterial activities. (Emelda, A. *et al*, 2020)

One of the many advantages of flavonoid compounds is their ability to lower cholesterol. The cholesterol that builds up on the inside of the coronary artery walls can be decreased by flavonoids. Consequently, it won't spread diseases like stroke, high blood pressure, and cardiovascular disease. (Anggraini & Ali, 2017). In previous studies, ginseng leaves have been shown to have an activity as antioxidants. (Lestario & Angelia Essi Christian, 2009). In addition to acting as antioxidants that can work to reduce LDL in the body, flavonoids can act as compounds that can lower triglycerides (TGA) and increase HDL (Ranti, *et al*., 2013).

MATERIAL S AND METHODS

Tools and Materials

The tools and instruments employed in this study comprised analytical balances (Kern), blenders, glassware (Pyrex®), a set of maceration tools, a set of rotavapor tools, pipettes, micropipette (Nesco), Vortex (Ika®), and a UV-Visible spectrophotometer (Thermo Scientific® Genesys 10S UV-Vis). Besides, 96% technical ethanol, cholesterol standard pa, chloroform (CHCl3) pa, acetic anhydrous acid (CH3CO)2O, and concentrated sulfuric acid (H2SO4) were among the materials used in this study.

Work Procedures

Simplicia Setup

Around 11 am, the samples of fresh ginseng leaves were collected from the plant in Makassar City, South Sulawesi. To remove any remaining dirt from the samples, they were sorted. After that, the samples were cleaned, chopped and allowed to air dry at room temperature. The simplicia was then thoroughly blended and extracted using the maceration method (Ilyas, *et al*., 2020).

Extract Manufacturing

50 grams of powdered ginseng leaf sample was macerated in up to 730 ml of 96% ethanol until the simplicia was completely dissolved. To maximize the results of the extraction, the simplicia was soaked for three days while being stirred occasionally. After that, it was filtered and remacerated. In order to create a thick extract, the filtrates obtained from the maceration results were combined and then concentrated using a rotary vacuum evaporator.(Ilyas, *et al*., 2020).

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Anticholesterol Potency Test

Preparation of 500 ppm Cholesterol Stock Solution

A 500 ppm cholesterol stock solution was prepared by dissolving 25 mg of cholesterol powder in chloroform to a volume of 50 ml.

Maximum Wavelength Determination

After incubating 5 ml of 500 ppm cholesterol stock solution in 2 ml of acetic anhydrous acid for 5 minutes, 0.1 ml of concentrated sulfuric acid was added and the mixture was vortexed for 2 minutes before being measured using a UV-Vis spectrophotometer at maximum wavelength from 400 to 800 nm for 5 minutes.(Ilyas, *et al*., 2020).

Determination of Operating Time

At 5-minute intervals from 5 to 30 minutes, 5 ml of 500 ppm cholesterol stock solution was pipetted, reacted with 2 ml of acetic anhydrous acid, incubated, then measured using a maximum wavelength of 669 nm to obtain cholesterol absorbance. The correlation between sample measurement time and absorbance was then observed. The goal was to ensure consistent measurement times.(Ilyas, *et al*., 2020).

Determination of Anticholesterol Potency in Extracts

The extract was created at a concentration of 10,000 ppm by weighing 500 mg and dissolving it in 50 ml of chloroform. The resulting solution was then diluted with pipettes containing 1.5, 2.5, 3.5, 4.5, and 5.5 ml each, before the volume was increased to 10 ml, resulting in concentrations of 1500, 2500, 3500, 4500, and 5500 ppm for the extract. 2 ml of each concentration was then pipetted into a test tube, to which 5 ml of a 500 ppm cholesterol standard was added, and the test tube was vortexed for 2 minutes. Following a 2 ml reaction with acetic anhydrous acid, it was incubated for 5 minutes. After that, 0.1 ml of highly concentrated sulfuric acid was added, and the mixture was vortexed for 2 minutes. A green color was produced after it was combined with 0.1 ml of concentrated sulfuric acid and vortexed for 2 minutes. Then, in the fifth minute, the solution was observed using a Uv-Vis spectrophotometer at a wavelength of 669 nm.(Ilyas, *et al*., 2020).

DATA ANALYSIS

The percentage of cholesterol reduction was calculated by comparing the absorbance measured from the ethanol extract of ginseng leaves with the absorbance measured from the standard cholesterol solution. The following calculation can be used to determine the percentage reduction in cholesterol levels:(Anggraini & Nabillah, 2018)

$$A = \frac{\mathrm{C} - \mathrm{B}}{\mathrm{C}} X\ 100\%$$

A = % reduction in cholesterol
B = Absorbance of the sample after treatment (ethanol extract + standard)
C = Absorbance of initial cholesterol standard

RESULTS AND DISCUSSION

Cholesterol is a natural compound resembling fat that has a steroid structure and plays an important role in the body. High cholesterol levels, on the other hand, can result in atherosclerosis, which can raise blood pressure, obstruct the arteries to the heart, brain, and limbs, and even result in death over an extended period of time.(Anggraini & Nabillah, 2018).

The aim of this study was to determine the in vitro anticholesterol activity of the ethanol extract of ginseng leaves at various concentrations. The plants used were ginseng leaves containing anticholesterol properties, namely flavonoids, saponins, and tannins(Lestario & Angelia Essi Christian, 2009).

Ginseng leaves contain the necessary flavonoid compounds, but because they are easily oxidized at high temperatures and are not heat resistant, the extraction method used is the cold method, specifically the maceration method. (Rompas, *et al*., 2012).

In this study, 96% ethanol was used as a solvent for extracting samples because it effectively extracts various active substances and can remove almost all organic solvents, both polar and semi-polar. This allows the isolation of active constituents such as flavonoids, which are normally insoluble in water(Ilyas, *et al*., 2020). Table 1 shows the percentage yield of the extract after evaporation of the extraction results at 50^{o}C using a rotary vacuum evaporator. The greater the yield of the extract, the more bioactive compounds are drawn into the raw material(Senduk, *et al*., 2020).

Table 1: Calculation of Percent Yield of Ginseng Leaf Ethanol Extract

Sample	Simple weight (g)	Extract weight (g)	Extract yield (%)
Ginseng Leaves	50	6,11	12,22

Anticholesterol activity is also assessed by the Lieberman-Burchard method. Cholesterol is one of the substances in the steroid class that can be measured using this method.(Anggraini & Nabillah, 2018). Both higher and lower plants contain steroid hormones. The most prevalent steroid in plants is a sterol.(Suryelita, *et al*., 2017). Since the goal of this study was to lower cholesterol levels, the Uv-Vis spectrophotometer was employed to measure anticholesterol activity. Cholesterol compounds have chromophore groups, and all of these chromophore groups are in organic compounds, which can strongly absorb light in the Uv-Vis region (Ganjar and Rohman, 2007). An alkene is an example of a chromophore for cholesterol.

This study started with a measurement of the maximum wavelength to determine the wavelength that results in the greatest absorbance.(Anggraini & Ali, 2017). 500 ppm of a cholesterol standard was used, and a

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wavelength of 669 nm was obtained. Additionally, the operating time was established in order to determine a reliable measurement time to track the progress of a reaction or change in color. The operating time in this experiment was used in the fifth minute.

The amount of cholesterol reduced in a sample of ginseng leaves extracted with ethanol was measured at concentrations of 1500, 2500, 3500, 4500, and 5500 ppm. This sample was then treated with the Lieberman-Burchard reagent, acetic anhydrous acid, to extract cholesterol while ensuring that the media was devoid of water and producing acetyl steroid derivatives. Finally, concentrated sulfuric acid was dripped through the walls to produce a green color for the steroid compounds, including cholesterol.(Anggraini & Nabillah, 2018).

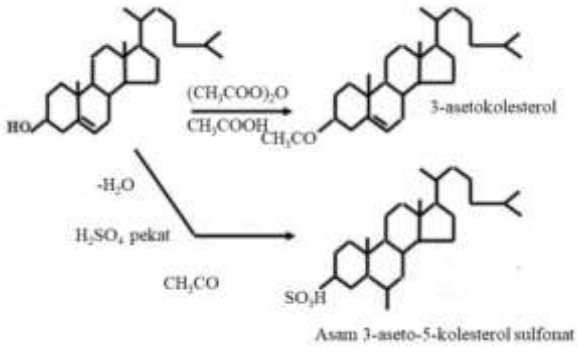

Figure 2: The green color formation reaction between cholesterol and the Lieberman-Burchard reagent(Anggraini & Nabillah, 2018)

Being photodegradable, the cholesterol solution needs to be wrapped in aluminum foil during processing because it needs to be shielded from light.(Ilyas, *et al*., 2020). In the fifth minute, the samples were measured at a maximum wavelength of 669 nm. Table 2 displays the results of the % reduction in cholesterol levels in the sample extract.

Table 2: Data on % reduction in cholesterol levels by sample extracts

Initial cholesterol absorbance 500 ppm	Concentration extract (ppm)	absorbance	% reduction in cholesterol levels
0.826	1500	0.751	9.08
	2500	0.663	19,733
	3500	0.498	39,709
	4500	0.356	56,900
	5500	0.186	77,481

Table 2 shows that ginseng leaf extract causes a decrease in absorbance. The absorbance value varies depending on the sample concentration, so the lower the absorbance obtained, the higher the sample concentration. This occurs because the anticholesterol content of the sample increases with sample concentration, meaning that as more cholesterol is inhibited, less cholesterol remains and the absorbance value decreases. Then, for each concentration, the percentage reduction in cholesterol levels was determined. The conclusion reached is that ginseng leaf extract reduces cholesterol levels by a higher percentage causing a lower absorbance value. The highest percent reduction, which was able to lower the initial cholesterol by 77.481%, was determined by the calculation of the percentage reduction in cholesterol levels at a concentration of 5500 ppm.

Figure 2 shows a plot of the data from Table 2 in the form of a model standard curve graph. The regression equation y = 0.0174x - 20.309 with r = 0.9952 is obtained. Because it satisfies the acceptance criteria, specifically the correlation coefficient value of $0.995 \leq r \leq 1$, the correlation coefficient obtained from this standard curve displays linear results (Rohman, 2016). The EC_{50} value is then calculated, and it is discovered to be 4,040 ppm. At the highest concentration of 5500 ppm, the EC_{50} value is capable of reducing 77.481% of initial cholesterol.

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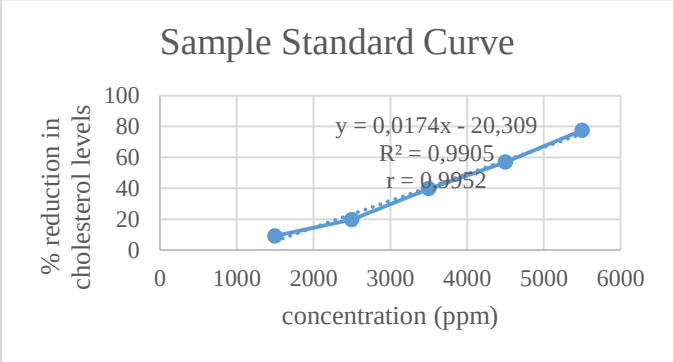

Figure 2: Standard curve for ethanol extract of ginseng leaves *(Talinum paniculatum)*

CONCLUSION

The results demonstrate that the ethanol extract of ginseng leaves has in vitro anticholesterol activity. The EC_{50} value of the ethanol extract of ginseng leaves is 4,040 ppm.

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