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The effect of salung leaf extract (*Psychotria viridiflora*) on blood glucose levels in mice

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Abstract: This research is crucial in the context of the rising global prevalence of diabetes, a metabolic disorder that significantly affects public health. This study aims to determine the effect of salung leaf extract (Psychotria viridiflora) on blood glucose levels in hyperglycemic mice (Mus musculus). The study used 25 mice (Mus musculus) weighing 20-40 grams, divided into 5 treatment groups (P). Each group consisted of 5 mice, including a negative control, positive control, and three experimental groups with the administration of P. viridiflora extract at various doses (0.0112; 0.0225; and 0.0337 g/kgBW). Blood glucose measurements were conducted four times, on days 0, 6, 12, and 18, which included an initial glucose measurement, post-alloxan induction, and subsequent measurements after treatment. Among the three tested doses, the highest dose (0.0337 g/kg BW) proved to be the most effective in reducing blood sugar levels in alloxan-induced M. musculus, with effectiveness comparable to the standard drug Glibenclamide. Thus, the extract of P. viridiflora leaves shows strong potential as an alternative herbal antidiabetic therapy for M. musculus.
Keywords: *psychotria viridiflora, leaf extract, blood glucose, mus musculus, antidiabetic, hyperglycemia.*

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INTRODUCTION

Indonesia has a biodiversity spread across various archipelagos, with 30,000 species of tropical forest plants, of which about 9,600 species are known to have medicinal properties, but only around 200 species are utilized as raw materials for medicine and are important medicines for the traditional medicine industry (Rahma, 2023). Medicinal plant leaves are all types of plants known to contain compounds that are beneficial and effective in preventing, alleviating, or curing a disease. The leaves of P. viridiflora are a medicinal plant that has been widely used by the people of North Bengkulu as a traditional medicine to lower blood glucose levels (See Figure 1). This is because the extract of P. viridiflora contains terpenoid, flavonoid, alkaloid, and tannin components based on the results of the TLC (Thin Layer Chromatography) test (Cristiandari, 2018)]. The consumption method is by drinking the boiled water of the leaves as an alternative medicine to glibenclamide. Glibenclamide is usually used as a medication to control high blood sugar levels in patients with type 2 diabetes (Irawan, 2022). Additionally, P. viridiflora leaves have benefits that are felt directly by the community, namely for body immunity, making the body healthier.

The P. viridiora plant is from the Rubiaceae family which is used by the Musi Rawas community as a traditional medicine passed down through generations to treat various diseases. The leaves of the plant can be seen in Figure 1.

Figure 1. Leaf of P. viridiflora
Source: https://toptropicals.com/catalog/image/3/psychotria_viridiflora.htm

P. viridiflora has the scientific classification asthe following.

Kingdom : *Plantae*
Subdivision : *Tracheophyta*
Division : *Angiospermae*
Kelas : *Eudikotil*
Subkelas : *Magnoliidae*

Family : *Rubiaceae*
Genus : *Psychotria*
Spesies : *P. viridiflora*

The results of the phytochemical tests conducted by researchers in the laboratory show that the secondary metabolite compounds that have the potential to reduce blood sugar levels, it shows the presence of active compounds in the form of alkaloids (229.22 mg/L), flavonoids (68.22 mg/L), and steroids (0.022 mg/L) identified using visible spectrophotometry method. The dominant alkaloid content has great potential as an antidiabetic because it can stimulate insulin secretion, inhibit carbohydrate-digesting enzymes, and increase insulin sensitivity (Ahmad, 2018). Flavonoids, which are also detected at a sufficiently high level, play an important role in lowering blood sugar levels through antioxidant activity, protection of pancreatic β cells, and inhibition of carbohydrate digestive enzymes (Kashyap, 2015). Although the steroid content is relatively low, this compound still contributes to glucose regulation through its influence on hormone and lipid metabolism.

The continuously increasing health problem is metabolic disorders such as diabetes mellitus, which is characterized by high blood glucose levels due to impaired insulin production or function. The treatment of diabetes generally uses synthetic drugs, but the long-term side effects often become a concern. Diabetes mellitus consists of two types, namely type 1 and type 2. Type 1 diabetes typically appears during childhood, characterized by damage to pancreatic B cells due to the immune system, resulting in the body being unable to produce insulin and requiring lifelong insulin injections. Meanwhile, type 2 diabetes is more common in adults and is associated with obesity, an inactive lifestyle, and poor diet. This type is characterized by insulin resistance, which is a decrease in the body's sensitivity to insulin, causing the pancreas to work harder until the beta cells become dysfunctional. In type 2 diabetes, insulin levels can be normal, but because the number of insulin receptors is low, glucose struggles to enter the cells, resulting in increased blood glucose levels. Normal blood sugar levels range from 72–108 mg/dL, and can be classified from low (1–70 mg/dL) to very high (180–900 mg/dL). If the body is in a normal state, then the concentration of glucose in both venous plasma and capillary blood is < 126 mg/dL (Soviana, 2019).

Glucose comes from two sources: food and production from the liver. Every time we eat, the pancreas responds by releasing insulin into the blood. Insulin acts as a key that will open the door (diffusion facility) so glucose can enter muscles and adipose tissue. The glucose then functions as an energy source for the muscles [Baharuddin, 2022)]. When insulin levels rise with the intake of food in the body, the liver will store glycogen. When blood sugar is low, glycogen in the liver will be converted into glucose and circulated out of the liver to target organs (Marbun, 2023).

Mice (Mus musculus) are often used as model animals in research because they have organ structures that are most similar to humans. Testing salung leaf extract on mice can provide an initial overview of the potential of this plant in controlling blood glucose levels before further testing is conducted on humans. There are many advantages that mice possess as animals for experimentation, namely they have physiological similarities to humans, a relatively short life cycle, a large number of births, high variability in traits, and are easy to handle Raihani, 2028)..

METHOD

Research Design

The research was conducted in January-February 2025. The study took place at the Biology Education Laboratory of UNIB and the Biological Sciences Learning Resource Center (SBIH) Ruyani Bengkulu. SBIH Ruyani is a place for students' research to seek new knowledge in order

to improve the quality of science education (Ruyani, 2018).

The method used in this research is the experimental method, while the design employed is a Completely Randomized Design (RAL). The research variable is the change in glucose levels of mice, consisting of 5 (five) treatments and 5 (five) repetitions. This study involves 25 mice, with each group consisting of 5 mice weighing between 20 - 40 grams, divided into 5 treatment groups (P). The treatments tested in this study are as follows:

Treatment 1 = Normal, as a negative control (only given feed and aquades)
Treatment 2 = Alloxan 10 mg/kg BW + glibenclamide dose 0.1 g/kg BW
Treatment 3 = Alloxan 10 mg/kg BW+ *P. viridiflora* leaf extract 0.0112 g/kg BW
Treatment 4 = Alloxan 10 mg/kg BW + *P. viridiflora* leaf extract 0.0225 g/kg BW
Treatment 5 = Alloxan 10 mg/kg BW+ *P. viridiflora* leaf extract 0.0337 g/kg BW.

Blood glucose measurements were carried out 4 times, specifically on days 0, 6, 12, and 18, consisting of initial glucose measurement, after alloxan induction, and followed by measurements after treatment. The alloxan used in each treatment is a compound that can generate Reactive Oxygen Species (ROS) through a reaction cycle. The reaction results in dialuric acid. This dialuric acid undergoes a reduction-oxidation (redox) cycle and forms superoxide radicals. Then, this radical will undergo mutase to become hydrogen peroxide and finally undergo iron catalysis to form hydroxyl radicals (Aini, 2019).

Tools and Materials

The tools used in this research include a blender, sieve, rotary evaporator (Heidolph Laborota 4000 Efficient with Vacum Laboxact), knife, analytical balance, oral syringe, insulin syringe, capillary tube, measuring cup, break glass, mouse scale, plastic tray, wire rack, and mouse drinking bottle. The materials used are as follows: *P.* viridiflora leaves, mice, alloxan, technical ethanol 96%, and fish pellets as food for the *M. musculus*.

Research Procedure

Extraction

The extraction of *P. viridiflora* leaves was conducted at SBIH Ruyani, Bengkulu City. The samples of *P. viridiflora* leaves were taken from mature leaves obtained from North Bengkulu region. The picked leaves were then washed with clean water to remove any dirt attached to them. Afterwards, the leaves were drained and dried by airing them out and sun-drying. The next process is the production of leaf powder. The *P. viridiflora* leaves that have been washed and dried for 4 days using sunlight are then crushed into a fine powder. The extraction process is carried out using the maceration method, which involves soaking 1 kg of *P. viridiflora* leaf powder in a glass bottle filled with 1000 mL of 96% ethanol. The bottle is tightly closed and left for three days, after which this process is repeated three times. The remaining ethanol solvent in the extract is then evaporated using a water bath until the extract is completely free from the solvent. The extract of *P. viridiflora* leaves obtained through the maceration process is then evaporated using a rotary evaporator (Rotavapor®) at a temperature of 40–50°C in a water bath.

Antidiabetic Activity

Preparation begins with the acclimatization stage of 25 mice that are kept for one week in plastic cages with wire mesh covers, aimed at allowing the mice to adapt to the new environment. The mice are 2-3 months old, weighing around 20-40 grams, have a good appetite, move actively, and are agile in healthy conditions. All mice are confirmed to have never been treated with drugs and are declared healthy with no physical defects. Then, the mice are initially tested for their blood

glucose levels and induced with alloxan. The choice of alloxan as a diabetes induction agent is based on its ability to condition test animals similarly to patients with diabetes mellitus (Indah, 2019).

M. musculus has characteristics such as a small body shape, white color (as seen in Figure 1), and a regular estrous cycle of 4-5 days. The housing conditions for maintaining *M. musculus* must always be clean, dry, and away from noise. The temperature in the housing must be kept between 18-19°C and humidity between 30-70%. *M. musculus* is frequently used in research considering that this animal has several advantages, including a regular and detectable estrous cycle, a relatively short gestation period, and a large number of offspring, as well as growth alignment with human conditions.

Figure 2. *Mus musculus*

In addition, alloxan can cause a permanent hyperglycemic state within a relatively short time, specifically 2-3 days after induction. Induction with alloxan at a dose of 3 mg/30g body weight of the mice was then observed for blood glucose levels over 24 hours. If the blood glucose level exceeds 200 mg/dl, the mice are said to be hyperglycemic. The test preparation was given orally for 18 days to the group of mice.

Data Analysis

Data on the changes in blood glucose levels of mice obtained from the results after induction were statistically analyzed using One Way ANOVA. The data obtained were initially tested for normality using the Shapiro-Wilk test. The data is said to be normally distributed if sig. > 0.05. Then, a homogeneity test was conducted using Levene's test; if the sig. value > 0.05, it means that the obtained data is homogeneous. Following this, a One Way ANOVA statistical test was performed at a 95% confidence level. If there is a difference between groups, it is followed by Post Hoc Duncan analysis to determine which treatment groups have significant differences.

FINDINGS AND DISCUSSION

Observations of blood glucose levels in mice were conducted before induction, after hyperglycemia, and during 18 days of treatment. On day 0, initial measurements of blood glucose levels and body weight of the mice were taken, followed by the induction of a combination of alloxan with leaf extract or a combination of alloxan with glibenclamide. Measurements were taken again on days 6, 12, and 18. Before conducting 4 measurements of blood glucose levels in each test group, the subjects were fasted. The initial blood glucose levels of the test animals were

measured and the results showed that the blood glucose levels of each mouse ranged around 100 mg/dL. This figure is within the normal range according to previous research (Indrawati, 2016). The normal range of blood sugar levels in mice is 62.8 - 176 mg/dl. The measurement results and the graph of the blood glucose profile in mice can be seen in Table 1.

Table 1. Changes in blood glucose levels in mice

Groups	N	Average Blood Glucose of *M. musculus* (mg/dL) (X±SD)				
		Day 0	Day 6	Day 12	18	H18-H6
P1	5	110.2±6.3	161.2±9.4	161.2±19.2	120.6±13.6^{bc}	-57
P2	5	100.2±32.4	174.4±24.5	155.4±18.5	125.2±15.3^{ab}	-49.2
P3	5	113±15.4	190.6±7.7	162.4±14	137.4±14.9^{c}	-53.2
P4	5	146.2±30.4	174.8±5.9	160.2±6.3	131.8±19.8^{c}	-43
P5	5	105.4±13	168.2±16.1	150.8±26.3	114.8±20.4^{a}	-54.2

Note: P1: Normal/control treatment,
P2: Glibenclamide treatment,
P3: Dose 1 treatment (0.0112 mg/kg BW),
P4: Dose 2 treatment (0.0225 mg/kg BW),
P5: Dose 3 treatment (0.0337 mg/kg BW).

Symbols on the values represented by the same letters indicate 'not significantly different' ($p > 0.05$), while different letters indicate 'significantly different' ($p < 0.05$) based on the Duncan post hoc test at a significance level of 5%.

The results of blood glucose levels can be seen in Table 1. The measurement data shows a varied decrease in blood glucose levels across each group from day 12 to day 18, which causes the data to have a relatively large standard deviation. This is due to the pathophysiological condition of the test animals, their ability to absorb the test substance, and the test animals' ability to adapt to hyperglycemic conditions (Cahyaningrum, 2019).

The results of the ANOVA test are used to determine whether there are differences in blood glucose levels among each group. In the treatments on days 6, 12, and 18, there are normal/negative control groups, glibenclamide/positive control groups, and various dosage/test control groups. In the normal group, also referred to as the negative control, treatment is done by only providing feed and aquades. Furthermore, the positive control group uses the diabetes medication in the form of glibenclamide at a dose of 0.1 g/mL body weight. The control test was conducted by administering three levels of doses: dose 1 consisting of P. viridiflora leaf extract 0.0112 g/mL body weight, dose 2 consisting of P. viridiflora leaf extract 0.0225 g/mL body weight, and dose 3 consisting of P. viridiflora leaf extract 0.0337 g/mL body weight. All treatment groups showed significantly different results with a significance value of $0.027 < 0.05$, indicating that the averages are different. Therefore, based on the ANOVA test, it is concluded that the treatment with P. viridiflora leaf extract has a significant effect on the glucose levels of mice in each treatment group.

The increase in the dosage of the extract given shows the lowest effect in lowering blood glucose levels. In contrast, at lower dosages, blood glucose levels tend to decrease more significantly. This is in accordance with research conducted on Becium grandiflorum (Gebremeske, 2020). The statement that increasing the drug dosage should enhance the response proportionate to the increased dosage. However, as the dosage increases, the response enhancement will eventually decline as the optimum dosage has been reached. This often occurs because the components of the compounds in natural medicinal materials are not singular, but consist of various bioactive compounds that work synergistically to produce effects. Therefore, the decrease in effectiveness at high doses may be due to an imbalance in the interaction of bioactive compounds that actually inhibit the pharmacological response to the reduction of glucose levels.

The extract of P. viridiflora leaves has hypoglycemic effects due to the synergistic effects of the bioactive compounds contained, including flavonoids, phenolics, saponins, and tannins (Cristiandari, 2018; Sok Yen, 2021). The mechanism of action of the extract in the organs is estimated to resemble the action of glibenclamide, which stimulates insulin secretion from pancreatic beta cells (Zhou, 2019).

CONCLUSION

The extract of *P. viridiflora* leaves contains active metabolite compounds such as alkaloids, flavonoids, and steroids that have the potential to lower blood glucose levels. Of the three doses tested, the highest dose (0.0337 g/mL) proved to be the most effective in lowering the blood sugar levels of alloxan-induced mice, with effectiveness comparable to the standard drug glibenclamide. Thus, this extract of *P. viridiflora* leaves has a strong potential as a viable herbal antidiabetic therapy alternative that deserves further research, especially on a clinical or human scale.

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