

Antidiabetic Activity of Ethanol Extract from Red Spinach Leaves (*Amaranthus tricolor* L.) on Mice

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ABSTRACT

This study aims to determine the antidiabetic activity of ethanol extract of red spinach leaves (*Amaranthus tricolor* L.) on male mice. The study was conducted experimentally using 25 male mice divided into 5 treatment groups, namely the negative control group (CMC Na 0.5%), positive control (glibenclamide 0.65 mg/kg BW), and 3 others groups given ethanol extract of red spinach leaves at doses of 100, 200 and 400 mg/kg BW. Mice were induced with streptozotocin (STZ) dose of 100 mg/kgbw intraperitoneally. After 72 hours, diabetic mice were treated for 15 days orally and blood glucose levels were checked every 5 days using a glucotest device. The results showed that Ethanol extract of Red Spinach Leaves (EERSL) doses of 100 and 400 mg/kg BW were not significantly different from the negative control CMC Na ($p > 0.05$) in reducing blood glucose levels. EERSL doses 200 mg/kgbw and positive control glibenclamide significantly reduce blood glucose levels compared to negative control CMC Na ($p < 0.05$). Ethanol extract of red spinach leaves has antidiabetic activity on male mice at a dose of 200 mg/kg BW, whose effect is not significantly different compared to the glibenclamide.

Keywords: Antidiabetic, *Amaranthus tricolor* L., ethanol extract, male mice, streptozotocin.

ABSTRAK

Penelitian ini bertujuan untuk mengetahui aktivitas antidiabetes ekstrak etanol daun bayam merah (*Amaranthus tricolor* L.) terhadap mencit jantan. Penelitian dilakukan secara eksperimental menggunakan 25 ekor mencit jantan yang dibagi menjadi 5 kelompok, yaitu kelompok kontrol negatif (CMC Na 0,5%), kontrol positif (glibenklamid 0,65 mg/kg BB) dan 3 kelompok lainnya diberikan ekstrak etanol daun bayam merah (EEDBM) dosis 100, 200 dan 400 mg/kg BB. Mencit diinduksi dengan streptozotocin dosis 100 mg/kg BB secara intraperitoneal. Setelah 72 jam, mencit yang diabetes diberikan perlakuan selama 15 hari secara per oral dan kadar glukosa darah dicek setiap 5 hari menggunakan alat glucotest. Hasil menunjukkan bahwa EEDBM dosis 100 dan 400 mg/kg BB tidak berbeda signifikan dengan kontrol negatif CMC Na ($p > 0,05$) dalam menurunkan kadar gula darah (KGD). EEDBM 200 mg/kg BB dan kontrol positif glibenklamid menurunkan KGD secara signifikan dibandingkan dengan kontrol negatif CMC Na ($p < 0,05$). Ekstrak etanol daun bayam merah memiliki aktivitas antidiabetes terhadap mencit jantan pada dosis 200 mg/kg BB, yang efeknya tidak berbeda signifikan dibandingkan dengan kontrol positif yaitu glibenklamid.

Kata Kunci: Antidiabetes, *Amaranthus tricolor* L., ekstrak etanol, mencit jantan, streptozotocin.



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1. Introduction

Diabetes mellitus (DM) is a metabolic disease characterized by hyperglycemia due to abnormalities in insulin secretion, impaired insulin action, or a combination of both. The disease can lead to various chronic complications in the nerves, kidneys, eyes, and blood vessels. There are two main types of diabetes mellitus, namely type I diabetes, known as insulin-dependent diabetes mellitus, and type II diabetes, known as non-insulin-dependent diabetes mellitus, which is caused by the decreased sensitivity of target tissues to the metabolic effects of insulin [1].

Pharmacological therapies, such as oral and non-pharmacological therapies with lifestyle modifications, have been carried out to manage treatments [2]. Although pharmacological therapy has been widely used, the side effects that are very likely to occur remain to be a consideration, mainly due to prolonged use. Therefore, many patients choose traditional medicine or herbal medicine as an alternative to diabetes treatment because the ingredients used are easily available, safer, affordable, and do not have significant side effects [3]. This is because the active compound components in one plant can cover each other's unwanted effects, making it safer to use [4].

The red spinach plant (*Amaranthus tricolor* L.) is one of the plants that can be used to treat diabetes. Tannin compounds in red spinach leaves can control blood glucose by preventing beta cell damage, inhibiting sugar absorption, and increasing insulin release by regenerating beta cells [5]. In addition to tannin compounds, flavonoids can also affect blood glucose levels. Flavonoids increase glucose utilization in tissues by increasing tyrosine kinase phosphorylation on insulin receptor substrates [6].

In a study conducted by Suryanita et al. (2022), ethanol extract from red spinach at 200 mg/kg BW showed a decrease in blood glucose levels in male white rats exposed to cigarette smoke. The average blood glucose level in the initial blood draw was 56 mg/dL. After exposure to cigarette smoke, this level increased to an average of 126.6 mg/dL. However, after the administration of ethanol extract, there was a significant decrease, with an average value down to 98 mg/dL [7].

Based on the description above, red spinach has the potential to be developed into an antidiabetic drug. In this study, we tested the antidiabetic activity of ethanol extract of red spinach leaves (*Amaranthus tricolor* L.) in male mice induced by streptozotocin.

2. Method

Materials

The materials used in this study were red spinach leaves, distilled water, CMC-Na, 70% ethanol, glibenclamide tablets (Indofarma), streptozotocin, and 0.9% NaCl infusion solution. The test animals used in this study were male mice (*Mus musculus*) aged 2-3 months with a weight of 20-30 g. The number of animals used was 25 animals for 5 test groups.

Research Procedure

Sampling

Sampling was done purposively without comparing the same samples from other places. The samples used were fresh red spinach leaves obtained from Tanjung Gusta village, Medan Helvetia District, Medan City, North Sumatra, Indonesia.

Preparation of Red Spinach Leaf Simplicia

Red spinach leaves were selected fresh and sorted to remove impurities and other foreign objects, then washed, drained, and weighed. Then, the red spinach leaves were dried in an oven at 40-50 °C until all the leaves dried, and then, they were weighed. The dried leaves were pulverized using a blender and sieved using a 40-mesh sieve.

Preparation of Ethanol Extract of Red Spinach Leaves (*Amaranthus tricolor* L.)

Red spinach leaf simplicia powder was extracted using the Microwave-Assisted Extraction (MAE) method. Weighed as much as 15 g of simplicia powder, 150 mL of 70% ethanol was added to the powder, with the ratio of simplicia and solvent 1:10. The extraction process uses a microwave with a power of 450 Watts, extracted for 8 minutes at a temperature of about 80°C. After the extraction process ended and the mixture cooled, then the mixture was filtered using Whatman filter paper No. 1. The filtrate from the filtration was evaporated using a rotary evaporator at 40°C until the solvent had no longer evaporated [8].

Phytochemical Screening

Phytochemical screening was performed on simplicia and extract, including alkaloids, flavonoids, glycosides, saponins, tannins, and steroids/triterpenoids.

Characterization of Simplicia and Extract

Characterization of simplicia included water content, water and ethanol soluble essence content, total ash content, and acid insoluble ash content. Characterization of extracts included water content, ash content, and total ash content.

Measurement of Blood Glucose Levels

The mice were fed with only drink for \pm 16-18 hours before testing was carried out. The blood glucose level was measured in each mouse using a glucometer. Blood was drawn through the tail vein, the first drop was discarded, and the subsequent drop was applied to the glucose strip on the tool. Blood glucose levels of healthy mice (normal) are 75-128 mg/dL [10].

Antidiabetic Activity Test of Ethanol Extract of Red Spinach Leaves (EERSL)

Male mice were subjected to diabetes induction with streptozotocin 100 mg/kg BW intraperitoneally. After 72 hours, blood glucose levels were measured. Mice were diabetic if the blood glucose level showed a value \geq 129 mg/dL or exceeded the normal mice blood glucose level.

Mice were divided into five groups; each consisted of 5 mice and were treated orally.

- Group I: CMC Na 0.5% suspension (negative control)
- Group II: Glibenclamide suspension 0.65 mg/kg BW (positive control)
- Group III: EERSL suspension dose of 100 mg/kg BW
- Group IV: EERSL suspension dose of 200 mg/kg BW
- Group V: EERSL suspension dose of 400 mg/kg BW

Treatment was given after mice tested positive for diabetes, and the treatment was given once a day for 15 days orally. Furthermore, blood glucose levels were measured on days 5, 10 and 15.

Data Analysis

Data obtained in this study were analyzed using SPSS software version 22. Data were analyzed statistically with preliminary tests to see the data's normality and homogeneity. Data were then analyzed using the One Way ANOVA test with a 95% confidence level, and Tukey's post-hoc test was continued to see the differences between treatments.

3. Results and Discussion

Phytochemical screening

Phytochemical screening test results found that simplicia and ethanol extract of red spinach leaves contain chemical compounds of alkaloid, flavonoid, glycoside, saponin, tannin, and steroid groups.

Characterization of Simplicia and extracts

The water content of red spinach leaves, simplicia, and extract were 6.64% and 7.3%, respectively. These results meet the requirements of water content in simplicia in general, where the requirement is at most 10% [11]. High water content causes the growth of microbes that can damage and affect the quality and stability of simplicia and extract [12].

Determination of water- and ethanol-soluble essence content aims to determine the amount of active compound content that is polar (soluble in water) and polar-non-polar (soluble in ethanol) [12]. Red spinach leaves' water- and ethanol-soluble essence contents were 30.08% and 11.71%, respectively. These results meet the Indonesian Herbal Pharmacopoeia Edition II requirements; water-soluble essence content is not less than 7.5%, while ethanol-soluble essence content is not less than 7.6% [11].

Determination of ash content aims to determine the mineral or metal content in simplicia and extracts. The total ash content in simplicia and ethanol extract of red spinach leaves was 5.68% and 5.44%, respectively. The results of acid-insoluble ash content in simplicia and ethanol extract of red spinach leaves were 0.39% and 0.50%, respectively. According to *Materia Medika Indonesia*, the requirement for total ash content is no more than 10%, and for acid-insoluble ash content is no more than 1%, so the results meet the requirements. The high ash content results can occur due to the less clean plant washing process, so it is still carried during extraction [13]. The higher the total ash content value, the higher the mineral content in the simplicia, while

the high results of acid-insoluble ash content indicate the presence of silicate content derived from soil or sand [14].

Antidiabetic Activity of Ethanol Extract of Red Spinach Leaf

Before testing, mice were starved (but still given water) to avoid the influence of other substances on fasting blood glucose levels [15]. The average blood glucose levels of fasting mice before streptozotocin induction are shown in Table 1.

Table 1. Average fasting blood glucose level of mice before streptozotocin induction

No.	Treatment Group	Mean Fasting Blood Glucose Level (mg/dL) ± SEM
1.	CMC Na 0.5%	127.4 ± 9.3
2.	Glibenclamide 0.65 mg/kgBW	152.6 ± 13.0
3.	EERSL 100 mg/kgBW	131.8 ± 15.8
4.	EERSL 200 mg/kgBW	148.6 ± 12.0
5.	EERSL 400 mg/kgBW	129.4 ± 14.2

After measuring the fasting blood glucose of mice, each mice was induced with a dose of 100 mg/kg BW STZ intraperitoneally. After 72 hours of induction, the blood glucose was measured. The average results of the blood glucose increase are shown in Table 2.

Table 2. Average blood glucose level of mice after three days of STZ induction

No.	Treatment Group	Mean Fasting Blood Glucose Level (mg/dL) ± SEM
1.	CMC Na 0,5%	171.6 ± 11.7
2.	Glibenclamide 0.65 mg/kgBW	205.0 ± 16.4
3.	EERSL 100 mg/kgBW	189.0 ± 19.8
4.	EERSL 200 mg/kgBW	201.0 ± 11.0
5.	EERSL 400 mg/kgBW	177.6 ± 18.6

There is a significant difference in blood glucose levels before and after STZ administration ($p < 0.05$). Thus, it can be concluded that administering an STZ dose of 100 mg/kg BW raised blood glucose levels in the mice.

After the mice were positive for diabetes, the mice were grouped into five treatment groups consisting of 5 mice and given oral treatment, namely control group 1 (negative control), which was given a 0.5% CMC Na suspension, group 2 (positive control) was given a glibenclamide suspension dose of 0.65 mg/kg BW, and a test group with three different treatment doses, namely EERSL suspension doses of 100, 200, and 400 mg/kg BW. The treatment began after the mice tested positive for diabetes and were given every day for 15 days orally. Blood glucose measurements were performed on days 5, 10, and 15. The results of the measurement of the decrease in blood glucose level on day five can be seen in Table 3.

Table 3. Percentage decrease in the average blood glucose of mice on day five after treatment

Test Group	% Blood glucose decrease ± SEM
CMC Na 0.5%	3.1 ± 2.6
Glibenclamide 0.65 mg/kgBW	10.6 ± 3.6
EERSL 100 mg/kgBW	8.6 ± 0.8
EERSL 200 mg/kgBW	9.8 ± 2.1
EERSL 400 mg/kgBW	5.8 ± 3.1

Table 3 shows no significant reduction in blood glucose levels between treatments ($p > 0.05$). Furthermore, blood glucose was measured on the 10th day.

The results of blood glucose levels on day ten can be seen in Table 4.

Table 4. Percentage decrease in the average blood glucose of mice on day ten after treatment

Test Group	% Blood glucose decrease ± SEM
CMC Na 0.5%	10.3 ± 2.8
Glibenclamide 0.65 mg/kgBW	27.5 ± 3.6*
EERSL 100 mg/kgBW	17.5 ± 2.1
EERSL 200 mg/kgBW	23.1 ± 2.4*

EERSL 400 mg/kgBW	20.1 ± 3.7
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Note: * = significantly different from the negative control CMC Na ($p < 0.05$)

Based on Table 4, using the ANOVA test, there are significant differences between treatment groups on the 10th day after treatment ($p < 0.05$). In the Post Hoc follow-up test using Tukey HSD, the percentage decrease in the EERSL group doses of 100 and 400 mg/kg BW was not significantly different compared to the negative control group CMC Na ($p > 0.05$).

In contrast, glibenclamide and EERSL 200 mg/kg BW significantly differed from that of the negative control group ($p < 0.05$), indicating that glibenclamide and EERSL 200 mg/kg BW reduced the blood glucose level on day 10. Furthermore, there is no significant difference in the decrease of blood glucose between EERSL 200 mg/kgBW and glibenclamide, indicating that EERSL 200 mg/kgBW has the same effectiveness as glibenclamide 0.65 mg/kgBW in reducing blood glucose levels.

The results of the 15th day of blood glucose reduction measurement can be seen in Table 5.

Table 5. Percentage decrease in the average blood glucose of mice on day 15 after treatment

Test Group	% Blood glucose decrease ± SEM
CMC Na 0.5%	20.7 ± 2.8
Glibenclamide 0.65 mg/kgBW	43.4 ± 3.7*
EERSL 100 mg/kgBW	32.8 ± 2.2
EERSL 200 mg/kgBW	38.7 ± 2.4*
EERSL 400 mg/kgBW	27.1 ± 4.2

Note: * = significantly different from the negative control CMC Na ($p < 0.05$)

Using the ANOVA test, there was a significant difference from the average value of the percentage of blood glucose reduction in each group on day 15 ($p < 0.05$). In the Post Hoc follow-up test using Tukey HSD, the percentage decrease in the EERSL 100 and 400 mg/kg BW was not significantly different from that of the negative control ($p > 0.05$). The EERSL 100 mg/kg BW could not reduce the blood glucose level, possibly due to the small dose, so the number of active compounds in the extract was not strong enough to reduce the blood glucose [16].

The results show that glibenclamide and EERSL 200 mg/kg BW significantly differ from negative control ($p < 0.05$), indicating that glibenclamide and EERSL 200 mg/kg BW can reduce the blood glucose level. The results also show no significant difference between glibenclamide and EERSL 200 mg/kgBW, indicating similar strength in reducing blood glucose levels. The chemical compounds in the extract may lower blood glucose levels, such as Tannin compounds in red spinach leaves, which can control blood glucose by inhibiting sugar absorption and increasing insulin release by regenerating beta cells [5]. In addition to tannin, flavonoid compounds may improve glucose utilization in tissues by increasing tyrosine kinase phosphorylation on insulin receptor substrates [6].

4. Conclusion

Ethanol extract from red spinach leaves has antidiabetic activity in male mice at a dose of 200 mg/kg BW, whose effect is not significantly different from the positive control, glibenclamide.

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