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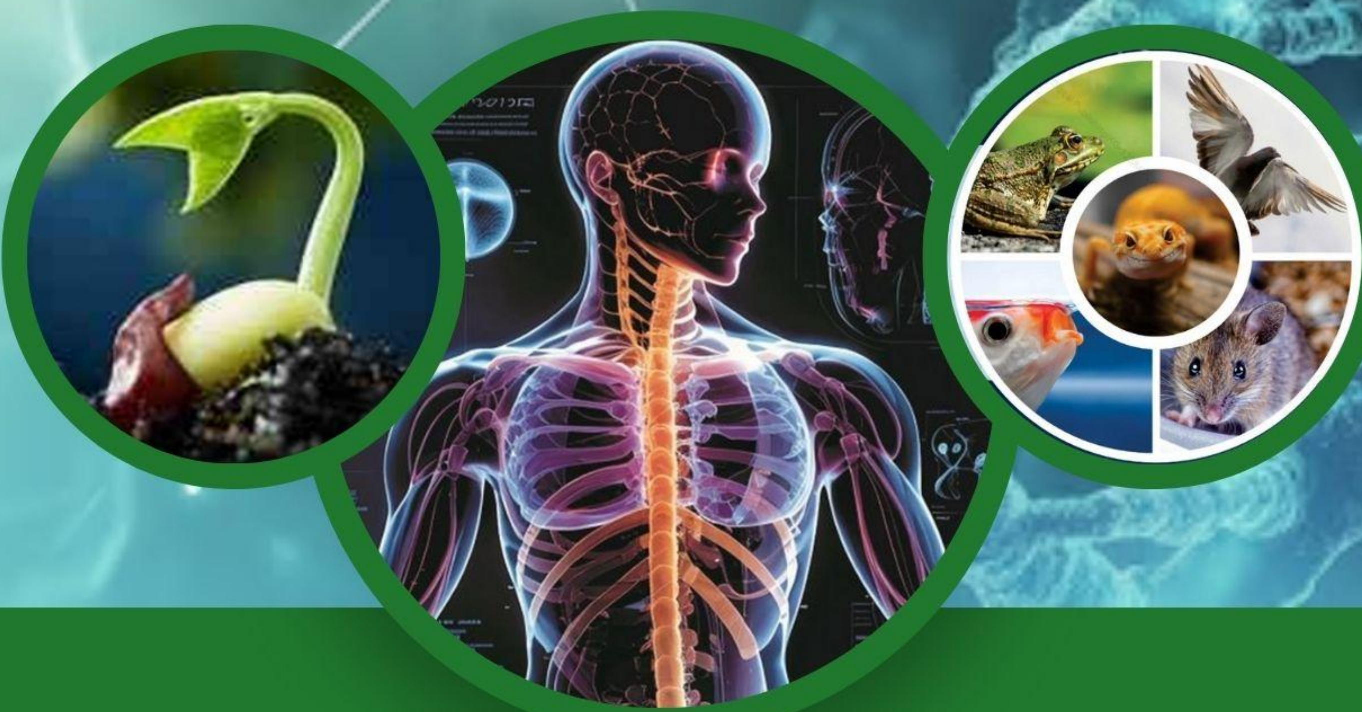

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EFFECTIVENESS OF METHANOL AND PETROLEUM BENZENE EXTRACTS OF *Catharantus roseus* LEAVES IN REDUCING BLOOD SUGAR LEVELS IN MICE (*Mus musculus*) DIABETES MELLITUS MODEL

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Abstract
Diabetes mellitus (DM) is one of the most common chronic diseases, and its global prevalence continues to rise. The prevalence of diabetes is projected to increase with population aging, from 111.2 million individuals aged 65–79 years. This number is expected to grow further, reaching 578 million by 2030 and 700 million by 2045. This disease affects the body's metabolism and can lead to serious complications. Although it can be managed with oral antidiabetic medications, there is a growing need for alternative therapies such as traditional medicines. One such alternative is the periwinkle plant (*Catharanthus roseus* (L.) G. Don), which has been reported to reduce blood glucose levels. The plant contains more than 70 types of alkaloids, which are believed to play a key role in lowering blood glucose. This study aims to investigate the effect of methanol and petroleum benzene extracts of *C. roseus* leaves on blood glucose levels in mice induced with diabetes mellitus. The study involved testing the pure methanol extract of *C. roseus* in diabetic mice to comprehensively verify its efficacy. Both methanol and petroleum benzene leaf extracts of *C. roseus* were diluted to a concentration of 5% and administered to the test animals at a dose of 1.5 mL. The experimental data were analyzed using ANOVA. The results demonstrated that administration of *C. roseus* extracts significantly reduced blood glucose levels in mice. These findings confirm the hypoglycemic effect of the methanol and petroleum benzene extracts of *C. roseus* leaves in lowering blood glucose levels in streptozotocin-induced diabetic mice.

INTRODUCTION

Diabetes mellitus is a non-communicable disease and a metabolic disorder commonly characterized by chronic hyperglycemia due to impaired insulin secretion, insulin response, or both (Ukratalo *et al*., 2022; Pangemanan *et al*., 2023; Kaihena *et al*., 2024). Elevated blood glucose (or blood sugar) levels are a hallmark of the chronic metabolic disease known as diabetes, which, over time, can lead to severe damage to blood vessels, eyes, kidneys, heart, and nerves (Moniharapon *et al*., 2023).

According to the International Diabetes Federation (2024), the statistics on diabetes show a growing burden on individuals, families, and countries worldwide. The IDF reports that 10.5% of adults aged 20-79 are affected by diabetes, with nearly half of them unaware of their condition. By 2045, the IDF projects that 1 in 8 adults, approximately 783 million people, will be living with diabetes, reflecting a 46% increase. More than 90% of type 2 diabetes cases are attributed to socio-economic, demographic, environmental, and genetic factors.

According to the Basic Health Research (2018), the prevalence of diabetes in Indonesia in 2013 was 1.5%. Still, by 2018, it had increased to 2.0%, indicating a 0.5% rise in the prevalence of diabetes in the country. One of the key factors driving the global increase in diabetes cases, including in Indonesia, is lifestyle and dietary changes (Suryanti, 2021). The shift toward consuming fast food has led to disturbances in human digestive systems, which results in higher blood sugar levels (Kaihena *et al*., 2023; Ukratalo *et al*., 2023). Diabetes occurs when an insulin deficiency cannot function properly (Setiawan, 2021). Insulin, produced by the body, lowers blood sugar levels by stimulating cells to absorb glucose (Triplett, 2012; Qaid & Abdelrahman, 2016).

The complications that may occur in patients with diabetes mellitus include both macrovascular and microvascular complications. Macrovascular complications include coronary artery disease, cerebrovascular disease, hypertension, vascular diseases, and infections. Meanwhile, microvascular complications that can occur in diabetes mellitus include retinopathy, nephropathy, sensorimotor neuropathy, and autonomic neuropathy affecting the pupils, heart, gastrointestinal, urogenital systems, as well as leg and foot ulcers (Saputri *et al*., 2025).

General treatment for diabetes mellitus includes education, medical nutrition therapy, physical exercise, and pharmacological therapy (Syuaib *et al*., 2025). Available diabetes treatment options have not provided satisfactory results for patients, as side effects may occur. The trend of using herbal-based antidiabetic treatments in developing countries has encouraged researchers to explore the hidden potential of plants found in nature (Tuhumury & Ukratalo, 2023; Ukratalo, 2023), as herbal antidiabetic treatments tend to be safer and have fewer side effects (Mahapatra & Bharti, 2019; Ukratalo *et al*., 2025).

Catharanthus roseus (commonly known as the periwinkle) is a small shrub in the evergreen category. The leaves of *Catharanthus roseus* are believed to relieve pain in the skin, especially in cases of open wounds (Kala *et al*., 2025). They are also used in treating diabetes mellitus, although traditionally. This plant, belonging to the Apocynaceae family, contains various chemical compounds.

The roots stems, leaves, and seeds of *Catharanthus roseus* contain over 70 types of alkaloids, including 28 bi-indole alkaloids throughout the plant. *Catharanthus roseus* also contains anticancer alkaloids, specifically the active components vinblastine and leurocristine (vincristine) (Paul *et al.* 2023). In addition to alkaloids, *Catharanthus roseus* also contains antioxidant compounds and flavonoids (Jayaraj *et al.,* 2020). Four key substances in *Catharanthus roseus* that can be utilized for alternative medicine are: Vinblastine is used in the treatment of leukemia, Vincristine used in the treatment of leukemia, breast cancer, and other malignant tumors, Vindesine used in the treatment of leukemia in children and pigmented tumors, and Vinorelbine is often used in treatments to prevent glandular division. Other compounds, such as neurotic (LR) and vincadioline, are alkaloids that can lower blood glucose levels.

Most free alkaloids are insoluble in water but dissolve in non-polar organic solvents, whereas alkaloid salts formed through acid reactions are typically soluble in polar solvents (Kumar, 2014; Thorat, 2018). Therefore, methanol, a polar solvent, is used to extract *Catharanthus roseus* leaves, while petroleum benzene, a non-polar solvent, is used in the extraction process. Due to their polar nature, active compounds that can be extracted by methanol and alkaloids include flavonoids and saponins. On the other hand, active compounds that can be extracted using petroleum benzene include free alkaloids, fats (triglycerides), sterols, phospholipids, carotene, fatty acids, and chlorophyll (El Aziz *et al*., 2019; Ng *et al*., 2020).

Leurosin (LR) and vincadioline, along with several alkaloids that reduce blood glucose levels, can be utilized as diabetes medications because they function as substitutes for insulin in lowering glucose levels. This is because leurosin (LR) and vincadioline can suppress blood sugar levels (Naroh, 2009). Due to their blood sugar-lowering capabilities, the extracts from *Catharanthus roseus* leaves are classified as oral hypoglycemic agents (OHA). However, before these extracts are widely used, comprehensive research must be conducted to prove that *Catharanthus roseus* leaf extracts can effectively lower blood glucose levels.

RESEARCH METHODS

Type and Research Design

This study is experimental research with a Post-Test Only Control Group Design, which compares the observation results between the control and treatment groups (Ukratalo *et al*., 2023).

Tools and Materials

The tools used in this study include an extraction reflux apparatus (Soxhlet), hotplate, separating funnel, pipette, a set of glassware, electronic balance, bottles, cloth, scissors, syringe, oral gavage (sonde), mouse confinement containers, and glucose.

The materials used include fresh *Catharanthus roseus* leaves, distilled water (aquades), methanol, petroleum benzene, streptozotocin (STZ), *Mus musculus* (test animals), aluminum foil, 70% alcohol, filter paper, cotton, tissue, and betadine.

Procedure

Extraction Procedure

The *Catharanthus roseus* leaves were first air-dried and ground into a fine powder. The ground leaves were then extracted using solvents (methanol and petroleum benzene) for 6 hours with a Soxhlet extractor, repeated three times until the extract no longer tasted bitter and the Soxhlet solvent was explicit. The extract was concentrated using an evaporator until the solvent was removed entirely.

Determination of Dose and Oral Administration of Test Solution

The dose for the test animals was determined based on the DL_{50} of *Catharanthus roseus* leaf extract. According to the Miller-Tainter method, the oral DL_{50} for male mice is 27.50 g/kg. The average weight of the mice is about 30 g, thus:

27.50 g/kg = 27.50 g / 1000 g = 0.0275 g

For a 30 g mouse, the DL_{50} would be:

30 x 0.0275 = 0.825 g

Three treatments were determined: 5% methanol extract, 5% petroleum benzene extract, and a control. A 1.5 ml dosage was administered once every 24 hours. For the 5% treatment, 5 g of extract was diluted in 100 ml of distilled water. Each treatment resulted in a dose below the DL50 of 0.825 g.

5% treatment = 1.5 ml x 5% = 0.075 g

Testing Procedure

Before testing the extracts on the mice, each mouse was injected with 0.1 ml of streptozotocin (STZ) and left for 2 weeks until the blood sugar level exceeded 150 mg/dl. The testing procedure was conducted on three groups, each consisting of three male mice with treatments of 5% methanol extract, 5% petroleum benzene extract, and control. Testing was conducted for 2 weeks, and during the last week, blood glucose levels were measured using a glucose.

Blood Glucose Measurement Procedure

After 2 weeks of administering the extracts, the test animals blood glucose levels were measured using glucose. The measurement was carried out by cutting the tip of the mouse's tail, and the blood from the tail was placed onto a glucose strip for testing. The measurement results were recorded. Measurements were taken before and after the extraction test to compare the differences in blood glucose levels.

Data Analysis

The blood glucose level data were analyzed using analysis of variance (ANOVA) at a 95% confidence level. If the ANOVA results indicate a significant difference, a post-hoc test will be conducted to identify the differences between groups (Kaihena *et al*., 2024a).

RESULTS AND DISCUSSION

The administration of methanol extract and petroleum benzene extract of *Catharanthus roseus* leaves to mice suffering from Type 1 diabetes mellitus was found to affect blood glucose levels. The average blood glucose measurements of diabetic mice before and after the administration of methanol extract and petroleum benzene extract of *Catharanthus roseus* leaves are presented in Table 1.

Table 1. Average Blood Glucose Levels of Mice (*Mus musculus*) Before (Initial), After Streptozotocin Induction, and After Treatment

	Group	Measurement Period Blood Glucose Levels (mg/dl			Average ± SD
		Initial	After STZ Induction	14 Days	
Treatment	Control -	116,67	121,33	119,33	119,11 ± 13,14
	Control +	115,67	411,33	552,33	359,78 ± 200,75
	Methanol	121,33	344,67	211,00	225,67 ± 122,79
	Benzene Treatment	123,00	278,00	185,33	195,44 ± 81,31
	Average	**119,17**	**288,83**	**267,00**	

Based on the results in Table 1, the blood glucose concentration in the negative control group was considered the baseline or starting point for the blood glucose concentration in each treatment group, as these mice were only provided with food and water as usual. In the positive control group (injected only with streptozotocin), the blood glucose concentration of the mice showed a continuous increase over the 14 days. In contrast, the groups treated with methanol and petroleum benzene extract exhibited decreased blood glucose levels by the second week of treatment.

The average blood glucose level of non-diabetic mice was 119.11 mg/dl. For diabetic mice induced with streptozotocin (STZ), the blood glucose level increased to an average of 359.78 mg/dl. After 14 days of treatment with methanol extract of *Catharanthus roseus* leaves, the blood glucose level of diabetic mice decreased to 225.67 mg/dl. Similarly, diabetic mice treated with petroleum benzene extract of *Catharanthus roseus* leaves had an average blood glucose level of 195.44 mg/dl after 14 days.

Based on these findings, it can be concluded that administering methanol and petroleum benzene extracts from *Catharanthus roseus* leaves can lower blood glucose levels in mice with diabetes mellitus induced by the diabetogenic agent streptozotocin. To further confirm whether there was a reduction in blood glucose levels, the change in blood glucose levels (ΔGD) was calculated by subtracting the post-treatment blood glucose level from the blood glucose level after streptozotocin induction. The average reduction in blood glucose was then determined and categorized according to the treatment group.

Table 2. Average Blood Glucose Reduction in Mice (*Mus musculus*)

Treatment Group	Average Blood Glucose Reduction (mg/dl)	Percentage Change (+) / Decrease (-) (%)
Control -	2	1.64%
Control +	-141	+34.27%
Methanol Extract 5%	133.67	-38.70%
Petroleum Benzene Extract 5%	92.67	-33.30%

The results from Table 2 show that both the methanol and petroleum benzene extracts of *Catharanthus roseus* significantly reduced blood glucose levels in diabetic mice. The methanol extract showed the highest reduction of 38.70%, followed by the petroleum benzene extract at 33.30%. Conversely, the positive control group (only STZ-induced) exhibited an increase in blood glucose levels by 34.27%.

The ANOVA test was used to determine whether significant differences existed in the means of more than two unrelated sample groups. The data used in this ANOVA test were transformed data that exhibited homogeneous variance, ensuring the validity of the ANOVA results. Based on the results, it was found that there were significant differences between the dosage groups, with a p-value of < 0.05. This indicates a meaningful reduction in blood glucose levels among the five treatment groups.

The two-way analysis of variance of blood glucose concentration in diabetic mice, comparing the negative control and positive control groups (exposed to streptozotocin), as well as the treatments with methanol extract and petroleum benzene extract at 5% concentration and a dose of 1.5 ml/weight, during the initial blood glucose measurement period and two weeks after treatment, revealed significant differences (statistically different) between the treatment groups, with $p < 0.05$. Additionally, the sampling period also showed significant results ($p < 0.05$), and the interaction between the factors was significant ($p < 0.05$).

A PostHoc test was conducted following the ANOVA test to determine which groups exhibited significant differences. The statistical analysis of the PostHoc test, with a significance level of $\alpha = 0.05$, showed significant differences ($p < 0.05$) between the negative control and positive control groups and between the 5% methanol and 5% petroleum benzene treatment groups.

The PostHoc results also indicated no significant difference between the 5% methanol treatment group and the 5% petroleum benzene treatment group. This statistically concludes that methanol 5% and petroleum benzene 5% extracts had similar effects. However, based on the percentage of average reduction in blood glucose levels (Table 2), the reduction in the methanol treatment group was 38.7%, while the petroleum benzene group showed a 33.3% reduction. The difference in the percentage reductions between the two groups was 5.4%.

The experimental animals used in this study were mice. Mice were chosen as test subjects because they offer several advantages as experimental animals, such as ease of maintenance and relatively low cost, in addition to having a physiological structure similar to humans. The mice used in this study were healthy male mice. According to Istiani (2008), female mice are more susceptible to diseases than male mice, so male mice were selected as the test subjects in this study.

Streptozotocin (STZ) is an alkylating agent that breaks the DNA strands in the cells of the pancreatic islets in mice (Zhu, 2022). Research has shown that the toxic effects of STZ are mediated by nitric oxide (NO) produced during STZ metabolism (Elamin *et al*., 2018). Streptozotocin was used for inducing diabetes mellitus in this study because it is a toxic substance capable of directly damaging the pancreatic β-cells, thereby inhibiting insulin production and secretion, leading the test animals to develop diabetes mellitus (Wu & Yan, 2015; Anastasiou *et al*., 2021).

In the pancreas, streptozotocin is presented by macrophages and recognized by helper T cells. The macrophages then secrete IL-1 to induce the proliferation and activation of helper T cells. Subsequently, helper T cells activate IFNγ to enhance macrophage activation. During activation, macrophages produce superoxide and NO, which, along with IL-1 and TNFα, damage the pancreatic β-cells due to free radicals (Lu *et al*., 2020; Li *et al*., 2022). A dose of 0.1 ml/weight of STZ given to the test animals over 14 days (2 weeks) stimulates the immune response of pancreatic cell mediators, resulting in necrosis of the β-cells.

According to Aksoy *et al*. (2003), in vivo administration of streptozotocin increases the levels of MDA (malondialdehyde). The rise in plasma MDA levels indicates increased oxidation of unsaturated fats, which are abundant in the pancreatic β-cells, causing disruption in insulin secretion and ultimately leading to hyperglycemia via the following mechanisms:

a. Streptozotocin breaks the DNA chains in the pancreatic islets and stimulates poly(ADP-ribose) synthetase, which decreases the intracellular levels of NAD+ and NADP+, inhibiting proinsulin synthesis and inducing hyperglycemia.
b. Streptozotocin activates reactive oxygen species (ROS), such as superoxide (O2), hydroxyl radical (OH), and hydrogen peroxide (H2O2).

Damage to the pancreatic β-cells by STZ results in reduced insulin production. The low levels of insulin produced disrupt the normal process of glucose glycogenolysis. As a result, the excessive glucose in the blood cannot be converted into glycogen for storage in the liver, leading to a hyperglycemic condition (Edgerton *et al*., 2001; Hatting *et al*., 2018).

The administration of methanol extract and petroleum benzene extract, both at a 5% concentration and a dose of 1.5 ml/weight for 2 weeks, reduced blood glucose levels in diabetic mice induced by streptozotocin. The hypoglycemic effect of the methanol extract showed a reduction of up to 38.7%, while the petroleum benzene extract produced a reduction of 33.3%. The hypoglycemic effect of the methanol extract was more pronounced than that of the petroleum benzene extract. However, according to the statistical data from the PostHoc test, both extracts effectively reduced blood glucose levels with similarly significant effects. Therefore, it can be concluded that the hypoglycemic compounds in *Catharanthus roseus* leaves can be extracted using both polar and nonpolar solvents.

Hypoglycemic Effect of Methanol Extract of *Catharanthus roseus* Leaves

The *Catharanthus roseus* leaf extract, obtained using the polar solvent methanol, can extract various compounds, including alkaloids, flavonoids, and saponins. According to Sasikumar *et al*. (2015), alkaloid compounds possess antioxidant properties that can reduce free radicals and reactive oxygen species (ROS), which may cause tissue and erythrocyte damage. Leurosine (LR), vincadioline, lochnerine, tetrahydroalstonine, vindoline, and vindolinine are some of the alkaloid compounds that act as antioxidants capable of suppressing the effects of reactive oxygen species and preventing the chain reactions of oxidants (Nisar *et al*., 2016; Paul *et al*., 2023). In addition to alkaloids, flavonoid compounds also act as antioxidants.

Several flavonoid compounds function as antioxidants, inhibit erythrocyte aggregation, and stimulate the production of oxidative nitrates that can dilate blood vessels. Flavonoids are a large group of polyphenolic compounds, including isoflavone. Isoflavones are flavonoid compounds that can capture free radicals (Umeno *et al*., 2016).

Isoflavones work by being oxidized by free radicals, forming isoflavone radicals. These isoflavone radicals then convert into more stable compounds. Isoflavones can stabilize reactive oxygen species because the highly reactive hydroxyl group of isoflavones changes the radical species into inactive forms. By capturing superoxide radicals, amplification reactions can be prevented, thereby reducing the reactivity of free radicals. The suppression of free radicals prevents the breaking of chains and DNA damage, ensuring that replication processes remain uninterrupted and preventing cellular mutations.

Saponins in the methanol extract of *Catharanthus roseus* leaves act as aglycones of glycosides, lowering blood glucose levels by inhibiting enzyme release in the small intestine, thereby hindering the conversion of polysaccharides into simple sugars. Inhibiting this system effectively reduces carbohydrate digestion and absorption, which can prevent the postprandial increase in glucose levels. As a result, glucose release becomes slower, and glucose absorption into the bloodstream becomes lower, slower, and more evenly distributed, thereby helping to avoid high blood sugar levels (Mir *et al*., 2018; Pham *et al*., 2019; Arulvendhan *et al*., 2024).

Hypoglycemic Effect of Petroleum Benzene Extract of *Catharanthus Roseus* Leaves

The *Catharanthus roseus* leaf extract, obtained using the nonpolar solvent petroleum benzene, can extract various compounds, including alkaloids, carotenoids, and chlorophyll these compounds in the petroleum benzene extract function as antioxidants. Carotenoids and chlorophyll antioxidants capture free radicals and prevent chain reactions (Pérez-Gálvez *et al*., 2020).

The hypoglycemic effects of methanol and petroleum benzene extract occur through two mechanisms: (a). Inhibition of the enzyme alpha-glucosidase, which catalyzes the breakdown of carbohydrates, and (b). antioxidant mechanisms that stop free radicals and regenerate pancreatic β-cells, allowing insulin production to return to normal.

The administration of *Catharanthus roseus* extract, which contains alkaloids and antioxidants, can neutralize free radicals caused by the chemical agent (streptozotocin), rendering them non-reactive. According to Alkadi (2020), antioxidant compounds donate one or more electrons to free radicals, thus halting the damage caused by these radicals. The free radicals quickly react with antioxidants to form stable and harmless molecules (Figure 1). This process contributes to the decrease in blood glucose concentration in diabetic mice, as it inhibits glucose absorption and improves the regeneration of β-cells in the pancreas.

a. **Reaction 1:**

$$\text{Reactant} \xrightarrow{\text{process}} \text{Product} + \text{OH}^-$$

$$\text{OH}^- + (\text{DNA, protein, lipid}) \xrightarrow{\text{reaction}} \text{Product} + \text{other free radicals}$$

The other free radicals will initiate the same reaction with nearby molecules, continuing the chain reaction and furthering the damage.

b. **Reaction 2:**

$$\text{Reactant} \xrightarrow{\text{process}} \text{Product}$$

Figure 1. Free Radical Activity Reaction (a) without antioxidants, and (b) with antioxidants (Widyatmoko, 2009)

The results from the analysis of variance (ANOVA) and PostHoc tests for blood glucose concentrations in diabetic mice indicate that the methanol extract and petroleum benzene extract of *Catharanthus roseus* leaves at a concentration of 5% and a dose of 1.5 ml/BW effectively reduce blood glucose levels in diabetic mice induced by streptozotocin. This suggests that the administration of *Catharanthus roseus* extract has a significant effect on lowering blood glucose levels.

The hypoglycemic effect of the methanol extract of *Catharanthus roseus* leaves is better than that of the petroleum benzene extract due to the difference in the content of antidiabetic compounds extracted by polar and nonpolar solvents in this study, namely methanol and petroleum benzene.

CONCLUSION

Based on the results of this study, it can be concluded that methanol and petroleum benzene extracts of *Catharanthus roseus* leaves can reduce blood glucose levels in diabetic mice (*Mus musculus*) induced by diabetes mellitus. The hypoglycemic effect in diabetic mice induced by the methanol extract of *Catharanthus roseus* leaves showed a reduction of up to 38.7%, while the petroleum benzene extract showed a reduction of 33.3%. According to the PostHoc test, no significant difference was observed between the hypoglycemic effects of the methanol and petroleum benzene extracts. However, based on the percentage of average blood glucose reduction, there is a 5.4% difference, with the methanol extract showing better hypoglycemic effects than the petroleum benzene extract due to the differences in the extracted compound content.

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