

CASHEW LEAF EXTRACT TEST (*Anacardium Occidentale L.*) AS AN INVIVO ANTI-CHOLESTEROL AGENT

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ABSTRACT

Objective: This study aims to assess the potential of cashew leaf extract (*Anacardium occidentale L.*) as an anti-cholesterol agent in vivo using mice (*Mus musculus*) as test animals. Extraction was carried out using a maceration technique with 70% ethanol solvent, then the extraction results were concentrated using a rotary evaporator to obtain a thick extract with a yield of 9.21% (16.05 g from 1000 g of simplicia). Fifteen mice were used and grouped into five parts (three mice each), consisting of a negative control group (0.5% NaCMC) and a positive control group (atorvastatin), and three treatment groups were given extracts with varying doses of 150 mg, 250 mg and 350 mg per kilogram of body weight. The test animals were first given a high-fat diet for seven days, then received treatment according to their group for the next seven days. Total cholesterol levels were measured using a cholesterol meter every day, the data were analyzed statistically using the One-Way ANOVA test with a significance level of 0.05. **Results:** showed that cashew leaf extract was proven to significantly reduce cholesterol levels in the medium and high dose treatment groups. The average cholesterol levels after each treatment were: negative control 170.40 mg/dL; positive control 103.20 mg/dL; 150 mg/kg extract 114.20 mg/dL; 250 mg/kg 108.00 mg/dL; and 350 mg/kg 103.80 mg/dL. Statistical analysis showed a significant difference between the negative control group and the group receiving the extract treatment at a high dose. **Conclusion:** cashew leaf extract has the potential to be a cholesterol-lowering agent in vivo, at a dose of 350 mg per kilogram of body weight showing the best effectiveness. Further research is recommended to evaluate the mechanism of action and chronic toxicity tests.

INTRODUCTION

Cashews (*Anacardium occidentale* L.) are among the industrial crops that have great potential to be cultivated, especially in marginal regions with dry climates. This is due to the high economic value of seeds or kernels as the main product of the plant. In addition, this plant also produces by-products in the form of CNSL oil extracted from seed shells and pseudo-fruits that can be processed into various products, such as syrups and jams. (Rusmin, 2007)

Cashew leaves are one of the types of plants that are widely cultivated in the Southeast Sulawesi region. Initially, this plant was used as a greening plant to help improve environmental conditions. However, along with increasing economic value and promising prospects, farmers began to cultivate this crop as an additional source of income that could support their economy. The enthusiasm of the people of Southeast Sulawesi for the cultivation of this plant is increasing from time to time. Cashew leaves are used as a superior commodity, both for export and import activities to support economic growth. The area of cashew leaf cultivation land in Southeast Sulawesi continues to increase significantly every year, in line with the increase in production. Consumption of cashew leaves is known to be able to reduce *low density lipoprotein* (LDL) levels and increase *high density lipoprotein* (HDL) levels in the body. (Wahyuningsih et al., 2021)

Research shows that high cholesterol levels in the blood can increase the risk of heart disease as well as disorders in blood vessels. Excessive levels of *low density lipoprotein* (LDL) can cause plaque buildup in the walls of blood vessels, which can eventually block blood flow and trigger a heart attack or stroke. Consumption of cashew leaves is known to be able to reduce *LDL low density lipoprotein* levels and increase *high density lipoprotein* (HDL) levels. This positive effect is obtained from the content of vitamin C and polyphenol compounds contained in cashew leaves (Pranowo & Purwanto, 2011).

Furthermore, the addition of 1% cashew leaf powder in the feed resulted in the lowest cholesterol level of 130.66. The highest *high density lipoprotein* (HDL) cholesterol value was recorded at 61.43, while *low density lipoprotein* (LDL) and triglycerides were lowest at 60.33 and 49.40, respectively. Cashew leaves contain active compounds saponins, which are known to lower *low density lipoprotein* (LDL) and triglyceride cholesterol levels and increase *high density lipoprotein* (HDL) cholesterol levels. Saponins work by binding to cholesterol and preventing its absorption, since the interaction between saponins and cholesterol forms an insoluble complex. The saponin content in cashew leaf powder (DJM) has been proven to be effective in lowering blood cholesterol levels. (Maharani et al., 2019)

Therefore, based on the background and results of previous research data, we are interested in conducting research on the saponin content in cashew leaf extract (*Anacardium occidentale*) from Central Buton Regency, Southeast Sulawesi, as well as its application as an anti-cholesterol substance.

METHODS

Types of Research

This research was carried out through experiments in the laboratory to test the ability of cashew leaf extract in lowering cholesterol levels in mice (*Mus musculus*).

Time and Place of Research

This research was conducted in August 2025 and samples were taken in Lombe Village, Central Buton, and sample testing was carried out at the Pharmaceutical Laboratory of Baubau Polytechnic and Bina Husada Polytechnic.

Tools and Materials

Tool

The equipment to be used in this study consists of a 40-mesh sieve, a stirring rod, and a maceration container, erlenmeyer, *easy touch* (cholesterol meter), a beaker, a measuring cup, a test animal cage, a drip pipette, a micro pipette, a *rotary evaporator*, a refrigerator, a 3 ml injection spool, a test tube and an analytical scale.

Material

The ingredients that will be used in this study are cashew leaves (*Anacardium occidentale L.*), mice (*Mus Musculus*) 15 heads, high-fat food consisting of goat fat oil, mouse feed, chicken egg yolk, NaCMC, atorvastatin 20 mg, ethanol 70%, viscosity.

Working Procedure

Sample Preparation

Cashew Leaf Sample (*Anacardium occidentale L.*) was taken from one place in Lombe, central Buton, Southeast Sulawesi. After the cashew leaf sample is collected, the cashew leaf sample is washed using running water to reduce the presence of microbes that can cause spoilage. After that, the sample is cut into small pieces and dried by aerating to remove the moisture content. From this process, 2 kg of dry simplicia was obtained. Then the dried simplicia is blended into a fine powder.

Process of Making Cashew Leaf Extract (*Anacardium occidentale L.*)

A total of 1000 grams of cashew leaf powder was weighed, then put into 2.5 liters of 70% ethanol. The mixture is tightly covered and left for 3 x 24 hours in a place protected from light, while stirring periodically. The extract is filtered using filter paper and residues and filtrates are obtained. Phytrate is concentrated using a rotary evaporator at a temperature of 50 degrees Celsius to obtain a thick extract.

Manufacturing Process of CMC Na Solution 0.5%

A total of 0.5 grams of Na CMC is weighed, then put into a beaker for a dissolution process containing 100 mL of warm aquadest, then stirred until evenly mixed.

Test Sample Creation (Groups 3, 4 and 5)

Weighing 150 mg, 250 mg and 350 mg of cashew leaf extract respectively, then placed in a lump and mashed using a pestle, 10 mL of Na CMC is measured using a measuring cup, then dissolved gradually little by little, at least 2 mL using a 0.5% solution of Na CMC as much as 10 mL, then stirred or crushed until evenly mixed then put in a beak.

Test Solution Manufacturing

Prepared as many as 10 tablets of Atorvastatin 20 mg, crushed using a mortar and pestle until homogeneous then weighed Atorvastatin which has been crushed by 0.052 mg then put into a beaker containing 0.5% Na CMC suspension as much as 5 mL, then stirred until evenly mixed.

Treatment Procedure in Test Animals of Mice (*Mus musculus*)

The test animals were randomly divided into 5 groups, each group consisting of 5 mice which include:

Group 1: the group of mice were treated with a 0.5% Na CMC suspension of 1 mL (as a negative control).

Group 2: a group of mice administered a 20 mg/kg dose of Atorvastatin suspension dissolved in 1 mL of 0.5% Na CMC solution (as a positive control).

Group 3: a group of mice were given a suspension of cashew leaf extract at a dose of 150 mg/kg BB dissolved in 1 mL of 0.5% Na CMC solution.

Group 4 : a group of mice that were given an extract suspension at a dose of 250 mg/kg BB dissolved in 1 mL of 0.5% Na CMC solution.

Group 5: a group of mice administered an extract suspension at a dose of 350 mg/kg BB dissolved in 1 mL of 0.5% Na CMC solution.

Anticholesterol Activity Test in Mice (*Mus musculus*) Test Animals

Anticholesterol testing in mice (*Mus musculus*) test animals was first weighed and then induced egg yolks adjusted subplantarily using 0.1 mL of 1% carrageenan solution. After that, the mice's blood was taken and measured using *easy touch* (cholesterol meter) in 1 day and given a suspension of the test material orally according to the group and the last step was taken to measure every 1 day for 7 days and the data was recorded.

In-Vivo Tests

Making Cashew Leaf Extract For In Vivo Test

Cashew leaf powder is weighed as much as 1000 grams, then 1000 grams of cashew leaf

powder is dissolved in 70% ethanol as much as 2.5 liters. Covered and then left for 3 x 24 hours to be kept protected from light exposure while stirring occasionally. The extract is then filtered using filter paper until residues and filtrate are obtained. The filtrate is then evaporated with a rotary evaporator at a temperature of 50 degrees Celsius until a thick extract is obtained. The results of cashew leaf extract were then diluted based on conversion doses for mice, so that the concentration of the extract as an anticholesterol adjuvant was obtained, namely 150 mg/20 kg BB, 250 mg/20 kg BB, and 350 mg/20 kg BB of mice. The result of conversion of atorvastatin dose as an anticholesterol treatment was 0.052 mg/20 kg of BB mice.

In Vivo Test of Cashew Leaf Extract against Mice (*Mus musculus*)

The *in vivo* test was carried out by randomly dividing mice test animals into five treatment groups, namely negative control group, positive control group, test group I given cashew leaf extract at a dose of 150 mg/kg BB, test group II with a dose of 250 mg/kg BB, and test group III with a dose of 350 mg/kg BB. Details of the division of groups are presented in Table 3.1.

Table 3.1 In Vivo Test of Extract on Mice (*Mus musculus*).

Yes	Action	Total Mint
1	Negative control, normal mice that were given only Na CMC solution	5
2	Positive control, mice administered anticholesterol treatment atorvastatin 0.052 mg/20kg BB and Na CMC solution	5
3	Test group I, mice were given cashew leaf extract at a dose of 150mg/20kg BB of mice	5
4	In test group II, mice were given cashew leaf extract at a dose of 250mg/20kg BB of mice	5
5	In test group III, mice were given cashew leaf extract at a dose of 350 mg/20kg BB of mice.	5

Remarks: Group I: Negative control given Na CMC 0.5%, Group II: Positive control given Atorvastatin 20 mg/kg BB, Group III: Administration of cashew leaf ethanol extract at a dose of 150 mg/kg BB, Group IV: Administration of cashew leaf ethanol extract at a dose of 250 mg/kg BB, Group V: Administration of cashmere leaf ethanol extract at a dose of 350 mg/kg BB.

Data Analysis

The data obtained in the form of total blood cholesterol levels from the research were then calculated and statistically analyzed using the One Way ANOVA test with a 95% confidence level. This test was used to evaluate the diameter of the inhibition zone in *in vitro* studies as well as anti-cholesterol amounts in *in vivo* studies.

RESULTS & DISCUSSION

This study is experimental and carried out by the maceration extraction method using 70% ethanol solvent. Next, the sample is concentrated using a rotary evaporator at a water bath temperature of 50°C to prevent the loss of heat-sensitive bioactive compounds. The yield of the condensed extract was concentrated using a rotary evaporator which was 16.05 grams, with a yield of 9.21%.

Extraction Results

Table 4.1 Results of extraction of cashew leaves (*Anacardium occidentale* L.Düssel

Powder weight (g)	Extract Weight (g)	Yields (%)
1000 grams	16,05 grams	9,21%

Premiere Date (2025)

Results of the test of the effectiveness of the extraction of cashew leaves (*Anacardium occidentale* L.) as an anti-cholesterol in mice test animals (*Mus musculus*)

Table 4.2 Results of the effectiveness of cashew leaf extraction (*Anacardium occidentale L.*) as an anti-cholesterol in mice test animals (*Mus musculus*).

Yes	Groups of Friends	Blood Cholesterol Level (mg/dL)		
		Before Treatment (Day 0)	After Induction the egg yolk is given (7 Days)	After the administration of cashew leaf extract (7 Days)
1	Group I	107.20mg/dL	175.20mg/dL	170.40mg/dL
2	Group II	109.60mg/dL	182.20mg/dL	103.20mg/dL
3	Group III	106.60mg/dL	178.80mg/dL	114.20mg/dL
4	Group IV	109.60mg/dL	176.60mg/dL	108.00mg/dL
5	Group V	109.80mg/dL	177.00mg/dL	103.80mg/dL

Remarks: Group I: Negative control administered Na CMC 0.5%; Group II: Positive control administered Atorvastatin 20 mg/kg BB; Group III: Administration of cashew leaf ethanol extract at a dose of 150 mg/kg BB; Group IV: Administration of cashew leaf ethanol extract at a dose of 250 mg/kg BB; Group V: Administration of cashew leaf ethanol extract at a dose of 350 mg/kg BB.

DISCUSSION

Cashew plants are nutritional content that is beneficial for the body. Cashew leaves, also known as guavas, have a fairly high calorie content and are rich in carbohydrates, protein, fat and fiber. In addition, these leaves contain vitamin K, vitamin E, folate, B vitamins, as well as essential minerals such as calcium, sodium, potassium, magnesium, phosphorus, zinc and iron. Various studies show that cashew leaves can help lower the risk of several diseases and support the overall health of the body. The health benefits of cashew leaves include lowering cholesterol levels, reducing the risk of heart disease, preventing anemia, being a source of energy, maintaining bone health, maintaining eye health, preventing the formation of gallstones, and helping with weight management (Markhamah, 2021).

In the body there are two types of cholesterol levels, namely LDL (Low Density Lipoprotein) and HDL (High Density Lipoprotein). LDL is often referred to as bad cholesterol because of its ability to stick to the walls of blood vessels, while HDL is known as good cholesterol because it can dissolve and transport LDL back to the liver. Total body cholesterol is the total amount of cholesterol in the blood. The balance of LDL and HDL levels is very important, because if LDL levels are higher than HDL, the body's metabolism and heart function can be disrupted. HDL plays a role in cleaning bad cholesterol from the walls of blood vessels, thereby supporting cardiovascular health (Ridayani *et al*, 2018).

Powder extraction process The process of extracting cashew leaves is carried out using the maceration method with 70% ethanol solvent. A total of 1000 grams of cashew leaf powder is weighed, then put in a glass container and added 2500 mL of 70% ethanol. Maceration lasts for 3 days with occasional stirring to ensure the active compounds are optimally extracted. When finished, the solution is filtered using filter paper or white cloth to separate the filtrate and residue. The collected filtrate is then evaporated using a rotary evaporator to remove the solvent so that a viscous extract is obtained. The extraction results showed that the condensed extract obtained weighed 16.05 grams with a yield of 9.21%. This yield shows that the extraction process has successfully taken about 9.21% of the initial sample weight of the extracted extract.

This study used 15 mice with 5 treatment groups, each group consisting of 3 mice. Before treatment, the mice test animals were first fed a feed that was high in cholesterol. Then they were given treatment according to their respective groups, namely negative control using 0.5% Na CMC, positive control using atorvastatin 20mg/20kgBB, extract dose group namely 150 mg/20kgBB extract of mice, 250 mg/20kgBB of mice and 350 mg/20kgBB of mice. The result of the amount of anti-cholesterol in mice for 7 days. It can be seen in table 4.2.

In group 1 (negative control), mice were given a 0.5% Na CMC suspension for 7 days. On the first day, which is day 0 before low cholesterol treatment, which is 107.20mg after being induced, high cholesterol feed is given and cholesterol measurement is carried out every day until the 7th day the cholesterol level in mice rises to 175.20mg and after the administration of CMC on the 7th day the

cholesterol level drops to 170.40mg. From the data, it can be seen that Na CMC functions as a carrier substance and does not have any effect on the mice test animals.

In group II (positive control), atorvastatin suspension was given. In this group, before treatment (day 0) cholesterol levels were 109.60mg after induction was given high cholesterol feed and cholesterol measurements were carried out every day until (day 7) cholesterol levels rose to 182.20 and after administration cholesterol levels dropped to 103.20. This proves that atorvastatin as an anticholesterol drug works effectively in inhibiting cholesterol levels, so that cholesterol can be significantly lowered. mg mg.

In group III, mice were given cashew leaf extract at a dose of 150 mg/kg BB. In this group, a decrease in cholesterol occurred but was not too significant. From day 0 before treatment, cholesterol levels were 106.60mg, then after induction were given high-cholesterol feed and cholesterol measurements were carried out every day until day 7 cholesterol levels increased to 178.80 and after the administration of cashew leaf extract of 150 mg/kgBB cholesterol levels decreased to 114.20. This indicates that at low doses (150 mg/kg BB), cashew leaf extract has not provided optimal anticholesterol effects. mg mg.

Group IV was given cashew leaf extract with a dose of 250 mg/kg BB. This group showed a more pronounced decrease in cholesterol. From before the treatment on day 0, which was 109.60, then after induction was given a high cholesterol feed and cholesterol measurements were carried out every day until day 7, which was 176.60 and after the 7th day of administration, the cholesterol level decreased to 108.00. This shows that the medium dose of cashew leaf extract began to provide a real anticholesterol effect, although it was not as effective as the positive control group. The effects tended to be stable and consistent throughout the observation time. mg mg.

Group V with a dose of cashew leaf extract of 350 mg/kg BB. This group showed the most potent cholesterol reduction among all the cashew leaf extract groups. Before the treatment, day 0, cholesterol in mice was 109.80mg, then after induction was given high-cholesterol feed and cholesterol measurement was carried out every day until day 7, cholesterol levels rose to 177.00mg and after administration cholesterol levels dropped drastically to 103.80mg. This shows that high doses of cashew leaf extract are very effective in lowering cholesterol.

CONCLUSION

Based on the results of the study, it can be concluded that cashew leaf extract can lower cholesterol in vivo using mice test animals and the most effective dose of cashew leaf extract in lowering blood cholesterol levels is dose 3 (350 mg/kgBB).

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