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ANTIDIABETIC ACTIVITY OF LEAF EXTRACT OF *Clerodenrum fragrans* Vent Willd IN *Rattus novergicus* INDUCED BY ALLOXAN

Aktivitas Antidiabetes Ekstrak Daun *Clerodenrum fragrans* Vent Willd Pada *Rattus novergicus* Yang Diinduksi Aloksan

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ABSTRACT
Sarang banua plants are grown in Simalungun, North Sumatra, Indonesia, and have been used by the community as traditional medicinal plants. Sarang banua plant is a type of Clerodendrum fragrans *Vent Willd, including the family Verbenaceae. This study aimed to determine the antidiabetic activity of the leaf extract of sarang banua (*C. fragrans *Vent Willd) in white male rats (*Rattus novergicus*) induced by alloxan. This study used a RAL design with seven treatments, namely (K0) standard feed, (K1) Na-CMC 0.5%), (K2) metformin, (K3) ethanol extract 100 mg/kg bw, 200 mg/kg bw (K4), 300 mg/kg bw (5), (K6) ethyl acetate extract 200 mg/kg bw and 300 mg/kg bw (K7). Groups K1 to K7 were induced by alloxan before being given treatment. Each treatment was replicated three times. The results showed that the application of leaf extract of the* C. fragrans *affected on reducing the blood glucose levels of alloxan-induced rats. The used of ethanolic extract of* C. fragrans *100 mg/kg bw resulted in the highest percentage decrease in blood glucose (54.46 ± 5.60%) of hyperglycemic rats induced by alloxan, close to a positive control (56.63 ± 1.86%).*

Keywords: *alloxan, antidiabetic activity,* Clerodendrum fragrans *Vent Willd, Indonesian medicinal plants,* Rattus novergicus

ABSTRAK
Tanaman sarang banua yang terdapat di Simalungun, Sumatera Utara, Indonesia telah dimanfaatkan oleh masyarakat sebagai tanaman obat tradisional. Tanaman Sarang banua (*Clerodendrum fragrans* Vent Willd), termasuk famili Verbenaceae. Penelitian ini bertujuan untuk mengetahui aktivitas antidiabetes ekstrak daun Sarang banua (*C. fragrans* Vent Willd) pada tikus putih jantan (*Rattus novergicus*) yang diinduksi aloksan. Penelitian ini menggunakan rancangan RAL dengan tujuh perlakuan yaitu (K0) pakan standar, (K1) Na-CMC 0,5%), (K2) metformin, (K3) ekstrak etanol 100 mg/kg bb, 200 mg/kg bb (K4), 300 mg/kg bb (5), (K6) ekstrak etilasetat 200 mg/kg bb dan 300 mg/kg bb (K7). Kelompok K1 sampai K7 diinduksi aloksan sebelum diberikan perlakuan. Hasil penelitian menunjukkan bahwa pemberian ekstrak daun *C. fragrans* berpengaruh terhadap penurunan kadar glukosa darah tikus yang diinduksi aloksan. Pemberian ekstrak etanol *C. fragrans* 100 mg/kg bb menghasilkan persentase penurunan glukosa darah tertinggi (54,46 ± 5,60%) tikus hiperglikemik yang diinduksi aloksan, mendekati kontrol positif (56,63 ± 1,86%).

Kata Kunci: aktivitas antidiabetes, aloksan, *Clerodendrum fragrans* Vent Willd, tanaman obat Indonesia, *Rattus novergicus*

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INTRODUCTION

The increase in various diseases such as diabetes, especially during the current covid-19 virus pandemic, can cause a decrease in body health (American Diabetes Association 2014). The number of people with diabetes in Indonesia continues to increase. By 2030 it is estimated that the number will increase to 21.3 million. This data shows that diabetes is one of the non-communicable diseases that need attention from the general public and the government regarding prevention and treatment efforts (Mahargyani 2019), especially during the current covid-19 pandemic. The need for herbal medicines, especially antidiabetic properties, is essential to increase immunity and body health.

Sarang banua plant (*Clerodendrum fragrans* Vent Willd), family Verbenaceae (LIPI Botanical Herbarium, Cibinong, June 2017), is a local plant that is widely available in the Simalungun area and is consumed by the community as traditional medicine (Simorangkir et al. 2019a) (Figure 1). The Sarang banua plant has antioxidant, antibacterial activity and contains secondary metabolites that have medicinal potential (Simorangkir et al. 2019a, Simorangkir et al. 2019b, Sapiun et al. 2020, Simorangkir et al. 2020, Simorangkir et al. 2021). People with diabetics have high blood sugar levels. Flavonoids and sesquiterpenes can inhibit the amylase enzyme, so blood sugar levels decrease (Sasmita et al. 2017). The active compounds suspected of having hypoglycemic activity are flavonoids (Mahargyani 2019). Flavonoid, phenolic, alkaloid, and terpenoid compounds have the potential to lower blood glucose levels. Flavonoids can inhibit glucose reabsorption from the kidneys and increase the solubility of blood glucose which is easily expelled through urine, which causes hypoglycemia (Lukacinova et al. 2008). Sarang banua plants contain alkaloids, steroids, saponins, and tannins (ethyl acetate extract), then flavonoids, triterpenoids, alkaloids, saponins, tannins, and quinones (ethanol extract) (Simorangkir et al. 2021). These contents causes the local plant sarang banua to have the potential as an alternative natural antidiabetic material.

Figure 1. Sarang banua (*Clerodendrum fragrans* Vent Willd) plant

Giving alloxan cause hyperglycemia in rats (Sasmita et al. 2017). Alloxan is commonly used to make diabetic experimental animals (Etuk and Muhammed 2010). Alloxan damage pancreatic cells resulting in impaired insulin production, so the blood glucose levels increase (Ajiboye et al. 2017, Ighodaro et al. 2017). Since the abundant natural product potential in Indonesia, exploration is needed to find alternative medicine as antidiabetic (Zhang et al. 2015, Gothai et al. 2016, Situmeang et al. 2019, Solikhah et al. 2020, Zega et al. 2021, Silaban et al. 2022). This study aimed to investigate the sarang banua leaf extract as an alternative raw material for traditional Indonesian herbal antidiabetic drugs.

MATERIALS AND METHODS

Location and time

This study was performed from April 2021–October 2021 at the Chemistry Laboratory, Faculty of Mathematics and Natural Science, Universitas Negeri Medan, Medan, Indonesia.

Plant sample

Fresh leaves of Sarang banua come from the Simalungun district, Province of North Sumatra, Indonesia. Fresh leaves were washed, dried without sunlight, mashed, and obtained *C. fragrans* simplicia powder.

Leaf extraction

Sarang banua leaves were extracted by maceration using solvents with graded polarity (n-hexane, ethyl acetate, and ethanol). *C. fragrans* Vent

Table 1. Average decrease in serum glucose levels in rats

No	Group	Decrease in Rat Serum Glucose Levels	
		(mg dL^{-1})	(%)
1	K0 (Standard Feed)	5.00	5.54 ± 1.18[a]
2	K1 (Negative control (Alloxan and Lar. Na-CMC 0.5%)	8.33	5.08 ± 0.82[a]
3	K2 Positive control (Alloxan and Metformin)	91.33	56.63 ± 1.86[b]
4	K3 (Alloxan and Ex. Ethanol *C. fragrans* 100mg/kg bw)	90.66	54.46 ± 5.60[b]
5	K4 Alloxan and Ex. Ethanol *C. fragrans* 200mg/kg bw)	77.33	45.40 ± 3.99[b]
6	K5 (Alloxan and Ex. Ethanol *C. fragrans* 300mg/kg bw)	66.00	39.92 ± 2.81[c]
7	K6 (Alloxan and Ex. Ethyl acetate *C. fragrans* 200mg/kg bw)	22.33	14.37 ± 1.99[a]
8	K7 (Alloxan and Ex. Ethyl acetate *C. fragrans* 300mg/kg bw)	38.00	23.21 ± 2.15[d]

Description: Values are shown as Mean ± SD, n=3. Different superscripts in the same vertical row showed significant differences ($P < 0.05$)

Willd Leaf Extraction Process shown in Figure 2. Each macerate was filtered and evaporated to obtain a concentrated extract (Simorangkir et al. 2021). In vivo antidiabetic test was performed on alloxan-induced white rats against ethyl acetate and ethanol extract.

Experimental animal preparation

The experimental animals used were male Wistar rats aged 6-8 weeks, body weight ± 150 - 200 g, and in healthy condition (active and not disabled). The experiment consisted of 8 groups, and each experimental group used three rats, so the total number of research subjects was 24. This research was permitted by Animal Research Ethics Committees/AREC, USU FMIPA No. 0413/KEPH-FMIPA/2021, 8 July 2021.

Alloxan monohydrate solution preparation

The dose of alloxan in rats to cause diabetes is 125 mg/kg bw inter peritoneal (IP) (Simorangkir and Hutapea 2016). A total of 0.125 g of alloxan monohydrate was dissolved with 0.9% NaCl in a 10 mL bottle.

Experimental procedure

White male rats were acclimatized for one week. On the 7th-day, initial blood was taken and on the same day, 1.25% alloxan was injected as much as 0.1 mL, except in the control group (K0). After three days of alloxan injection, the rat's blood was taken, and the rat's blood glucose was measured. Furthermore, the rats were given *C. fragrans* extract daily for 14 days and metformin for the positive control rat group. The treatments were: K0 (normal feed, without alloxan and without extract), K1 (alloxan and Na-CMC 0.5%), K2 (alloxan and metformin), K3 (alloxan and ethanol extract of *C. fragrans* 100 mg/kg bw, K4 (alloxan and ethanol extract 200 mg/kg bw), K5 (alloxan and ethanol extract 300 mg/kg bw, K6 (alloxan and ethyl acetate extract 200 mg/kg bw, K7 (alloxan and ethyl acetate extract 300 mg/kg bw). On day 22, the blood of rats was taken, serum was prepared and glucose levels were examined. The data obtained were analyzed by ANOVA followed by the LSD test.

RESULTS AND DISCUSSION

After three days of alloxan administration, there was an increase in rat serum glucose except in the control group (K0), and after the application of *C. fragrans* extract, there was a decrease in blood glucose, except in the control group (K0) and the negative control group K1 (Na-CMC 0.5 %) (Figure 3).

Alloxan induces hyperglycemia in rats (Sasmita et al. 2017, Sinaga et al. 2021). In this study, hyperglycemia occurred in rats after 72 hours of injection of 120 mg/kg bw via inter peritoneal. Alloxan is an essential ingredient used to make diabetic

experimental animals in rats, mice, rabbits, and dogs (Etuk and Muhammed 2010). The mechanism of alloxan is to damage pancreatic ß-cells, starting with alloxan entering through the glucose transporter GLUT2 and reacting with glutathione. The dialuric acid and other products in the form of reactive oxygen species (ROS) in the form of superoxide radicals, hydrogen peroxide, and finally hydroxyl radicals resulting from the alloxan application process. ß-pancreatic cells have meager antioxidant defense capabilities, so that hydroxyl radicals cause death/damage to ß-pancreatic cells (Lenzen 2008, Sharma et al. 2013), resulting in decreased insulin production. This increase in blood glucose levels (Ajiboye et al. 2017).

The application of *C. fragrans* leaf extract had a significant effect on decreasing serum glucose ($p < 0.01$ (Table 1). The application of *C. fragrans* ethanol extract 100 mg/kg bw showed the highest decrease in serum glucose (54.46 ± 5.60%), not different from the administration of metformin 56.63 ± 1.86% ($p > 0.01$), but significantly different from other treatments (< 0.05). The selection of mice as experimental animals for hyperglycemia is due to the similarity of the structure and physiology of the pancreas of mice with humans (Dolensek et al. 2015).

Previous studies reported that histopathological damage to the diabetic pancreas was indicated by changes in the shape of the pancreas in the form of shrinkage and reduction in the size of the islets of Langerhans and in addition a swelling of the cell nucleus in the islets of Langerhans (Khamchan et al. 2018).

The application of metformin 5 mg/kg bw in hyperglycemic rats showed a decrease in serum glucose levels (Tabel 1 and Figure 3). Metformin is an oral hypoglycemic sulfonylurea derivative that works actively lower blood glucose levels (Balsells et al. 2015, Johns et al. 2018). Metformin works mainly in increasing insulin secretion and repair of pancreatic ß-cells. ATP-sensitive potassium channels in pancreatic ß-cells are inhibited by metformin, so that cell membrane is depolarized, causing the opening of voltage-dependent calcium channels. This causes Ca^{2+} to enter the cytosol so that intracellular calcium increases in pancreatic ß-cells, stimulating insulin release and pancreatic ß-cell repair (Balsells et al. 2015, Lorincz et al. 2018). Application of *C. fragrans* extract at treatment of 100 mg/kg

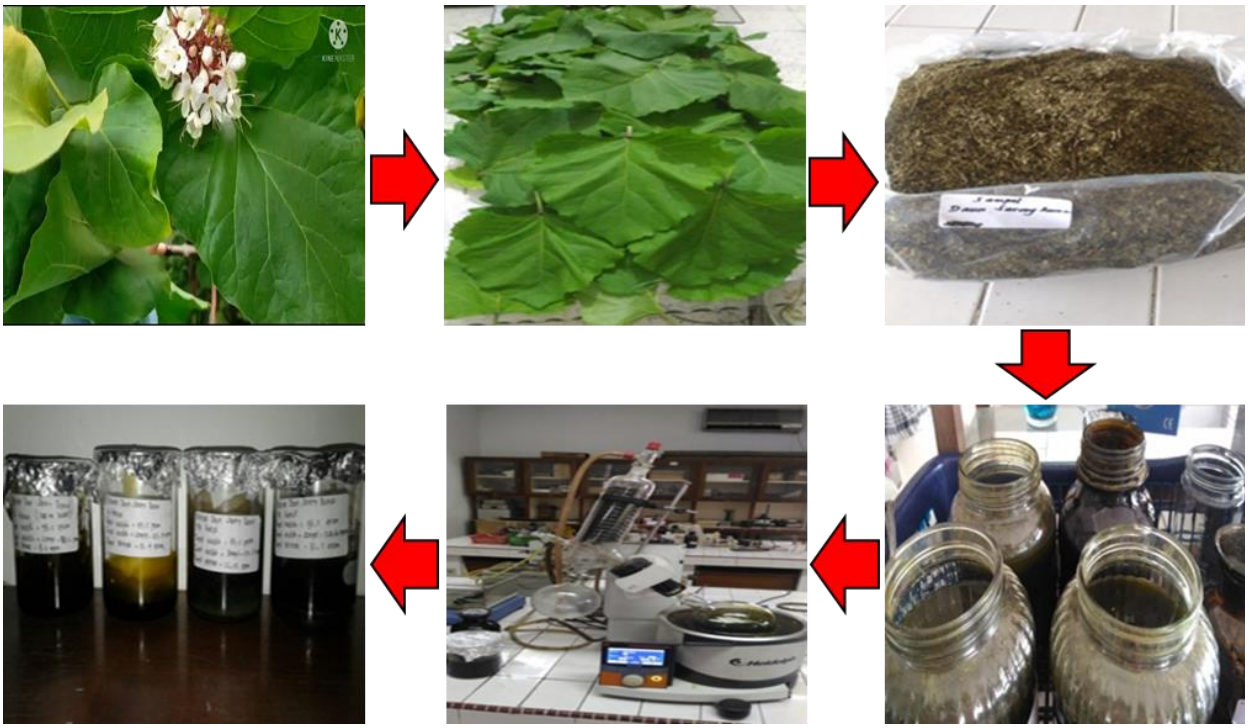

Figure 2. *C. fragrans* Vent *Willd* leaf extraction process

bw in hyperglycemic rats has shown improvement towards normal. However, the damage is still visible, namely the abnormal shape of the island of Langerhans. Yet at this dose, there was an improvement in pancreatic cells compared to the pancreas of hyperglycemic rats. Meanwhile, a treatment of 100 mg/kg bw showed a histological picture similar to that of the metformin and control groups. This indicates that the treatment of 100 mg/kg bw is better than 200 or 300 mg/kg bw and ethyl acetate extract of *C. fragrans* 200 and 300 mg/kg bw. The application of polyphenolic extract of *S. cumini* in hyperglycemic rats increase the activity of superoxide dismutase, catalase, and glutathione peroxidase (a natural antioxidant) against oxidative stress, which causes pancreatic cell repair (Ibitoye and Muhammed 2018).

The function of pancreatic ß-cells to secrete insulin and reduce oxidative stress can also be improved by the flavonoids quercetin, gallic acid, and anthocyanins from *S. samarangense* (Khamchan et al. 2018). In this study, the ability of *C. fragrans* leaf extract to reduce blood glucose was possible because of its phytochemical content and antioxidant activity that can bind hydroxyl radicals and reduce oxidative stress that causes pancreatic ß-cell death in hyperglycemic rats, thus providing the ability to repair and maintain pancreatic ß-cells damaged by alloxan treatment.

CONCLUSION

The application of the leaves extract of sarang banua (*C. fragrans* Vent Willd) had a significant effect on reducing blood glucose levels of hyperglycemic rats induced by alloxan. The treatment of ethanolic extract of *C. fragrans* 100 mg/kg bw resulted in the highest percentage decrease in blood glucose (54.46 ± 5.60%) of hyperglycemic rats induced by alloxan, close to positive control (56.63 ± 1.86%).

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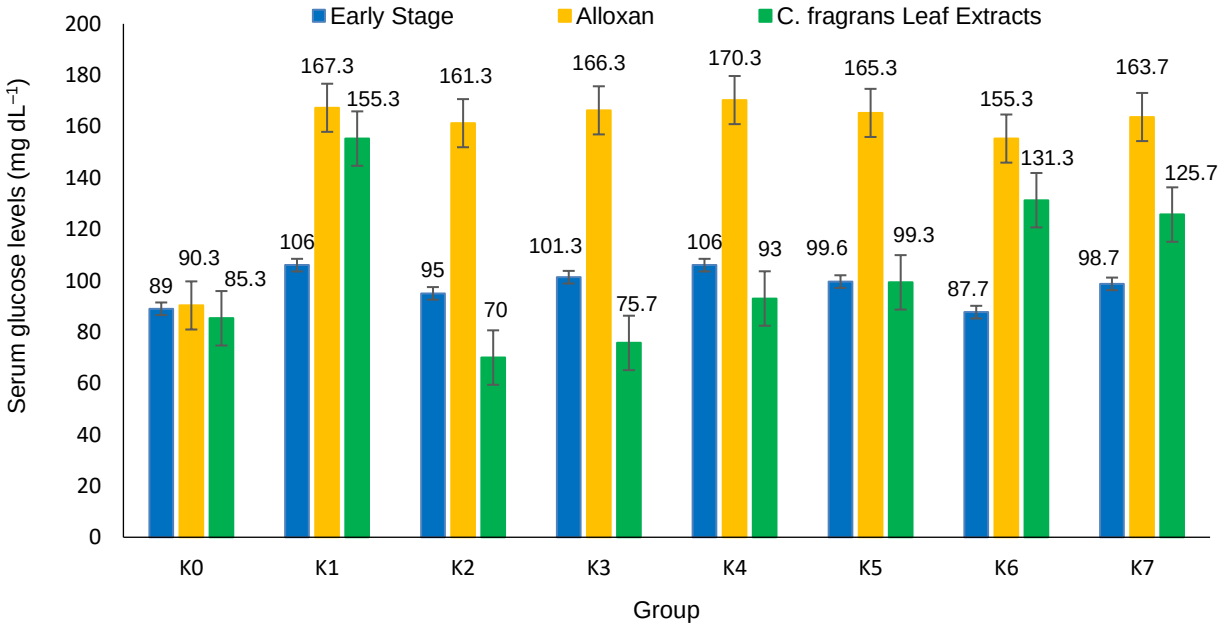

Figure 3. Serum glucose levels of rat at an early stage, alloxan and *C. fragrans* leaf extracts treatments. K0 (Standard Feed), K1 Negative control (Alloxan and Lar. Na-CMC 0.5%), K2 Positive control (Alloxan and Metformin), K3 (Alloxan and Ex. ethanol *C. fragrans* 100 mg/kg bw), K4 (Alloxan and Ex. ethanol *C. fragrans* 200 mg/kg bw), K5 Alloxan and Ex. ethanol *C. fragrans* 300 mg/kg bw), K6 Alloxan and Ex. ethyl acetate *C. fragrans* 200 mg/kg bw) and K7 (Alloxan and Ex. ethyl acetate *C. fragrans* 300 mg/kg bw)

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