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Antidiabetic Activity Test of Ethanol Extract of Bandotan Leaves (*Ageratum conyzoides* L.) in Alloxan-Induced White Rats

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Abstract

Diabetes mellitus (DM) is a chronic metabolic disorder characterized by elevated blood glucose levels, causing significant morbidity and mortality globally. Indonesia is one of the countries with the highest prevalence of diabetes. This study aimed to evaluate the antidiabetic activity of ethanol extract from Bandotan leaves (*Ageratum conyzoides* L.) in alloxan-induced diabetic white rats. This experimental research used 30 white rats divided into six groups: normal control (distilled water), negative control (distilled water+alloxan), positive control (glibenclamide 0.65 mg/kg BW+alloxan), and three groups receiving ethanol extract of Bandotan leaves (EEBL) at doses of 100, 200, and 400 mg/kg BW with alloxan induction. Extraction of Bandotan leaves was carried out using the maceration method using 96% ethanol, resulting in a yield of 37,35%. Phytochemical screening showed the extract contained alkaloids, steroids, quinones and saponins. Induction of diabetes was carried out by intraperitoneal injection of alloxan 30mg/kgBB. Blood sugar levels were measured on days 5, 10, 15, 20, 25, 30 after induction. The results showed that administration of EEDB significantly ($p<0,005$) reduced blood sugar levels with the best effect at a dose of 400mg/kgBB. The ethanol extract of bandotan leaves was proven to have effective antidiabetic activity in alloxan-induced mice, with the potential for development as an affordable and easily accessible alternative diabetes therapy.

Keywords: *Ageratum conyzoides* L., antidiabetic, alloxan, ethanol extract, hyperglycemia

Abstrak

Diabetes melitus (DM) merupakan gangguan metabolik kronis yang ditandai dengan peningkatan kadar glukosa darah, menyebabkan morbiditas dan mortalitas signifikan secara global. Indonesia termasuk negara dengan prevalensi diabetes tertinggi. Penelitian ini bertujuan untuk mengevaluasi aktivitas antidiabetes ekstrak etanol daun bandotan (Ageratum conyzoides L.) pada tikus putih yang diinduksi aloksan, yang menggunakan 30 tikus putih dibagi menjadi enam kelompok: kontrol normal (aquades), kontrol negatif (aquades+aloksan), kontrol positif (glibenklamid 0,65 mg/kg BB+aloksan), dan tiga kelompok ekstrak etanol daun bandotan (EEDB) dengan dosis 100, 200, dan 400 mg/kg BB yang diinduksi aloksan. Ekstraksi daun bandotan dilakukan dengan metode maserasi menggunakan etanol 96%, menghasilkan rendemen 37,35%. Skrining fitokimia menunjukkan ekstrak mengandung alkaloid, flavonoid, tanin, steroid, kuinon, dan saponin. Induksi diabetes dilakukan dengan injeksi aloksan 30 mg/kg BB secara intraperitoneal. Pengukuran kadar gula darah dilakukan pada hari ke-5, 10, 15, 20, 25, dan 30 setelah induksi. Hasil penelitian menunjukkan bahwa pemberian EEDB secara signifikan ($p<0,05$) menurunkan kadar gula darah dengan efek terbaik pada dosis 400 mg/kg BB. Ekstrak etanol daun bandotan terbukti memiliki aktivitas antidiabetes yang efektif pada tikus yang diinduksi aloksan, dengan potensi pengembangan sebagai alternatif terapi diabetes yang terjangkau dan mudah diakses.

Kata kunci: *Ageratum conyzoides L., antidiabetes, aloksan, ekstrak etanol, hiperglikemia*

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INTRODUCTION

Diabetes Mellitus (DM) is a chronic metabolic disorder marked by elevated blood glucose levels due to impaired insulin secretion or action, leading to serious health impacts worldwide[1]. Type 2 diabetes, the most prevalent form in adults, causes complications in multiple organs and presents a growing global health and economic challenge[2]. The limitations of current synthetic antidiabetic drugs — including side effects, cost, and adherence issues—have driven interest in plant-based alternatives with fewer adverse effects. Natural compounds are increasingly studied for diabetes management, showing

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mechanisms such as enhanced insulin secretion, improved glucose absorption, and inhibition of carbohydrate-digesting enzymes[3,4]. One underexplored plant is *Ageratum conyzoides* L. (bandotan), a common weed with notable traditional medicinal uses. Native to tropical and subtropical regions, this plant has been traditionally used to treat infections[5], wounds[6], and inflammation[7].

Bandotan leaves are rich in bioactive compounds like alkaloids, flavonoids, tannins, saponins, and steroid/ terpenoids. Flavonoids and alkaloids, in particular, have antioxidant properties that protect and regenerate pancreatic β-cells, potentially restoring insulin production. Compounds such as precocene I and II, ageratochromene, and coumarin derivatives also contribute to its anti-inflammatory and antioxidative effects[8].

Although initial studies show the hypoglycemic potential of *Ageratum conyzoides* L., research on its efficacy, dosage, and mechanisms is still limited. This study used alloxan-induced diabetic mice to evaluate the antidiabetic effects of its ethanol leaf extract. By simulating type 1 diabetes through β-cell damage, the study assessed the extract's effectiveness and dose-response, supporting the potential of bandotan as a natural diabetes treatment[9].

METHODOLOGY

This study took place at the Integrated Laboratory of Prima Indonesia University, Medan. Bandotan leaves were collected from Jl. Marelan VI, cleaned, dried, and re- sorted before extraction. Research steps included extraction, phytochemical screening, animal preparation, alloxan-induced diabetes, treatment, and blood sugar measurement.

Tools and Materials

The tools used included analytical scales, blenders, beakers, pipettes, rotary evaporator, water bath, glucometer, and related lab equipment. Materials consisted of *Ageratum conyzoides* L. leaves (4–5 kg), distilled water, 96% ethanol, n-hexane, test animals and feed, alloxan, glibenclamide, syringes, filter paper, and reagents such as HCl, H_2SO_4, Mg powder, $FeCl_3$, ethyl acetate, Mayer's, Dragendorff's, and Bouchardat's reagents.

Preparing the Sample

Sample preparation begins with collecting fresh and clean bandotan leaves, then washing them with running water. After that, the samples were dried in a drying cabinet and ground using a blender. The ground samples were carried out by a maceration process using 96% ethanol, which was soaked for 3 times 24 hours. The extract is filtered using filter paper, then evaporates the solvent is evaporated using a rotary evaporator at a temperature of 60°C[10,11].

The yield of the extract is calculated using the formula:

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Yield (%) = (Weight of extract obtained / Weight of dry simplicia) × 100%

Preparation of Test Animals

This study used healthy adult white rats (*Rattus norvegicus*) that were 8-12 weeks old and weighed 150-200 grams. The rats were acclimatized for 7 days with unlimited food and water, and their weight was taken every day[12].

Diabetes Induction and Treatment Administration

Diabetes is induced by administering 30 mg/kg body weight of alloxan monohydrate intraperitoneally following an 8-hour fast. After that, the rats are fed and given 10% glucose to drink for two days before being replaced with ordinary water. Diabetic rats have fasting blood sugar levels of more than 200 mg/dL.The dose is estimated using the human to rat conversion factor (0.018) for rats weighing 200 g. Aloxan dosage is 0.54 mg per rat (30 mg x 0.018). Bandotan leaf extract doses are 1.8 mg (100 mg/kg), 3.6 mg (200 mg/kg), and 7.2 mg (400 mg/kg). The extract was given orally for 30 days via a gastric tube[13].

Blood Sugar Level Measurement

Blood sugar levels were measured on days 5, 10, 15, 20, 25, and 30 after diabetes induction by collecting blood from the rat's tail tip. A glucometer was used, displaying results within 10 seconds. The main parameter observed was the change in blood sugar levels before and after administering the ethanol extract of bandotan leaves.

Data Analysis

The data was analyzed using One-Way ANOVA to determine differences between groups, followed by post-hoc testing and Independent T- tests to compare two groups. The study was performed using SPSS version 25 (MacOS) with a 95% confidence level (α= 0.05). Data is given as tables and graphs. The efficiency of blood glucose reduction is determined by the percentage drop in blood sugar levels, which is then compared to normal, negative, and positive control groups.

Tabel 1. Grouping of Test Animals And The Treatments Given

Group	O	Treatment
1	-	Normal control: Aquades
2	K	Negative control: Alloxan 30mg/kg body weight
3	K	Positive control: Glibenclamide 0.65mg/kg body weight + Alloxan
4	K	EEDB 100 mg/kg body weight + Alloxan
5	-	EEDB 200 mg/kg body weight + Alloxan
6	K	EEDB 400 mg/kg body weight + Alloxan

Note: EEDB = Ethanol Extract of Bandotan Leaves

RESULT AND DISCUSSION

Plant Identification

The plant used in this research is Bandotan leaves (*Ageratum conyzoides* L.)

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which have been identified at the Integrated Laboratory of Prima Indonesia University.

Extraction of Bandotan Leaves (Ageratum conyzoides L.)

Bandotan leaves are extracted by collecting young and old leaves, washing 4.7 kg of leaves, drying for two days, and grinding into powder. A total of 500 grams of powder were macerated in 5 liters of 96% ethanol for three days, with stirring every 15 minutes. A thick extract of 186.79 grams was obtained by evaporation with a rotary evaporator (60°C) and a water bath (90°C).

Yield Calculation

The calculation of the yield of ethanol extract from bandotan leaves resulted in the following outcomes:

Table 2. Calculation of The Yield of Ethanol Extract from Bandotan Leaves

Sample	Wet Simplicia Weight	Dry Simplicia Weight	Extract Weight	% Yield
Bandotan Leaves (*Argeratum conyzoides* L.)	4.7 kg	500 gr	186.79 gr	37.35%

Results of Phytochemical Screening Test

Phytochemical screening shows that the ethanol extract of bandotan leaves (*Ageratum conyzoides* L.) contains various secondary metabolite compounds as follows:

Tabel 3. Phytochemical Screening Shows That The Ethanol Extract of Bandotan Leaves (*Ageratum conyzoides* L.)

No	Phytochemical Test	Reagents	Observations	Results
1	Alkaloid	Dragenfroff	Red deposits formed	+
2	Flavonoid	Mg + Thick HCL	Dark red color formed	+
3	Tanin	FeCL3	Blackish green color formed	+
4	Steroid	H2SO4 2N	Green color formed	+
5	Quinon	NaOH	Blackish red-orange color formed	+
6	Saponin	HCL 1M	Stable foam formed in approximately 10 minutes	+

Note: (+) = Contains a group of secondary metabolite compounds

Blood Sugar Level Test

The study found that an extract of bandotan leaves (*Ageratum conyzoides* L.) is effective at lowering blood sugar levels in white rats stimulated with alloxan. In group K-IV (100 mg/kg body weight), blood sugar levels dropped from 243 mg/dL to 157 mg/dL. After 30 days of extract administration, group K-V (200 mg/kg body weight) decreased from 216 mg/dL to 152 mg/dL, whereas group K- VI (400 mg/kg body weight) decreased from 236 mg/dL to 150 mg/dL. This demonstrates the hypoglycemic effects of bandotan leaf extract[14]. Diabetes mellitus is a metabolic

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illness that can cause long-term problems such as neuropathy and vascular issues[15]. Uncontrolled diabetes can cause hyperglycemia, which can harm numerous body systems such as blood vessels and neurons. Diabetes is classified into Types 1 and 2, with Type 2 induced by insulin resistance and Type 1 by insulin shortage. Type 2 diabetes is diagnosed based on blood pressure <140/<90 mmHg and specific blood glucose levels[16].

The bandotan leaf (Ageratum conyzoides L.) includes health- promoting chemical components such as terpenoids, flavonoids, and alkaloids and has the potential to be used as a traditional medicine[17]. Bandotan an activities possesses antibacterial and anti-hemorrhagic properties and has the ability to treat disorders including fever, rheumatism, and diabetes. In this investigation, glibenclamide was employed as a positive control to treat diabetes mellitus, increasing insulin release via potassium channels in pancreatic beta cells[18].

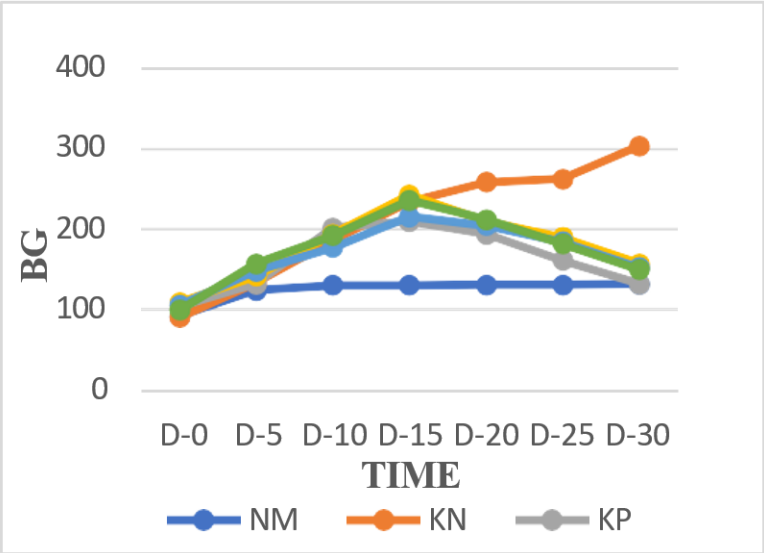

Figure 1. Graph of Blood Sugar Level Decrease

The ethanol extract test of bandotan leaves (*Ageratum conyzoides* L.) resulted in a significant drop in blood sugar levels in white rats induced with alloxan. Over 30 days, group K-IV (100 mg/kg BW) saw a decline from 243 mg/dL to 157 mg/dL, K-V (200 mg/kg BW) from 216 mg/dL to 152mg/dL, and K-VI (400 mg/kg BW) from 236 mg/dL to 150 mg/dL. This drop reveals the bandotan extract's hypoglycemic potential when compared to the negative control (K-II), and it is comparable to the efficacy of the positive control glibenclamide (K- 3). A study of 30 rats found that an ethanolic extract of bandotan leaves is helpful at lowering blood sugar levels. After 30 days of treatment, a dose of 100 mg/kg body weight lowered blood

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sugar from 243 mg/dL to 157 mg/dL; a dose of 200 mg/kg body weight reduced it from 216 mg/dL to 152 mg/dL; and a dose of 400 mg/kg body weight reduced it from 236 mg/dL to 150 mg/dL. Statistical research revealed substantial decreases at a dose of 200 mg/kg body weight on day 20 and 400 mg/kg body weight on day 30, with active chemicals in bandotan leaves believed to lower blood sugar via free radical scavenging. A dose of 400 mg/kg body weight proved to be the most effective in controlling hyperglycemia[19,20]

Data Analysis

Data analysis was conducted using One Way ANOVA technique in SPSS with Shapiro-Wilk test to check the data normality. The results of the analysis showed at table 4.

Table 4. Shapiro-Wilk Test Results

Treatment	N*A	NC*B	PC*C	EEDB 100*D mg/kgBW	EEDB 200*E mg/kgBW	EEDB 400*F mg/kgBW	P - Value
Aloxan-Induced	130.20 ± 21.92*	233.60 ± 71.74	210.20 ± 75.29	243.00 ± 37.59*	215.80 ± 58.08	235.60 ± 56.64	0.045
EEDB 100 mg/KgBW D20	131.40 ± 20.83*	258.80 ± 55.82*	194.00 ± 64.15	210.40 ± 49.12	204.60 ± 60.23	212.00 ± 50.70	0.026
EEDB 200 mg/KgBW D25	131.40 ± 20.83*	263.00 ± 57.85*	160.80 ± 43.40*	189.80 ± 47.36	184.40 ± 48.28	182.00 ± 41.53	0.004
EEDB 400 mg/KgBW D30	132.00 ± 21.72*	303.80 ± 67.62*	132.40 ± 35.20*	156.80 ± 39.73*	151.60 ± 51.40*	149.80 ± 42.61*	0.000

The data show significant results (P<0.05) on day 25 and day 30 after the administration of EEDB at doses of 200 mg/kg body weight and 400 mg/kg body weight. A Post Hoc LSD analysis was performed to identify treatment groups that showed significant differences.

Note:

A = Significantly different from the normal group (K-I) (P<0.005)
B = Significantly different from the negative control (K-II) (P<0.005)
C = Significantly different from the positive control (K-III) (P<0.005)
D = Significantly different from the dose of 100 mg/kg body weight (K-IV) (P<0.005)
E = Significantly different from the dose of 200 mg/kg body weight (K-V) (P<0.005)
F = Significantly different from the dose of 400 mg/kg body weight (K-VI) (P<0.005)

CONCLUSION

The ethanol extract of bandotan (*Ageratum conyzoides* L.) leaves significantly reduced blood sugar levels in alloxan-induced rats, with the highest dose of 400 mg/kgBW showing the most substantial effect (P<0.05). This dose demonstrated a dose-dependent antihyperglycemic effect. The antidiabetic potential is attributed to its phytochemical content, particularly flavonoids and alkaloids, which may support β-cell regeneration and enhance insulin secretion. These findings support

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bandotan's traditional use in herbal medicine and suggest it as a potential natural alternative for managing diabetes. Further studies are needed to investigate its mechanism, long-term safety, and efficacy in humans.

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Nusantara Scientific Medical Research Journal (NSMRJ)
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